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Manisha Talim

Breast-Feeding and Risk for Childhood Obesity: Response to Mayer-Davis et al.
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Errata

Diabetes Care 2007 30: 455.
Physical Activity in U.S. Adults With Diabetes and At Risk for Developing Diabetes, 2003

Elaine H. Morrato, MPH, DrPH
James O. Hill, PhD
Holly R. Wyatt, MD
Vahram Ghushchyan, PhD
Patrick W. Sullivan, PhD

OBJECTIVE — Given the risk of obesity and diabetes in the U.S., and clear benefit of exercise in disease prevention and management, this study aimed to determine the prevalence of physical activity among adults with and at risk for diabetes.

RESEARCH DESIGN AND METHODS — The Medical Expenditure Panel Survey is a nationally representative survey of the U.S. population. In the 2003 survey, 23,283 adults responded when asked about whether they were physically active (moderate or vigorous activity, ≥30 min, three times per week). Information on sociodemographic characteristics and health conditions were self-reported. Additional type 2 diabetes risk factors examined were age ≥45 years, non-Caucasian ethnicity, BMI ≥25 kg/m², hypertension, and cardiovascular disease.

RESULTS — A total of 39% of adults with diabetes were physically active versus 58% of adults without diabetes. The proportion of active adults without diabetes declined as the number of risk factors increased until dropping to similar rates as people with diabetes. After adjustment for sociodemographic and clinical factors, the strongest correlates of being physically active were income level, limitations in physical function, depression, and severe obesity (BMI ≥40 kg/m²). Several traditional predictors of activity (sex, education level, and having received past advice from a health professional to exercise more) were not evident among respondents with diabetes.

CONCLUSIONS — The majority of patients with diabetes or at highest risk for developing type 2 diabetes do not engage in regular physical activity, with a rate significantly below national norms. There is a great need for efforts to target interventions to increase physical activity in these individuals.


The incidence of diagnosed diabetes increased 41% between 1997 and 2003, with rising obesity a major contributing factor (1). Physical activity is a cornerstone of lifestyle modifications aimed at preventing and managing type 2 diabetes and its related morbidities (2). Epidemiological studies have shown that physical activity reduces the risk of type 2 diabetes by 30% in the general population (3). Evidence from randomized controlled trials (4,5) has demonstrated that maintenance of modest weight loss through physical activity and diet reduces the incidence of type 2 diabetes in high-risk individuals by as much as 40–60% over 3–4 years. The risk of mortality among individuals with diabetes is also inversely related to fitness level (6,7).

Regular activity is also an important component in public health efforts addressing the rising obesity epidemic and is one of the leading Healthy People 2010 indicators in the U.S. (8–10). The Surgeon General’s Report on Physical Activity and Health (11) outlined the health benefits of physical activity, which include not only achieving weight reduction and reducing the risk of developing diabetes but also reducing the risk of developing high blood pressure and dying from heart disease and enhancing overall psychological well being. Recent evidence suggests that aerobic exercise at levels consistent with public health recommendations is as effective as antidepressant medications in treating mild to moderate depression (12), a common comorbidity affecting approximately one-quarter of patients with diabetes (13) and hindering optimal diabetes self-care (14).

In 2003, an estimated 46% of Americans achieved recommended levels of daily moderate physical activity (15), which is nearing the 2010 goal of 50% (10). However, data on the prevalence of inactivity in people with diabetes and at highest risk for developing type 2 diabetes is limited (14,16–18). In a large health maintenance organization, 29% of patients with diabetes engaged in physical activity (≥30 min) once a week or less (14). In a survey of adults aged ≥55 years with type 2 diabetes, 55% of respondents reported no weekly physical activity (17). Recent data (18) from the National Health and Nutrition Examination Survey found that less than one-third of diabetic adults who can exercise voluntarily met recommended levels of physical activity. Yet, the awareness of the need for physical activity appears high among adults with diabetes, as approximately three-quarters recalled having been told at least once by a health care professional that they needed to exercise more (19).

The purpose of this research was to evaluate the prevalence of physical activity among all adults with diabetes and at risk for developing diabetes using a recent nationally representative sample and, importantly, to identify patient characteristics associated with the likelihood of being physically active.

RESEARCH DESIGN AND METHODS — The Medical Expenditure Panel Survey (MEPS) is cosponsored by the Agency for Healthcare Research
and Quality and the National Center for Health Statistics and is a nationally representative survey of the U.S. civilian non-institutionalized population, collecting detailed information on demographic characteristics, income and education status, and self-reported health conditions and use of medical care services (20).

The sampling frame for the MEPS Household Component is drawn from respondents to the National Health Interview Survey. The MEPS supplements and validates information on medical care and pharmacy events at the person level. Medical condition diagnoses are based on ICD-9-CM codes (21,22). The sample design of the MEPS includes stratification, clustering, multiple stages of selection, and disproportionate sampling (23). MEPS sampling weights incorporate adjustment for the complex sample design and reflect survey nonresponse and population totals from the Current Population Survey (23). Adult respondents to the year 2003 survey who reported about their physical activity were eligible for this study. Of 23,519 adult participants (aged ≥18 years) in 2003, 23,283 (99%) responded when asked about their physical activity.

### Table 1—Physical activity recommendations

<table>
<thead>
<tr>
<th>Population</th>
<th>Physical activity measures</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>All adults</td>
<td>Reduce the proportion of adults who engage in no leisure-time physical activity.</td>
<td>Healthy People 2010 Physical Activity and Fitness Objectives (10)</td>
</tr>
<tr>
<td></td>
<td>Increase the proportion of adults who engage regularly, preferably daily, in moderate physical activity for at least 30 min per day.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increase the proportion of adults who engage in vigorous physical activity that promotes the development and maintenance of cardiorespiratory fitness ≥3 days per week for ≥20 min per occasion.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increase the proportion of adults who perform physical activities that enhance and maintain muscular strength.</td>
<td></td>
</tr>
<tr>
<td>All adults</td>
<td>Recommended physical activity: moderate-intensity activities in a usual week (i.e., brisk walking, bicycling, vacuuming, gardening, or anything else that causes small increases in breathing or heart rate) for at least 30 min per day, at least 5 days per week; or vigorous-intensity activities in a usual week (i.e., running, aerobics, heavy yard work, or anything else that causes large increases in breathing or heart rate) for at least 20 min per day, at least 3 days per week or both. This can be accomplished through lifestyle activities (i.e., household, transportation, or leisure-time activities). Insufficient physical activity: ≥10 min total per week of moderate- or vigorous-intensity lifestyle activities but less than the recommended level of activity. Inactivity: &lt;10 min total per week of moderate- or vigorous-intensity lifestyle activities.</td>
<td>Centers for Disease Control and Prevention (50)</td>
</tr>
<tr>
<td>Prevention/delay of type 2 diabetes</td>
<td>Modest physical activity (30 min daily).</td>
<td>2006 Standards of Medical Care in Diabetes (2)</td>
</tr>
<tr>
<td>Diabetes management</td>
<td>To improve glycemic control, assist with weight maintenance, and reduce risk of cardiovascular disease, at least 150 min per week of moderate-intensity aerobic physical activity (50–70% of maximum heart rate) is recommended and/or at least 90 min per week of vigorous aerobic exercise (&gt;70% of maximum heart rate). The physical activity should be distributed over at least 3 days per week and with no more than 2 consecutive days without physical activity. In the absence of contraindications, people with type 2 diabetes should be encouraged to perform resistance exercise three times a week, targeting all major muscle groups, progressing to three sets of 8–10 repetitions at a weight that cannot be lifted &gt;8–10 times.</td>
<td>2006 Standards of Medical Care in Diabetes (2)</td>
</tr>
</tbody>
</table>
Physical activity

To ascertain physical activity, all adult respondents were asked if they “spend half an hour or more in moderate or vigorous physical activity at least three times a week.” The general context of the questionnaire is “on average.” The MEPS glossary states, “moderate physical activity causes only light sweating or a slight or moderate increase in breathing or heart rate and would include activities such as fast walking, raking leaves, mowing the lawn, or heavy cleaning. Vigorous physical activity causes heavy sweating or large increases in breathing or heart rate and would include activities such as running, race walking, lap swimming, aerobic classes, or fast bicycling” (24). The MEPS criterion for physical activity (level and duration) was consistent with 2003 recommendations for a “regular physical activity program, adapted to the presence of complications” (25) but less stringent than current public health measures (Table 1). Self-reported physical activity has been shown to have moderate validity in other national surveys (26).

Table 2—Unadjusted rates of self-reported physical activity among U.S. adults*

<table>
<thead>
<tr>
<th>Selected characteristics</th>
<th>Unweighted</th>
<th>Physically active</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All adults</td>
<td>23,226</td>
<td>56.4 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>12,649</td>
<td>52.8 ± 0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>10,577</td>
<td>60.3 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Age-groups (year)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–29</td>
<td>5,555</td>
<td>62.3 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30–39</td>
<td>4,566</td>
<td>57.7 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>40–49</td>
<td>4,689</td>
<td>55.8 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>50–59</td>
<td>3,564</td>
<td>54.9 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>60–69</td>
<td>2,265</td>
<td>55.9 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>70–79</td>
<td>1,723</td>
<td>52.2 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>⩾80</td>
<td>864</td>
<td>36.8 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>18,234</td>
<td>57.5 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Black</td>
<td>3,778</td>
<td>50.9 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>5,940</td>
<td>49.5 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>994</td>
<td>54.5 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Geographic region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northeast</td>
<td>3,434</td>
<td>55.5 ± 1.3</td>
<td>0.01</td>
</tr>
<tr>
<td>South</td>
<td>9,109</td>
<td>54.5 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Midwest</td>
<td>4,631</td>
<td>57.6 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>West</td>
<td>6,052</td>
<td>59.0 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Education levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than high school</td>
<td>6,181</td>
<td>48.3 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High school</td>
<td>11,153</td>
<td>56.0 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Some college (&lt;4 years)</td>
<td>1,440</td>
<td>59.2 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>College degree (4 years)</td>
<td>2,899</td>
<td>61.7 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Graduate school (&gt;4 years)</td>
<td>1,407</td>
<td>64.7 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Income level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>3,986</td>
<td>46.9 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Near poor</td>
<td>1,351</td>
<td>46.9 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>Low income</td>
<td>3,832</td>
<td>52.3 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Middle income</td>
<td>6,873</td>
<td>56.2 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>High income</td>
<td>7,184</td>
<td>61.4 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (18.5–24.9)</td>
<td>8,079</td>
<td>63.0 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Overweight (25.0–29.9)</td>
<td>7,977</td>
<td>58.4 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Obese, classes 1 and 2 (30.0–39.9)</td>
<td>5,290</td>
<td>47.3 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Obese, class 3 (&gt;40)</td>
<td>835</td>
<td>34.1 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>21,047</td>
<td>57.6 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>2,179</td>
<td>45.1 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Physical functioning limitations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>19,076</td>
<td>60.1 ± 0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>4,128</td>
<td>38.5 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Ever advised to exercise more</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>7,957</td>
<td>49.0 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14,997</td>
<td>60.7 ± 0.7</td>
<td></td>
</tr>
</tbody>
</table>

Data are % ± SE or n. Tests were conducted to test for variation in rates of physical activity across subgroups.

*All data are based on the Medical Expenditure Panel Survey, 2003.

Ascertainment of diabetes and diabetes risk factors

Self-reported information from the MEPS survey was used to determine whether a respondent had diabetes or risk factors for developing type 2 diabetes. Respondents were asked if they had ever been diagnosed with diabetes (excluding gestational diabetes). Adults with type 2 diabetes were not differentiated from type 1 diabetes, although it is estimated that >90% of adults with diabetes have type 2 diabetes (27). For type 2 diabetes risk factors, we selected clinical and demographic variables available in the MEPS survey, which were included in the American Diabetes Association’s list of risk factors (28). In addition to physical inactivity, other risk factors included age ≥45 years, non-Caucasian ethnicity, BMI ≥25 kg/m², diagnosis of hypertension (diagnosed on two or more different medical visits with high blood pressure), and history of cardiovascular disease (diagnosed with angina or angina pectoris, heart attack or myocardial infarction, or stroke or any other kind of heart disease or condition).

In the analyses, we defined cardiovascular risk factors as the presence of one or more of the following clinical conditions: history of cardiovascular disease, a diagnosis of hypertension, and/or hyperlipidemia. In the MEPS, 259 mutually exclusive clinical classification categories were mapped from ICD-9-CM codes to create clinically homogenous groupings (22). The current research used clinical classification categories 053 “disorders of lipid metabolism” to identify individuals with hyperlipidemia.
Physical activity in adults

Table 3—Unadjusted rates of self-reported physical activity among U.S. adults diagnosed with diabetes or at risk for developing type 2 diabetes*

<table>
<thead>
<tr>
<th>Health condition</th>
<th>Unweighted</th>
<th>Physical active</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>1,825</td>
<td>38.5 (35.7–41.3)</td>
<td></td>
</tr>
<tr>
<td>No cardiovascular risk factors†</td>
<td>469</td>
<td>46.0 (39.9–52.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>With cardiovascular risk factors†</td>
<td>1,355</td>
<td>36.1 (32.9–39.4)</td>
<td></td>
</tr>
<tr>
<td>No diabetes</td>
<td>21,401</td>
<td>57.8 (56.6–58.9)</td>
<td></td>
</tr>
<tr>
<td>No diabetes risk factors‡</td>
<td>4,741</td>
<td>64.9 (62.7–67.1)</td>
<td></td>
</tr>
<tr>
<td>One diabetes risk factor‡</td>
<td>8,743</td>
<td>59.8 (57.3–60.4)</td>
<td></td>
</tr>
<tr>
<td>Two diabetes risk factors‡</td>
<td>4,790</td>
<td>54.3 (30.0–32.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Three diabetes risk factors‡</td>
<td>2,432</td>
<td>52.1 (42.4–46.6)</td>
<td></td>
</tr>
<tr>
<td>Four diabetes risk factors‡</td>
<td>648</td>
<td>42.0 (37.7–46.2)</td>
<td></td>
</tr>
</tbody>
</table>

Data are % (95% CI) or n. *All data are based on the Medical Expenditure Panel Survey, 2003. †Cardiovascular risk factors were history of cardiovascular disease, diagnosis of hypertension, and/or diagnosis of hyperlipidemia. ‡Type 2 diabetes risk factors were age ≥45 years, non-Caucasian ethnicity, BMI >25 kg/m², diagnosis of hypertension, and history of cardiovascular disease.

Assessment of BMI and other covariates

We used self-reported information from the MEPS Household Component survey for the assessment of BMI, medical advice to exercise, and other covariates. Respondents were asked to estimate their current body weight and height; if a “doctor or other health professional ever advised you to exercise more?”; if they had “difficulties walking, climbing stairs, grasping objects, reaching overhead, lifting, bending or stooping, or standing for long periods of time”; and to report on current smoking status, age, sex, race, ethnicity, years of schooling, and income level (22). The Centers for Disease Control and Prevention formula was used to calculate BMI (29), and the National Heart, Lung, and Blood Institute classification scheme was used to define normal, overweight, and obese categories (30).

Because depression is common among individuals with diabetes (13) and is associated with physical inactivity (14), the relationship of depression with physical activity was also assessed in this study. A respondent was classified as having depression if they had a medical encounter coded with the three-digit ICD-9 code of 311 (depressive disorder) or 296 (episodic mood disorders, including major depression).

Data analysis

To adjust for the complex sample design, the current research used the MEPS person-level and variance adjustment weights using STATA 9.1 in all analyses to ensure nationally representative estimates. Given the MEPS sample design, F tests were conducted to test for variation in unadjusted rates of physical activity across selected subgroups. Multiple logistic regression analysis was used to estimate the adjusted odds of being physically active among adults with and without diabetes after controlling for sex, age, race/ethnicity, education and income levels, region, BMI, cardiovascular risk factors, depression, physical limitation status, and receiving advice to exercise more.

RESULTS — Overall, 56% of adults reported that they were moderately to vigorously physically active three or more times a week (Table 2). Regular activity decreased with increasing BMI and varied with age. Physical activity was higher among respondents who were male, white, had higher education and income levels, reported previous medical advice to exercise more, and had no limitations in physical functioning. Among adults with diabetes, 39% reported they were physically active compared with 58% of those without diabetes (Table 3). The proportion of respondents without diabetes who reported being physically active decreased as the number of type 2 diabetes risk factors increased, until approximating the prevalence reported among individuals with diabetes. After adjusting for demographic, socioeconomic, and clinical characteristics, the most notable associations with regular activity, regardless of diabetes status, were the negative correlations with mental and physical health and the positive correlation with family income (Table 4).

The association of physical activity with several demographic and clinical factors varied between adults with versus without diabetes. For example, the association of sex, race/ethnicity, and education status was evident in adults without diabetes but not in those with diabetes. Normal-weight individuals with diabetes were no more likely to be active than overweight or obese adults; whereas, in adults without diabetes, the likelihood of being active incrementally declined with each increasing BMI category. Lastly, prior advice from a health professional to exercise more was positively associated with current physical activity levels in nondiabetic individuals but had no association in those with diabetes.

CONCLUSIONS — The most concerning news from this study is that at a time when the prevalence of the disease is increasing, <40% of adults with diabetes reported being regularly engaged in moderate or vigorous physical activity. These results confirm recent findings from National Health and Nutrition Examination Survey 1999–2002 (18) and suggest that no substantial improvement in physical activity has occurred over the last decade (16). This is disturbing because there is clear evidence of the health benefits of physical activity for the management of type 2 diabetes (2). Further, despite increased public health attention on the need for being physically active, the prevalence of physical activity reported by adults with diabetes in 2003 was no different from rates seen the year before (31). Moreover, the level of physical activity reported by respondents with diabetes was significantly lower on average than national norms for adults without diabetes.

The news is not particularly encouraging even in individuals without diabetes. While more than half of adults without diabetes reported being physically active, activity levels declined with increasing BMI and with increasing numbers of cardiovascular disease risk factors. Since there is a general trend toward increasing BMI and increasing cardiovascular disease risk factors. Since there is a general trend toward increasing BMI and increasing cardiovascular disease risk factors, the U.S. population, this could suggest that physical activity levels will decrease in the future.

Because this is a cross-sectional analysis, it is impossible to determine why adults with diabetes are less active than their peers without the disease. Less physical activity may reflect the inertia of a lifetime of habits. These individuals likely have the same motivational barriers, including lack of interest and not enough time, as adults without diabetes (17,32,33). However, those with diabetes often have physical disabilities (34), per-
Diabetes Care, Volume 30, Number 2, February 2007

**Table 4—Factors associated with self-reported physical activity among U.S. adults with and without diabetes* **

<table>
<thead>
<tr>
<th>Selected characteristics</th>
<th>Physically active†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diabetes</td>
</tr>
<tr>
<td>Sex (Ref. = female)</td>
<td>1.14 (0.89–1.46)</td>
</tr>
<tr>
<td>Age-groups (years)</td>
<td></td>
</tr>
<tr>
<td>20–29</td>
<td>1.00 (Ref.)</td>
</tr>
<tr>
<td>30–39</td>
<td>0.85 (0.39–1.87)</td>
</tr>
<tr>
<td>40–49</td>
<td>0.92 (0.44–1.91)</td>
</tr>
<tr>
<td>50–59</td>
<td>0.80 (0.41–1.56)</td>
</tr>
<tr>
<td>60–69</td>
<td>0.86 (0.42–1.75)</td>
</tr>
<tr>
<td>70–79</td>
<td>0.85 (0.42–1.70)</td>
</tr>
<tr>
<td>≥80</td>
<td>0.54 (0.24–1.21)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>1.00 (Ref.)</td>
</tr>
<tr>
<td>Black</td>
<td>1.11 (0.75–1.64)</td>
</tr>
<tr>
<td>Asian</td>
<td>1.39 (0.42–4.57)</td>
</tr>
<tr>
<td>Hispanic (Ref. = no)</td>
<td>1.43 (0.98–2.07)</td>
</tr>
<tr>
<td>Geographic region</td>
<td></td>
</tr>
<tr>
<td>Northeast</td>
<td>1.00 (Ref.)</td>
</tr>
<tr>
<td>South</td>
<td>0.98 (0.65–1.48)</td>
</tr>
<tr>
<td>Midwest</td>
<td>1.04 (0.67–1.63)</td>
</tr>
<tr>
<td>West</td>
<td>1.52 (0.99–2.32)</td>
</tr>
<tr>
<td>Education levels</td>
<td></td>
</tr>
<tr>
<td>Less than high school</td>
<td>1.00 (Ref.)</td>
</tr>
<tr>
<td>High school</td>
<td>1.15 (0.87–1.51)</td>
</tr>
<tr>
<td>Some college (&lt;4 years)</td>
<td>1.22 (0.68–2.20)</td>
</tr>
<tr>
<td>College degree (4 years)</td>
<td>1.14 (0.69–1.89)</td>
</tr>
<tr>
<td>Graduate school (&gt;4 years)</td>
<td>0.89 (0.45–1.76)</td>
</tr>
<tr>
<td>Income level</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>1.00 (Ref.)</td>
</tr>
<tr>
<td>Near poor</td>
<td>1.54 (0.88–2.70)</td>
</tr>
<tr>
<td>Low income</td>
<td>1.53 (1.00–2.34)</td>
</tr>
<tr>
<td>Middle income</td>
<td>1.60 (1.05–2.42)</td>
</tr>
<tr>
<td>High income</td>
<td>2.03 (1.32–3.14)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
</tr>
<tr>
<td>Normal (18.5–24.9)</td>
<td>1.00 (Ref.)</td>
</tr>
<tr>
<td>Overweight (25.0–29.9)</td>
<td>1.08 (0.77–1.53)</td>
</tr>
<tr>
<td>Obese, classes 1 and 2 (30.0–39.9)</td>
<td>0.79 (0.54–1.17)</td>
</tr>
<tr>
<td>Obese, class 3 (≥40)</td>
<td>0.39 (0.24–0.64)</td>
</tr>
<tr>
<td>Cardiovascular risk factors (Ref. = none)‡</td>
<td>0.88 (0.65–1.20)</td>
</tr>
<tr>
<td>Depression (Ref. = no)</td>
<td>0.66 (0.46–0.95)</td>
</tr>
<tr>
<td>Physical function limitations (Ref. = none)</td>
<td>0.47 (0.37–0.61)</td>
</tr>
<tr>
<td>Ever advised to exercise more (Ref. = no)</td>
<td>0.99 (0.73–1.34)</td>
</tr>
</tbody>
</table>

Data are odds ratio (95% CI). *All data are based on the Medical Expenditure Panel Survey, 2003. †Odds ratios were obtained from logistic regression models adjusting for sex, age, race/ethnicity, education and income levels, region, BMI, cardiovascular risk factors, depression, physical limitation status, and receiving advice to exercise more. ‡Cardiovascular risk factors were history of cardiovascular disease, diagnosis of hypertension, and/or diagnosis of hyperlipidemia. Ref., reference.

Individuals with diabetes are undiagnosed diabetes. Recent estimates suggest that one-third of adults with diabetes among individuals with diabetes was reduced by half. However, the association with education status was not observed among adults with diabetes. In addition, the data also suggest that rates of physical activity were not lower among Hispanic compared with non-Hispanic adults with diabetes. It is not clear why correlates of physical activity would be different in those with diabetes, but these differences may be important in developing strategies to increase physical activity in this population.

In this study, the rate of physical activity among adults without diabetes, while disappointing, is consistent with other national surveys (15). The highest rates reported were among the youngest, most educated, and most economically advanced adults, but even then over a third was inactive. Reinforcing the value of life-long physical activity for young, sedentary adults can help curb the rising obesity and diabetes epidemics as young adults gain, on average, an estimated 2 lb per year (36), with a long-term risk of becoming overweight exceeding 50% (37).

The results of this research are subject to limitations. All variables relied on self-reports, including disease status and the diagnosis of diabetes. While diabetes and risk factor estimates presented here are consistent with other U.S. survey-based national estimates (38–40), it may be that the self-reported rates of diabetes and diabetes risk factors in this study are underestimated, leading to a bias toward the null when assessing differences in physical activity by disease status. Self-reported health conditions can be underreported in general (41), and blacks, whites, and Hispanics differ in reporting of diseases and levels of illness and disability (42,43). Previous studies (44) have also shown that overweight respondents tend to underestimate their weight and overweight their height so BMI scores are underestimated. However, excellent concordance between medical records and patient self-report has been observed for several medical diagnoses, including history of diabetes, obesity, and history of acute myocardial infarction (45).

MEPS also does not contain information on undiagnosed diabetes. Recent estimates suggest that one-third of individuals with diabetes are undiagnosed (27). Respondents in this study...
with multiple risk factors for developing type 2 diabetes may have undiagnosed diabetes, which may explain why their rates of physical activity were similar to those patients with diabetes. Finally, several known environmental factors associated with physical activity were also unavailable for study using the MEPS data so that environmental barriers to physical activity could not be assessed.

Self-report was also used to ascertain physical activity in the MEPS due to the challenges of measuring cardiorespiratory fitness on a large national scale. Self-reported physical activity has moderate validity with individuals tending to over-report activity (26). On the other hand, while the MEPS definition of moderate and vigorous physical activity included domestic household and leisure-time activity, it did not specifically query other sources of physical exertion undertaken by adults, such as through employment (24), and therefore may underestimate total physical activity. For example, the International Physical Activity Questionnaire measures more contributors toward total physical activity and has been shown to lead to higher physical activity prevalence estimates compared with the Behavior Risk Factor Survey Surveillance (46). Also, the extent of sedentary behavior, such as longer television viewing, was not assessed in the MEPS. Recent epidemiological evidence (47) suggests that increased sedentary behavior is a predictor of diabetes risk independent of leisure-time physical activity. Nevertheless, physical activity estimates from national public health surveys, such as the MEPS, can provide valuable information to guide national policy and program decisions (48).

Caution should also be taken in directly comparing results from this study with other studies as part of the apparent differences in the prevalence of physical activity may be attributable to differences in how physical activity was defined (49) and changing public health recommendations (Table 1). In the MEPS, physical activity was defined as “moderate/vigorous activity, ≥30 min, three or more days per week.” The American Diabetes Association’s recommendations have become more specific as scientific understanding has evolved, i.e., from “regular physical activity” in 2003 (25) to “150 min per week of moderate-intensity (50–70% of maximum heart rate)” in 2006 (2). The Centers for Disease Control and Prevention similarly defines recommended physical activity as “moderate-intensity activities in a usual week of 30 min per day for at least 5 days per week.” (50) Therefore, values reported in the 2003 MEPS data may be an overestimation of the proportion of adults achieving “therapeutic levels” of exercise based on current public health guidelines.

It is difficult to be optimistic about addressing the twin epidemics of obesity and diabetes without success in increasing physical activity in the population. The results of this study provide very pessimistic data about achieving this goal. Physical activity is least likely to be present in those who already have diabetes and in those most at risk for developing diabetes. There is a great need for intensive efforts to target interventions to increase physical activity in these individuals.

References
22. MEPS HC-078: 2003 medical conditions, November 2005 [article online]. Avail-


Effect of Adjunctive Pramlintide Treatment on Treatment Satisfaction in Patients With Type 1 Diabetes

David G. Marrero, PhD
John Crean, PhD
Bei Zhang, MD
Terrie Kellmeyer, PhD
Maurice Gloster, MD

Kathrin Herrmann, PhD
Richard Rubin, PhD
Naomi Fineberg, PhD
Orville Kolterman, MD

OBJECTIVE — To assess the effect of adjunctive pramlintide treatment on treatment satisfaction in patients with type 1 diabetes treated with intensive insulin regimens.

RESEARCH DESIGN AND METHODS — Intensively treated (multiple daily injection [MDI] or continuous subcutaneous insulin infusion [CSII]) patients with type 1 diabetes completed a study-specific treatment satisfaction questionnaire following 29 weeks of either placebo (n = 136) or pramlintide (n = 130) treatment in a double-blind, noninferiority pramlintide dose titration trial. End points included patient reported outcomes, their relationship to insulin treatment regimen, A1C, weight, and insulin use.

RESULTS — Pramlintide-treated patients reported greater treatment satisfaction in most questionnaire responses. Treatment satisfaction was similar for pramlintide-treated patients regardless of intensive insulin regimens (MDI versus CSII). Mean A1C was reduced to a similar extent in both pramlintide (-0.39 ± 0.07%) and placebo-treated (-0.45 ± 0.07%) patients. However, pramlintide treatment was associated with reductions in mean body weight (-1.50 ± 0.33 kg; P < 0.0001) and mealtime insulin use (-19.05 ± 5.17%; P < 0.005) over 29 weeks, while placebo treatment resulted in weight gain (1.28 ± 0.25 kg) and a smaller reduction in mealtime insulin use (-2.20 ± 3.33%).

CONCLUSIONS — Despite similar reductions in A1C, pramlintide treatment resulted in greater treatment satisfaction compared with placebo treatment. This was independent of insulin delivery method.


Recent epidemiological data report that ~35% of patients with type 1 diabetes are in poor glycemic control (1). Examining the daily experience of the insulin-treated patient highlights the motivational challenges of intensive therapy. Beyond avoiding future complications, there are few discernable incentives to intensify insulin. However, there are clearly tangible disincentives, including increased risk for severe hypoglycemia and weight gain (2–6). Additionally, clinical trials using continuous glucose monitoring devices recently have documented that the typical 24-h blood glucose profile of patients achieving near normoglycemia is characterized by profound, and often rapid and frequent, fluctuations (7–9). For example, Boland et al. (7), in a study of patients using intensive insulin regimes, demonstrated a clear dissociation between diurnal blood glucose control and A1C level. Almost 80% of recorded postmeal blood glucose values in subjects with A1C levels of ~7.5% were in the moderate-to-severe hyperglycemic range, whereas the majority of nocturnal values were in the hypoglycemic range.

Considering these issues, it is understandable that patient adherence may gradually diminish with long-term intensive therapy. A recent study (10) reported that most patients with type 1 and 2 diabetes experienced symptoms of depression, anxiety, and burnout that interfered with diabetes self-management. Thus, a therapy providing tangible improvement in day-to-day diabetes control might represent a valuable clinical tool for insulin-using patients, particularly for motivated patients failing to achieve optimal glycemic control with intensive insulin therapy.

The discovery of amylin has led to the development of a medication that has been shown to improve glycemic control in insulin-using patients with diabetes (11). Amylin is a hormone that is colocated and cosecreted with insulin from pancreatic β-cells (11). Like insulin, amylin is absent in patients with type 1 diabetes and deficient in patients with late-stage type 2 diabetes (12). Animal models have demonstrated that amylin regulates gastric emptying, postprandial glucagon secretion, and food intake (11). These effects complement insulin’s effect on glucose disposal by limiting the appearance of glucose in the circulation following meals. Pramlintide, a synthetic amylin analog indicated as an adjunctive treatment to insulin in patients with type 1 and 2 diabetes, acutely reduces postprandial glucose fluctuations and enhances satiety (13–15). Long-term, adjunctive pramlintide therapy decreases A1C with concomitant reductions in insulin use and body weight in patients with type 1 and type 2 diabetes (16,17).

The primary aim of this end-of-study survey was to evaluate, under double-blind conditions, the effects of premeal subcutaneous pramlintide versus placebo injections on aspects of treatment satisfac-
tation including perceived improvement in blood glucose predictability, appetite, and weight control. Additional survey items assessed if perceived benefits represented a significant improvement over insulin alone and if they outweighed the burden of extra injections.

**RESEARCH DESIGN AND METHODS**

**Survey analysis**
Survey data were analyzed on a post hoc basis for 266 of 296 intensively treated (multiple daily injection [MDI] or continuous subcutaneous insulin infusion [CSII] pump therapy) patients with type 1 diabetes who completed a 29-week, double-blind, noninferiority pramlintide dose-titration trial. The 30 subjects who did not complete the questionnaire (12% of the experimental group and 8% of the control subjects) were lost to follow-up in the study. Details of the parent study design are reported in full elsewhere (18). In brief, patients were randomized to receive either placebo or pramlintide injections before meals (30/60 μg) in addition to their insulin. The study began with a 4-week initiation period followed by a 25-week maintenance period. During initiation, it was recommended that mealtime insulin be reduced 30–50%, reflecting the decreased demand for mealtime insulin with pramlintide treatment. While this recommended insulin dose reduction applied to placebo-treated patients, insulin dosing was always adjusted according to clinical judgement. Pramlintide was introduced at a dose of 15 μg and titrated in 15-μg increments as tolerated (nausea) to a final dose of 30 or 60 μg at the end of the 4-week initiation period. For placebo-treated patients, injection volumes increased to the same volume equivalent as the pramlintide-treated patients to maintain blinding.

Patients remained on a stable dose of pramlintide for the 25-week maintenance period of the study, with insulin doses adjusted to optimize glycemic control. Throughout the study all patients, pramlintide, as well as placebo treated, optimized insulin usage using the same criteria. During initiation, patients who reduced or increased basal insulin if preprandial glucose concentrations were <130 or >180 mg/dl, respectively. Patients also reduced or increased mealtime insulin if postprandial glucose concentrations were <160 or >240 mg/dl, respectively. During the maintenance period, patients reduced or increased basal insulin if preprandial glucose concentrations were <110 or >140 mg/dl, respectively. Patients also reduced or increased mealtime insulin if postprandial glucose concentrations were <140 or >180 mg/dl, respectively. Subjects were asked to perform glucose measurements before and after every meal for the entire 29-week study period as part of the study protocol.

**Patients**
Inclusion criteria included ≥18 years of age, insulin use for ≥1 year, an A1C between 7.5 and 9.0%, stable body weight (±2.5 kg within 2–6 months before screening), and no symptoms of severe hypoglycemia for 6 months before screening. Female subjects were postmenopausal, surgically sterile, or using adequate contraception. Approximately 50% of patients used MDI (three or more injections per day), and 50% used CSII in conjunction with self-monitored blood glucose testing (prepost each major meal and at bedtime). There were slightly more subjects using CSII assigned to the experimental group, but the difference was not statistically significant (P = 0.14). Patients were excluded if they had clinically significant comorbid conditions or used oral antidiabetes agents, bile acid–sequestering agents, antiobesity agents, or medications affecting gastrointestinal motility. Baseline characteristics were well matched between treatment groups with similar insulin delivery methods (Table 1).

**Treatment satisfaction survey measurements**
To address treatment satisfaction, a 14-item questionnaire was created specifically for this trial. The majority of items are consistent with those found on previously validated instruments, including the Diabetes Treatment Satisfaction Questionnaire and the Treatment Satisfaction component of the Diabetes-Specific Quality of Life Scale (19,20). Additional items were added to evaluate whether the unique aspects of pramlintide therapy impacted treatment satisfaction. For example, “7) Study medication provided benefits that insulin alone has not provided me; 10) Study medication provided me with enough benefit to outweigh the extra injections.” Each item was coded on a six-point Likert scale, with one representing strongly disagree and six representing strongly agree. All patients completed the 14-item treatment satisfaction questionnaire at the conclusion of their exposure to either pramlintide or placebo at 29 weeks.

**Statistical analyses**
Efficacy end point data for the subjects who completed the patient satisfaction survey (n = 266) were summarized de-

---

**Table 1—Baseline characteristics**

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Placebo</th>
<th>Pramlintide</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>136</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>Sex (%)(male/female)</td>
<td>40/60</td>
<td>49/51</td>
<td>0.096</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41 ± 12</td>
<td>41 ± 14</td>
<td>0.218</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.6 ± 17.0</td>
<td>81.8 ± 17.4</td>
<td>0.479</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.7 ± 4.7</td>
<td>27.7 ± 4.7</td>
<td>0.866</td>
</tr>
<tr>
<td>A1C (%)</td>
<td>8.1 ± 0.8</td>
<td>8.1 ± 0.7</td>
<td>0.959</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>21 ± 12</td>
<td>20 ± 12</td>
<td>0.465</td>
</tr>
<tr>
<td>Race (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>92</td>
<td>94</td>
<td>0.946</td>
</tr>
<tr>
<td>Hispanic</td>
<td>5</td>
<td>3</td>
<td>0.878</td>
</tr>
<tr>
<td>African American</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Asian American</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Average daily insulin dose (units)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mealtime</td>
<td>27.8 ± 16.1</td>
<td>26.1 ± 14.3</td>
<td>0.318</td>
</tr>
<tr>
<td>Basal</td>
<td>27.3 ± 16.2</td>
<td>29.6 ± 19.8</td>
<td>0.651</td>
</tr>
<tr>
<td>Total</td>
<td>55.1 ± 27.3</td>
<td>55.7 ± 28.8</td>
<td>0.801</td>
</tr>
<tr>
<td>Insulin regimen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDI (three or more injections)</td>
<td>68 (50)</td>
<td>55 (42)</td>
<td></td>
</tr>
<tr>
<td>CSII</td>
<td>68 (50)</td>
<td>75 (58)</td>
<td>0.141</td>
</tr>
</tbody>
</table>

Data are means ± SD and n (%) unless otherwise indicated. *P value of between-group analysis (pramlintide versus placebo).
Effect of pramlintide on treatment satisfaction

Table 2—Change from baseline A1C, weight, and insulin use in patients who answered the treatment satisfaction questionnaire

<table>
<thead>
<tr>
<th>End point</th>
<th>Placebo</th>
<th>Pramlintide</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>136</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>A1C (%)</td>
<td>-0.45 ± 0.07</td>
<td>-0.39 ± 0.07</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>1.28 ± 0.25</td>
<td>-1.50 ± 0.33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Average daily insulin dose (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mealtime</td>
<td>-2.20 ± 3.33</td>
<td>-19.05 ± 5.17</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Basal</td>
<td>18.88 ± 6.80</td>
<td>13.18 ± 6.07</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>4.44 ± 3.39</td>
<td>1.24 ± 8.57</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Mean postprandial glucose (mg/dl)</td>
<td>172.7 ± 2.1</td>
<td>151.3 ± 2.2</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data are means ± SD unless otherwise indicated. *P value of between-group analysis (pramlintide versus placebo). NS, not significant.

RESULTS

Primary efficacy and safety end points

Reductions in A1C following 29 weeks of pramlintide treatment were similar for the pramlintide- and placebo-treated patients who answered the treatment satisfaction questionnaire (Table 2). Placebo-treated patients used significantly more mealtime insulin and more insulin overall. This reflects protocol instructions to increase basal insulin if fasting plasma glucose >140 mg/dl and to increase mealtime insulin if postprandial glucose >180 mg/dl. At week 29, the average percent change from baseline for basal insulin was +19.0 and +13% for placebo- versus pramlintide-treated patients, respectively; at week 29, the average percent change from baseline for mealtime insulin was −2.0 and −19% for placebo- versus pramlintide-treated patients, respectively. Thus, placebo-treated patients required considerably more insulin to achieve equivalent overall glycemic control (per A1C). In addition, pramlintide-treated patients had significantly lower postprandial glucose excursions while having equivalent overall glycemic control as placebo-treated patients, as measured by mean postprandial glucose concentrations (Table 2). Finally, placebo-treated patients gained weight, whereas pramlintide-treated patients lost weight over the course of the 29-week trial (Table 2).

The most common adverse events observed were reduced appetite, vomiting, sinusitis, nausea, and severe hypoglycemia (reporting criteria: ≥10% and at least twofold greater incidence in any pramlintide-treated group [30/60 μg] than in the placebo-treated group). These adverse events were similar to those in the original study, with the exception of an increased incidence of somnolence and asthenia in the 30-μg pramlintide-treated group of the treatment satisfaction cohort. Adverse events observed ≥10% and with at least twofold greater incidence in any pramlintide-treated group (30 or 60 μg) than in the placebo-treated group (i.e., reduced appetite, vomiting, sinusitis, nausea, severe hypoglycemia) were similar to those observed in the population as a whole (18), with the exception of an increased incidence of somnolence and asthenia in the 30-μg pramlintide-treated group of the treatment satisfaction cohort.

Survey outcomes

Mean survey ratings for the pramlintide-compared with placebo-treated patients were highly significant (reflecting stronger agreement) on the following items: “study medication made my blood glucose control more even or predictable,” “provided me with more flexibility in what I can eat,” “made it easier to control my weight,” and “made it easier to control my appetite.” The magnitude of these differences is highlighted by the substantially higher percentage of pramlintide-treated patients with ratings of agree/strongly agree (Table 3) on each of these items.

Substantially more pramlintide-treated patients agreed or strongly agreed that study medication provided benefits that insulin alone had not and that these benefits outweighed the burden of extra injections. Most patients, regardless of treatment assignment, indicated that study medication did not make it easier to avoid hypoglycemia but that side effects, including hypoglycemia, would not prevent them from using it on a long-term basis (Table 3). Nearly twice as many pramlintide- than placebo-treated patients agreed or strongly agreed that study medication reduced worries about having diabetes, increased confidence about managing diabetes, improved how they felt overall, and improved functioning at home, work, or school (Table 3).

In analyses adjusting for possible differences in age, sex, duration of diabetes, quality of glycemic control (as measured by A1C), and BMI, neither sex nor duration of diabetes was significantly related to any of the 14 questions, while patient age, quality of glycemic control, and BMI did show limited interaction with treatment. However, each of these interactions was modest and related only to question 13, “I would like to continue taking the study medication.”

Influence of insulin delivery method

Placebo-treated patients on CSII reacted more negatively to the patient satisfaction questionnaire compared with their placebo-treated counterparts on MDI (Table 4). However, patient satisfaction scores for pramlintide-treated patients were not different when categorized by mode of insulin delivery (CSII versus MDI).

Patients were given the opportunity to use pramlintide in an open-label extension following completion of the 29-week blinded trial. Of 266 patients that completed the survey during the double-blind
Effectiveness of pramlintide therapy was assessed in a group of patients with diabetes management, including blood glucose predictability, appetite, and weight control, in a group of patients with type 1 diabetes receiving intensive insulin therapy. These outcomes are consistent with the pharmacodynamic profile of pramlintide, including attenuated diurnal and postprandial glycemic excursions, enhanced satiety, and reduced food intake (13–15). Improvements in daily symptom control may account for positive responses by pramlintide-treated patients who reported increased confidence in their ability to manage their diabetes and function at home, work, or school and an increased overall sense of well being.

Patients’ positive perceptions of pramlintide therapy did not appear to be affected by age, sex, duration of diabetes, or mode of insulin delivery, suggesting a genuine drug effect. Moreover, the benefits of pramlintide therapy appeared to outweigh the potential burden of extra injections associated with using the drug. This was particularly evident for patients using CSII, a population that might be expected to be more troubled by extra injections but who, nonetheless, had satisfaction scores comparable with those administering insulin through MDIs. One might expect that insulin pump users would have less glucose variation between basal and postmeal states and, thus, be less influenced by the positive effects of pramlintide. However, pump users who took pramlintide during the blinded portion of the trial reported significantly greater satisfaction than those pump users treated with placebo.

In previous studies in which pramlintide was initiated at a fixed dose and mealtime insulin was not proactively reduced, there was an increased incidence of insulin-induced severe hypoglycemia in pramlintide-treated patients. In this study, pramlintide dose escalation with concomitant insulin dose reduction during initiation lowered rates of severe hypoglycemia in pramlintide-treated patients to levels similar to placebo-treated patients using insulin (18). Consistent with this, patients receiving pramlintide or placebo treatment reported similar experiences with respect to study treatment’s effect on their ability to avoid hypoglycemia. While pramlintide has been associated with transient nausea, this effect appeared to be mitigated by the dose titration schedule utilized in the present study (18). In response to the questionnaire, patients using pramlintide reported more side effects associated with the drug, but these differences did not negatively impact patients’ desire to continue using pramlintide or their willingness to recommend it to others.

The study data did not directly assess why patients using pramlintide consistently reported improved satisfaction with their diabetes treatment. One possibility is that pramlintide’s acute effects on improving postmeal glucose excursions (13,14) and enhancing postmeal satiety (13,14) and improving patients’ perception of general control over their diabetes. The inability to approximate physiologic insulin secretory patterns and location with exogenous insulin, although much improved with the availability of rapid- and long-acting insulin analogs, is reportedly far from optimal (21). As illustrated by recent continuous glucose monitoring system studies (7,8) showing excessive postprandial glucose excursions even when insulin has been optimized with pump therapy, controlling postmeal hyperglycemia remains one of the more difficult aspects of intensive insulin management. Unfortunately, simply increasing the insulin dose at mealtime in an attempt to compensate for excessive postprandial peaks typically falls short of the desired effect and increases the risk of hypoglycemia and weight gain (21,22). Several trials have demonstrated that improved flexibility in the daily the-
tic regimen, particularly increased dietary freedom, corresponds to enhanced treatment satisfaction in patients with diabetes engaging in intensified insulin treatment (23–25). Data from cross-sectional studies (26,27) have also indicated that poor postprandial glucose control leads to increased self-reported deterioration of mood and cognitive function in type 1 and type 2 diabetes. A recent double-blind, placebo-controlled investigation (28) evaluated the effects of acutely raising glucose using a hyperinsulinemic glucose clamp in a group of patients with type 2 diabetes. Intriguingly, performance on a series of cognitive tasks and self-reported mood state worsened after blood glucose was acutely raised to the hyperglycaemic range compared with the euglycemic range. Since patients were blinded to glucose readings, the results suggest the inability to control acute hyperglycemia after meals might affect well-being.

Conclusions about treatment satisfaction in this study involve several limitations. Most importantly, the data reported are postintervention only, with no baseline for comparison. As such, we are not able to ascertain whether treatment

### Table 4—Patient-reported outcomes by treatment group for insulin delivery method subgroups

<table>
<thead>
<tr>
<th>Question</th>
<th>Placebo (MDI n = 68; CSII n = 68)</th>
<th>Pramlintide (MDI n = 54; CSII n = 76)*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Made my blood sugar control more even or predictable</td>
<td>MDI 3.45</td>
<td>Pramlintide (MDI n = 54; CSII n = 76)*</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td></td>
<td>CSII 2.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) Provided me with more flexibility in what I can eat</td>
<td>MDI 3.35</td>
<td>Pramlintide (MDI n = 54; CSII n = 76)*</td>
<td>0.012‡†</td>
</tr>
<tr>
<td></td>
<td>CSII 2.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) Made it easier to control my weight</td>
<td>MDI 2.68</td>
<td>Pramlintide (MDI n = 54; CSII n = 76)*</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td></td>
<td>CSII 2.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4) Made it easier to avoid low blood sugar reactions (hypoglycemia)</td>
<td>MDI 3.01</td>
<td>Pramlintide (MDI n = 54; CSII n = 76)*</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>CSII 2.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5) Made it easier to control my appetite</td>
<td>MDI 3.04</td>
<td>Pramlintide (MDI n = 54; CSII n = 76)*</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td></td>
<td>CSII 2.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6) Had some side effects that would keep me from using it on a long-term basis</td>
<td>MDI 1.77</td>
<td>Pramlintide (MDI n = 54; CSII n = 76)*</td>
<td>0.004‡</td>
</tr>
<tr>
<td></td>
<td>CSII 1.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7) Provided benefits that insulin alone has not provided me</td>
<td>MDI 3.14</td>
<td>Pramlintide (MDI n = 54; CSII n = 76)*</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td></td>
<td>CSII 2.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8) Reduced at least some of my worries about having diabetes</td>
<td>MDI 2.94</td>
<td>Pramlintide (MDI n = 54; CSII n = 76)*</td>
<td>0.003‡</td>
</tr>
<tr>
<td></td>
<td>CSII 2.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9) Made me feel more confident about managing my diabetes</td>
<td>MDI 3.51</td>
<td>Pramlintide (MDI n = 54; CSII n = 76)*</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td></td>
<td>CSII 2.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10) Provided me with enough benefit to outweigh the extra injections</td>
<td>MDI 3.29</td>
<td>Pramlintide (MDI n = 54; CSII n = 76)*</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td></td>
<td>CSII 2.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11) Improved my ability to function at home, at work, or at school</td>
<td>MDI 3.00</td>
<td>Pramlintide (MDI n = 54; CSII n = 76)*</td>
<td>&lt;0.001‡†</td>
</tr>
<tr>
<td></td>
<td>CSII 2.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12) Improved how I feel overall</td>
<td>MDI 3.26</td>
<td>Pramlintide (MDI n = 54; CSII n = 76)*</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td></td>
<td>CSII 2.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13) I would like to continue taking the study medication</td>
<td>MDI 4.22</td>
<td>Pramlintide (MDI n = 54; CSII n = 76)*</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td></td>
<td>CSII 4.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14) I would recommend the study medication to other people with diabetes</td>
<td>MDI 4.29</td>
<td>Pramlintide (MDI n = 54; CSII n = 76)*</td>
<td>0.004‡</td>
</tr>
<tr>
<td></td>
<td>CSII 4.21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*One pramlintide-treated subject changed their baseline insulin regimen (from MDI to CSII) during the study. †Significant interaction. ‡Significant treatment effect of delivery method. NS, not significant.
groups differed significantly with respect to baseline treatment satisfaction measures. The relatively large sample size, however, helps mitigate this effect. These data are preliminary, using a measure that has not yet been validated. Despite these limitations, the treatment satisfaction results reported here are compelling and suggest that use of pramlintide potentially offers insulin-requiring patients a promising adjunct to traditional insulin therapy. Notable are the improvements in perceived control of both weight and appetite and improved predictability in glucose values that correspond to actual clinical outcomes (18). These elements address areas that are often difficult and stressful for patients with diabetes to manage. In this regard, the extent to which use of pramlintide might enhance patient efforts to achieve more optimal glycemic control is an interesting issue that deserves further investigation.

References

terman OG: Adjunctive therapy with the amylin analogue pramlintide leads to a combined improvement in glycemic and weight control in insulin-treated patients with type 2 diabetes. *Diabetes Technol Ther* 4:51–61, 2002


Comparison of Vildagliptin and Rosiglitazone Monotherapy in Patients With Type 2 Diabetes

**A 24-week, double-blind, randomized trial**

**OBJECTIVE** — To compare the efficacy and tolerability of vildagliptin and rosiglitazone during a 24-week treatment in drug-naïve patients with type 2 diabetes.

**RESEARCH DESIGN AND METHODS** — This was a double-blind, randomized, active-controlled, parallel-group, multicenter study of 24-week treatment with vildagliptin (100 mg daily, given as equally divided doses; n = 519) or rosiglitazone (8 mg daily, given as a once-daily dose; n = 267).

**RESULTS** — Monotherapy with vildagliptin and rosiglitazone decreased A1C (baseline = 8.7%) to a similar extent during the 24-week treatment, with most of the A1C reduction achieved by weeks 12 and 16, respectively. At end point, vildagliptin was as effective as rosiglitazone, improving A1C by −1.1 ± 0.1% (P < 0.001) and −1.3 ± 0.1% (P < 0.001), respectively, meeting the statistical criterion for noninferiority (upper-limit 95% CI for between-treatment difference ≤0.4%). Fasting plasma glucose decreased more with rosiglitazone (−2.3 mmol/l) than with vildagliptin (−1.3 mmol/l). Body weight did not change in vildagliptin-treated patients (−0.3 ± 0.2 kg) but increased in rosiglitazone-treated patients (+1.6 ± 0.3 kg; P < 0.001 vs. vildagliptin). Relative to rosiglitazone, vildagliptin significantly decreased triglycerides, total cholesterol, and LDL, non-HDL, and total-to-HDL cholesterol (−9 to −16%, all P ≤ 0.01) but produced a smaller increase in HDL cholesterol (+4 vs. +9%, P = 0.003). The proportion of patients experiencing an adverse event was 61.4 vs. 64.0% in patients receiving vildagliptin and rosiglitazone, respectively. Only one mild hypoglycemic episode was experienced by one patient in each treatment group, while the incidence of edema was greater with rosiglitazone (4.1%) than vildagliptin (2.1%).

**CONCLUSIONS** — Vildagliptin is an effective and well-tolerated treatment option in patients with type 2 diabetes, demonstrating similar glycemic reductions as rosiglitazone but without weight gain.


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_A promising new approach to treating type 2 diabetes is the augmentation of glucagon-like peptide (GLP)-1 receptor signaling by increasing endogenous GLP-1 through inhibition of the dipeptidyl peptidase IV (DPP-4) enzyme (1). Vildagliptin is a potent and selective DPP-4 inhibitor that improves islet function by increasing both α- and β-cell responsiveness to glucose (2,3). Vildagliptin has been shown in 12-week studies to decrease A1C when given as monotherapy (4,5) or in combination with metformin (6). Head-to-head comparison studies recently have been recommended to better establish the efficacy and safety of investigational therapies, such as vildagliptin monotherapy, relative to other current therapies (7). Several classes of drugs are approved for the pharmacological treatment of type 2 diabetes, including the thiazolidinediones (TZDs), rosiglitazone, and pioglitazone, which are among the most recent additions to the therapeutic armamentarium. Accordingly, the present multicenter, 24-week, double-blind, randomized, controlled clinical trial was conducted to compare the efficacy and tolerability of monotherapy with vildagliptin (100 mg daily) versus rosiglitazone (8 mg daily) in drug-naïve patients with type 2 diabetes._

**RESEARCH DESIGN AND METHODS** — This was a 24-week, double-blind, randomized, active-controlled, parallel-group study conducted at 202 centers in 11 countries in the Americas and Europe. Eligible patients were randomized to receive vildagliptin 100 mg daily (given as equally divided doses) or rosiglitazone 8 mg daily (given as a once-daily dose) in a ratio of 2:1. Efficacy and tolerability were assessed at weeks 4, 12, 16, and 24 of active treatment.

The study enrolled type 2 diabetic patients with A1C in the range of 7.5–11.0%. These patients had received no pharmacologic treatment for at least 12...
weeks before screening and no antidiabetic agent for >3 consecutive months at any time in the past and were considered to be representative of a drug-naive population. Male and female patients (nonfertile or of childbearing potential using a medically approved birth control method), aged 18–80 years, with BMI 22–45 kg/m² and with fasting plasma glucose (FPG) <15 mmol/l were eligible to participate.

Patients were excluded if they had a history of type 1 diabetes or secondary forms of diabetes; acute metabolic diabetes complications; myocardial infarction, unstable angina or coronary artery bypass surgery within the previous 6 months; congestive heart failure; liver disease, such as cirrhosis or chronic active hepatitis; and any contraindications and warnings according to the country-specific label for rosiglitazone. The following laboratory abnormalities were also excluded: alanine aminotransferase or aspartate aminotransferase >2.5 times the upper limit of normal, direct bilirubin >1.3 times the upper limit of normal, serum creatinine levels >220 μmol/l, clinically significant abnormal thyroid-stimulating hormone, or fasting triglycerides >7.9 mmol/l.

A1C, FPG, body weight, and vital signs were measured at each study visit. Standard hematology and biochemistry laboratory assessments were made at each visit except on week 16. Fasting lipid profiles were measured and electrocardiograms were performed at screening and at weeks 0, 12, and 24. All adverse events were recorded. Edema was assessed by the investigator as part of the normal adverse event-reporting process, either as a new occurrence or worsening of an existing condition. Patients were provided with glucose-monitoring devices and supplies and instructed on their use. Hypoglycemia was defined as symptoms suggestive of low blood glucose confirmed by a self-monitored blood glucose measurement <3.1 mmol/l plasma glucose equivalent. Severe hypoglycemia was defined as any episode requiring the assistance of another party.

All laboratory assessments were made by central laboratories. All assessments, except A1C, were performed by BARC (Biopharmaceuticals Research Corporation). Assays were performed according to standardized and validated procedures in accordance with good laboratory practice. A1C measurements were performed by either BARC-EU (Ghent, Belgium) for European patients or by Diabetes Diagnostics Laboratory (Columbia, MO) or Covance-US (Indianapolis, IN) for patients from the Americas. All samples from any single patient were measured by the same laboratory.

Analysis populations and data analysis
The primary intention-to-treat (ITT) population consists of all randomized patients who 1) had a screening A1C value ≥ 7.4%, 2) received at least one dose of study medication, and 3) had a baseline as well as at least one postbaseline A1C measurement. A total of 89 randomized patients were excluded from the primary ITT population for the following reasons: 4 received no intervention and 13 had no postbaseline A1C measurement; in addition, 61 patients were inappropriately randomized with screening A1C < 7.4%, and 11 patients had no baseline A1C assessment due to a systematic error in the measurement of A1C by the U.S. laboratory originally used for the study. The U.S. laboratory was subsequently changed, and no measurements performed by the initial laboratory are used in the analyses. The statistical power of the study was preserved by recruitment of additional patients, and all samples from any single patient were measured by the same laboratory throughout the study. The safety population consists of all patients who received at least one dose of the study drug and had at least one postbaseline A1C measurement. The primary efficacy variable was the change from baseline in A1C at study end point using the last observation carried forward for patients who discontinued early. Secondary efficacy parameters included changes in FPG, fasting plasma lipids, and body weight. The efficacy analyses were performed with data from the primary ITT population, which was prespecified as the main efficacy population. Change from baseline in primary and secondary end points were analyzed using an ANCOVA model, with treatment and pooled center as the classification variables and baseline as the covariate. A test for the noninferiority of vildagliptin to rosiglitazone in A1C was carried out through a CI approach. Noninferiority for A1C was established if the upper limit of the 95% CI for the between-treatment difference in the adjusted mean change from baseline to end point obtained from the ANCOVA model did not exceed 0.4%. For the secondary efficacy variables, tests of superiority were conducted at the two-sided significance level of 0.05. In addition, prespecified subanalyses of A1C changes were conducted based on initial (baseline) A1C and on BMI category.

Ethics and good clinical practice
All participants provided written informed consent. The protocol was approved by the independent ethics committee/institutional review board at each study site, and the study was conducted in accordance with the Declaration of Helsinki, using Good Clinical Practice.

RESULTS — A total of 786 patients were randomized, and 697 patients comprised the primary ITT population (459 patients randomized to receive vildagliptin 100 mg daily and 238 patients randomized to rosiglitazone 8 mg daily); 782 patients comprised the safety population. Figure 1 summarizes the disposition of patients from screening through study end point, and Table 1 reports the demographic and baseline metabolic characteristics of the patients in the primary ITT population. The groups were well balanced, with A1C varying 8.7% and FPG averaging 10.3 mmol/l in both treatment groups. One-third of patients had an A1C > 9%. Participants were predominantly Caucasian and obese (30% with BMI ≥ 35 kg/m²), with a mean age of 54 years and mean disease duration of 2.4 years. More than 85% of all patients randomized to either treatment completed the 24-week study.

Efficacy
Figure 2A depicts the time-course of mean A1C during the 24-week treatment with vildagliptin 100 mg daily or rosiglitazone 8 mg daily. Baseline A1C values were identical in the two treatment groups (8.7 ± 0.1%). A1C decreased with vildagliptin treatment over the entire 24-week study period, with most of the reduction attained by week 12. Rosiglitazone treatment appeared to have a somewhat slower onset of effect, with nearly maximum reduction reached at week 16. In the primary ITT population, the adjusted mean change in A1C from baseline to study end point was −1.1 ± 0.1% (P < 0.001) in patients receiving vildagliptin (n = 459) and −1.3 ± 0.1% (P < 0.001) in patients receiving rosiglitazone (n = 238). Noninferiority of vilda-
gliptin 100 mg daily to rosiglitazone 8 mg daily was established, as the upper limit of the 95% CI for the between-group difference in adjusted mean change in A1C (−0.01 to 0.39) did not exceed the prespecified noninferiority margin.

The decrease in A1C with either agent was substantially larger in patients with baseline A1C >9.0%, with mean A1C reductions of −1.8 ± 0.1% (P < 0.001) from a baseline of 10.0% (n = 166) with vildagliptin and of −1.9 ± 0.2% (P < 0.001) from a baseline of 9.9% (n = 88) with rosiglitazone. In the vildagliptin group, patients with BMI >30 kg/m² showed a somewhat greater reduction in A1C (ΔA1C = −1.3 ± 0.1%, n = 184) compared with obese patients with BMI ≥30 kg/m² (ΔA1C = −1.0 ± 0.1%, n = 275). Rosiglitazone, on the other hand, was somewhat more efficacious in patients with BMI ≥30 kg/m² (ΔA1C = −1.4 ± 0.1%, n = 155) than in leaner patients (ΔA1C = −1.1 ± 0.2%, n = 83). FPG also decreased significantly during the 24-week treatment with either agent.

<table>
<thead>
<tr>
<th>Table 1—Baseline characteristics of the primary ITT population*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Vildagliptin 100 mg daily</td>
</tr>
<tr>
<td>n ( )</td>
</tr>
<tr>
<td>Age (years) 54.5 ± 11.7</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male 264 (57.5)</td>
</tr>
<tr>
<td>Female 195 (42.5)</td>
</tr>
<tr>
<td>Race</td>
</tr>
<tr>
<td>Caucasian 365 (79.5)</td>
</tr>
<tr>
<td>Hispanic or Latino 51 (11.1)</td>
</tr>
<tr>
<td>Black 27 (5.9)</td>
</tr>
<tr>
<td>All other 16 (3.5)</td>
</tr>
<tr>
<td>BMI (kg/m²) 32.2 ± 5.7</td>
</tr>
<tr>
<td>BMI group (kg/m²)</td>
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<tr>
<td>&lt;30 184 (40.1)</td>
</tr>
<tr>
<td>≥30 275 (59.9)</td>
</tr>
<tr>
<td>≥35 132 (28.8)</td>
</tr>
<tr>
<td>A1C (%) 8.7 ± 1.1</td>
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<td>A1C group (%)</td>
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<tr>
<td>≤9.0 293 (63.8)</td>
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<tr>
<td>&gt;9.0 166 (36.2)</td>
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<tr>
<td>FPG (mmol/l) 10.3 ± 2.7</td>
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<tr>
<td>Disease duration (years) 2.3 ± 3.4</td>
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<td>Data are means ± SD or n (%). *Baseline characteristics were similar in the randomized population.</td>
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Figure 2—A: Mean ± SE A1C during the 24-week treatment with vildagliptin (100 mg daily; ▲) or rosiglitazone (8 mg daily; ○) in patients with type 2 diabetes (primary ITT population: vildagliptin, n = 434 at week −2, n = 397 at week 24; rosiglitazone, n = 221 at week −2, n = 209 at week 24). B: Adjusted mean change from baseline to end point in body weight in the primary ITT population and in subgroup of patients with BMI ≥35 kg/m². ▾, vildagliptin 100 mg daily; □, rosiglitazone 8 mg daily. *P < 0.05; **P < 0.01; ***P < 0.001 vs. rosiglitazone. C: Adjusted mean change from baseline to end point in fasting lipid parameters in the primary ITT population. ■, vildagliptin 100 mg daily; □, rosiglitazone 8 mg daily. *P < 0.05; **P < 0.01; ***P < 0.001 vs. rosiglitazone.
agent. In the primary ITT population, the mean baseline FPG was 10.3 mmol/l in both treatment groups. The FPG reduction (adjusted mean change) was -1.3 ± 0.1 mmol/l (P < 0.001) in patients receiving vildagliptin and -2.3 ± 0.2 mmol/l (P < 0.001) in patients receiving rosiglitazone (P < 0.001 vs. vildagliptin).

**Lipids and body weight**

Figure 2 also depicts changes in body weight (B) and fasting lipid parameters (C) after the 24-week treatment with vildagliptin 100 mg daily or rosiglitazone 8 mg daily in the primary ITT population. Body weight at baseline averaged 91.2 ± 0.9 kg in the vildagliptin group and 93.1 ± 1.3 kg in the rosiglitazone group. Body weight did not change during 24-week treatment with vildagliptin but increased significantly during rosiglitazone monotherapy. The between-treatment difference in body weight was -1.9 ± 0.3 kg (P < 0.001). In addition, a post hoc analysis indicated that in the more severely obese population (BMI ≥ 35 kg/m²; mean body weight of ~111 kg; n = 208), a larger decrease in body weight was seen with vildagliptin, while the increase seen with rosiglitazone monotherapy was similar to the overall cohort. The between-treatment difference in body weight in this subpopulation was -2.8 ± 0.6 kg (P < 0.001).

In the primary ITT population, fasting lipid levels were similar in the two treatment groups at baseline, averaging 2.3 mmol/l for triglycerides, 5.3 mmol/l for total cholesterol, 3.1 mmol/l for LDL, 1.2 mmol/l for HDL, and 4.1 mmol/l for non-HDL cholesterol in the combined cohort, with a total-to-HDL cholesterol ratio of 4.7. Relative to rosiglitazone, vildagliptin produced significant decreases in triglycerides (-9%, P = 0.010) and total (-14%, P < 0.001), LDL (-16%, P < 0.001), and non-HDL cholesterol (-16%, P < 0.001) but less improvement in HDL cholesterol (+4 vs. +9% from baseline, P = 0.003 for between-group difference). Relative to rosiglitazone, vildagliptin decreased total-to-HDL cholesterol by 9.1 ± 1.9% (P < 0.0001).

**Tolerability**

During the 24-week treatment, one or more adverse event was reported by 61.4% of patients receiving vildagliptin 100 mg daily and by 64.0% of patients receiving rosiglitazone 8 mg daily. In patients receiving vildagliptin, the most frequent specific adverse events (≥4% in either group) were nasopharyngitis (6.8%), dizziness (6.0%), headache (5.0%), and upper respiratory tract infection (4.5%). In rosiglitazone-treated patients, the most common adverse events were nasopharyngitis (7.5%), headache (5.2%), dizziness (4.1%), and peripheral edema (4.1%). The incidence of peripheral edema with vildagliptin was 2.1%. Increased body weight was reported as an adverse event in 0.8% of vildagliptin-treated patients and in 2.6% of rosiglitazone-treated patients. One patient in each group reported one mild hypoglycemic event, and no serious hypoglycemic events occurred in either group.

The proportion of patients experiencing any serious adverse event in the two treatment groups was comparable (2.9% vs. 3.0%), and no specific serious adverse event was reported by more than one patient within a treatment group. The frequency of discontinuations due to adverse events was also similar in the vildagliptin (2.9%) and the rosiglitazone (3.4%) groups. There was one death during the study. This was a 70-year-old male subject randomized to vildagliptin who died from postsurgical complications.

With the exception of a slightly higher proportion of patients with notables hematocrit and hemoglobin abnormalities in the rosiglitazone group, there were no major changes from baseline to end point nor were there any between-treatment differences observed for any laboratory parameter or vital signs. The frequency of treatment-emergent electrocardiogram abnormalities was low and comparable in the two treatment groups.

**CONCLUSIONS** — This study demonstrated that in patients representative of a drug-naive population, vildagliptin was well tolerated and caused no weight gain despite a significant and clinically meaningful decrease from baseline in A1C that was similar to that with rosiglitazone. As expected, both vildagliptin and rosiglitazone produced more substantial reductions in A1C in the subgroup of patients with a high baseline level, and as in the whole cohort, the improvement in glycemic control was similar in patients with high baseline A1C receiving vildagliptin (Δ = -1.8%) or rosiglitazone (Δ = -1.9%). Vildagliptin appeared to be slightly more effective than rosiglitazone in patients with BMI <30 kg/m², and rosiglitazone was slightly more effective in obese patients (BMI ≥ 30 kg/m²).

Although the two agents had similar overall efficacy to reduce A1C, the different mechanism of action of the two agents likely underlies several differences noted regarding secondary efficacy end points as well as in tolerability profiles. Vildagliptin inhibits the enzyme DPP-4, causing an increase in active plasma levels of the incretin hormones GLP-1 and gastrointestinal polypeptide (3). Vildagliptin has been shown to improve islet function by increasing the ability of both α- and β-cells to sense and respond appropriately to glucose (2,8). These effects are thought to be mediated by GLP-1 (9). In contrast, the TZDs target insulin resistance acting by activation of peroxisome proliferator-activated γ receptors, which results in enhanced peripheral and hepatic insulin action (10). Furthermore, TZDs stimulate differentiation of preadipocytes into new, small, and highly insulin-sensitive fat cells (10). This promotes storage of free fatty acids in adipose tissue, thus relieving the liver and muscle from lipotoxicity and reducing gluconeogenesis (11). The decrease in FPG in vildagliptin-treated patients seen in the present study was significantly less than that in patients receiving rosiglitazone, but the A1C improvements were similar, suggesting a more pronounced effect of vildagliptin on plasma glucose levels in the postprandial period and throughout the day.

Many effective antidiabetes agents lead to some weight gain as a result of improved glycemic control (12), and this is a particular limitation of (13) and potential safety concern about TZDs, due to their tendency to cause fluid retention and edema (14). In this study, the increase in body weight and incidence of edema in patients receiving rosiglitazone that was observed is consistent with previous reports, whereas vildagliptin achieved a comparable improvement in glycemic control with no weight gain and a low incidence of edema. The ability of vildagliptin to improve glycemic control without weight gain needs to be further clarified mechanistically.

In the present study, relative to rosiglitazone, vildagliptin treatment was associated with a significant improvement in triglycerides; total, LDL, and non-HDL cholesterol; and, importantly, the total-to-HDL cholesterol ratio. The changes in fasting lipids seen in rosiglitazone-treated patients were consistent with those re-
Vildagliptin versus rosiglitazone monotherapy

ported in previous studies (15–17). The mechanism underlying the improvement in lipid profile seen in vildagliptin-treated patients is unknown but could reflect a chronic improvement in postprandial lipids, as suggested by a recent report that found decreased postprandial lipemia primarily through a reduction in intestinally derived apolipoprotein B-48–containing particles after a 4-week treatment with vildagliptin (8).

With the exception of a higher incidence of edema in rosiglitazone-treated patients, the two agents were similarly well tolerated in this 24-week study of vildagliptin 100 mg daily versus rosiglitazone 8 mg daily, and there was a very low incidence of hypoglycemia.

In conclusion, similar A1C efficacy can be achieved using the DPP-4 inhibitor vildagliptin or a TZD as monotherapy in drug-naïve patients with type 2 diabetes. Vildagliptin is well tolerated, and, despite the improvement in glycemic control, it does not cause weight gain, which is an important consideration in the decision-making process for selecting first-line therapy in type 2 diabetes.

Acknowledgments — This study was funded by Novartis Pharmaceuticals. A list of investigators is provided in the APPENDIX.

The authors gratefully acknowledge the investigators and staff at the 202 participating sites and the editorial assistance of and helpful discussion with Beth Dunning Lower, PhD, who is a subcontractor for Novartis.

APPENDIX

List of investigators
Argentina: Dr. Alfredo Lozada, Dr. Adriana Osorio, Dr. Gustavo Frechtel, Dr. Marcelo Ruscullela, Dr. Maria Isabel Klyver de Salme, Dr. Guillermo Marcucci, Dr. Jorge Gomez. Austria: Prim. Doz. Dr. Attila Dunky, Dr. Franz Winkler, Prof. Hermann Toplak, Univ. Doz. Dr. Raimund Weinig, Prim. Univ. Prof. Rudolf Prager. Brazil: Dr. Maria Cereque, Dr. Freedy Eliaschewitz, Dr. Maria Zanella, Dr. Marcos Tambascia. Canada: Dr. Chantal Godin, Dr. Kevin Saunders, Dr. Dennis O’Keefe, Dr. Errol Raff, Dr. Gottesman, Dr. Jean-Francois Yale, Dr. Ronnie Aronson, Dr. Yaw Twum-Barima, Dr. Andre Nadeau, Dr. Stuart Ross, Dr. Ronald Goldenberg. Finland: Dr. Jaakko Tuomilehto, Dr. Johan Eriksson, Prof. Sirkka Kemanen-Kikuartaaniemi, Dr. Veli Sillanpaa, Dr. Jorma Lahtela, Dr. Pasi Nevalainen, Dr. Pentti Jarvinen. France: Dr. Christian Faugere, Dr. Guillaume Dantin, Dr. Jean Francois Ravaud, Dr. Gerard Lalanne, Dr. Philippe Blanchard. Germany: Dr. Manfred Reiss, Dr. Med. Michael Ziegler, Dr. Reinhold Schneider, Dr. Wolfgang Boerner, Dr. Hans Seibert, Dr. Med. Juergen Matthes, Dr. Doris Boehme, Dr. Matthias Rovenich, Dr. Hans-Joachim Herrmann, Dr. Frank Klein, Dr. Med. Arthur Sterzing, Dr. Med. Edmond Homisy, Dr. Daniel Ayasse, Dr. Gerhard Krehan, Dr. Karin Todoroff, Dr. Med. Rudolf Fuchs. Hungary: Dr. Istvan Wittmann, Dr. Janos Penzes, Dr. Peter Torzza, Dr. Jozsef Rinfel, Dr. Tibor Fulop, Dr. Judit Simon, Dr. Sandor Palla, Dr. Horten Karolyi, Dr. Gyula Neuwirth, Dr. Gyozo Vandorfi. Italy: Dr. Iskra Liguerre, Dr. Manuel Munoz, Dr. Carlos Almendro, Dr. Belen Fraile, Dr. Jose Gonzalez Clemente, Dr. Magdalena Hernandez, Dr. Fernando Quirce, Dr. Nidia Ruiz, Dr. Ildefonso Espinosa, Dr. Luis De Teresa. Sweden: Prof. Ulf Adamsson, Dr. Arvo Hanni, Dr. Mona Landin-Olsson, Dr. Bo Polhem, Prof. Stephan Rossner, Dr. Anders Sjogren, Dr. Mats Dahl, Dr. Gunnar Stromblad, Dr. Anders Nilsson, Dr. Anders Norrby. U.S.: Dr. Donald Huffman, Dr. David Colan, Dr. Ronald Graf, Dr. Richard Cherlin, Dr. Terry Poling, Dr. Kashif Latif, Dr. Robert Hippet, Dr. Anna Jackson, Dr. Terence Isakov, Dr. Usah Lilavivat, Dr. Neal Shealy, Dr. Jeffrey Newman, Dr. Monica Perlman, Dr. Vin Tangpricha, Dr. Gerard Stanley, Dr. Paul Dudley, Dr. James Lehman, Dr. Christopher Case, Dr. George Handey, Dr. Dobivenkalata Bali, Dr. Francis Yemofio, Dr. Arthur Mullen, Dr. Flor Geola, Dr. Eric Klein, Dr. J. Forsythe, Dr. Raymond Grenfell Jr., Dr. Abraham Areepanthu, Dr. F. Lester, Dr. Larry Stonesifer, Dr. Kristine Bordeneave, Dr. George Ryckman, Dr. Thomas Moretto, Dr. Jay Shubrook, Dr. Danny Sugimoto, Dr. Othman Shemisa, Dr. Joseph Aloii, Dr. William Long Jr., Dr. Frank Civitarese, Dr. Richard Cook, Dr. Matthew Portz, Dr. Anthony Bartkowiak, Dr. Steven Levine, Dr. Rajeev Kumar Jain, Dr. David Wright, Dr. Carl Griffin, Dr. Alan Garber, Dr. Sherywn Schwartz, Dr. Harold Fields, Dr. Jerry Mitchell, Dr. Jayaram Naidu, Dr. Kenneth Hershon, Dr. Gregory Gottschlich, Dr. Curtis Brown, Dr. Berto Zamora, Dr. Deborah Thompson, Dr. Scott Yates, Dr. Angela Adelizzi, Dr. Michael Lai, Dr. Garland Thorn Jr., Dr. Timothy Howard, Dr. Larry Cowan, Dr. Deborah Cole-Sedivy, Dr. Richard Egelhof, Dr. John Thomson, Dr. John Agabiy, Dr. Stephen Hippler, Dr. Thomas Higgins, Dr. Thomas Wade, Dr. Terence Hart, Dr. Andrew Slaski, Dr. John McGintigan, Dr. Kimy Charani, Dr. Natalie Shemonsky, Dr. John Wadleigh, Dr. Michael McAdoo, Dr. Pedro Velasquez-Mier, Dr. Arthur Pitterman, Dr. Alan Wynne, Dr. S. Archer, Dr. Howard Ellison, Dr. Anicia Villafrua, Dr. A. Clifton Cage, Dr. David Damian, Dr. John Sibille, Dr. Peterman Prosser, Dr. Richard Ferreras, Dr. Ruth Smothers, Dr. W. David Clark, Dr. Mark Runde, Dr. Chet Monder, Dr. Matthew Acampora, Dr. Paul Fiacco, Dr. Brian Kauth, Dr. Ronald Sockolov, Dr. Gregory Smith, Dr. John Devlin, Dr. Daniel Sheerer, Dr. Lawrence Levinson, Dr. Eli Ipp, Dr. David Mansfield, Dr. Paul Davis, Dr. Michael Campolo.

References


Effect of a Nurse-Directed Diabetes Disease Management Program on Urgent Care/Emergency Room Visits and Hospitalizations in a Minority Population

Mayer B. Davidson, MD1
Adeela Ansari, MD1
Vicki J. Karlan, MPH2

OBJECTIVE — To evaluate whether nurse-directed diabetes care reduced preventable diabetes-related urgent care/emergency room visits and hospitalizations in a minority population.

RESEARCH DESIGN AND METHODS — Diabetic patients who receive care in a county public health clinic were randomly selected for a Diabetes Managed Care Program (DMCP) in which a specially trained nurse followed detailed treatment algorithms to provide diabetes care for 1 year. Preventable diabetes-related urgent care/emergency room visits and hospitalizations for these patients incurred during the intervention year and the year before enrollment were compared. Preventable diabetes-related causes were defined as metabolic (diabetic ketoacidosis, hyperglycemia, or hypoglycemia) or infection (cellulitis, foot ulcer, osteomyelitis, fungal infection, or urinary tract infection).

RESULTS — Use of the urgent care/emergency room and hospitalizations during the intervention year and the year prior were available for 331 patients who completed the DMCP intervention. There were 94 total urgent care/emergency room visits and hospitalizations in the year before entering the DMCP and 46 during the DMCP year, a 51% reduction. Preventable diabetes-related episodes were far fewer. During the prior year, 14 patients made 15 urgent care/emergency room visits and 3 patients incurred 6 hospitalizations. During the DMCP year, 4 different patients made 5 emergency room/urgent care visits and one other patient was hospitalized. Preventable diabetes-related use was significantly (P < 0.001) lower during the intervention year compared with the prior year. Total charges for urgent care/emergency room visits and hospitalizations only (not other charges related to diabetes care) during the year before entering the DMCP were $129,176 compared with $24,630 during the DMCP year.

CONCLUSIONS — When compared with usual care, nurse-directed diabetes care resulted in significantly fewer urgent care/emergency room visits and hospitalizations for preventable diabetes-related causes. Policy makers seeking to improve diabetes care and conserve resources should seriously consider adopting this approach.


The American Diabetes Association (ADA) has promulgated evidence-based guidelines that lead to improved diabetes care processes and outcome measures (1). Disappointingly, all three outcome measures (A1C, LDL cholesterol, and blood pressure levels) are met in <10% of people with diabetes (2–4). Unfortunately, most approaches used to improve diabetes outcome measures have been ineffective in practice. These include: 1) reminding patients about appointments (5,6); 2) providing feedback information on patients to their treating physicians (7–10), even when treatment recommendations for the patient were included (11,12); 3) case management (when the case manager could not make treatment decisions) (13,14); 4) physician education (15,16); and 5) multifaceted quality improvement interventions in the practice setting (17,18).

Outcomes of diabetes care are generally worse in minority populations (19), though there are very few intrinsic racial/ethnic differences that can account for the increased complications of diabetes in these populations. With the exception of a slight increase in renal disease, complications among minorities were similar to those in Caucasians when everyone had access to the same medical care (20,21).

To improve diabetes care in an inner-city population, we completed and recently published the results of a Diabetes Managed Care Program (DMCP) in which a specially trained registered nurse treated 367 patients following detailed treatment algorithms for diabetes care for 1 year (22). An endocrinologist (M.B.D.) was available by phone and met with the nurse once a week. ADA process measures were met 98% of the time, and the mean and median A1C levels were 7.0 and 6.7%, respectively. Sixty percent of the patients met the ADA A1C goal and 82% the LDL cholesterol goal.

This article describes the effect of nurse-directed diabetes care on preventable diabetes-related urgent care/emergency room visits as well as hospitalizations in the 331 diabetic patients who completed the DMCP intervention and also received usual care in the same clinic for the year before program enrollment.

RESEARCH DESIGN AND METHODS — A total of 367 diabetic patients were randomized and followed by a specially trained nurse in the DMCP for 1 year (22). Of those, 331 patients had
been followed in this county clinic during the year before entering the DMCP. The algorithms used to treat patients enrolled in the DMCP covered glycemic control including those for diet therapy alone; sulfonylurea agents and metformin, either alone or in combination; a glitazone added to maximal (tolerated) doses of metformin and a sulfonylurea agent; bedtime NPH insulin plus daytime oral anti-hyperglycemia drugs; and a split-mixed insulin regimen with NPH and regular insulin. There were also algorithms and protocols for evaluating and managing lipid disorders, evaluating nephropathy, and treating microalbuminuria (see online appendix, available at http://dx.doi.org/10.2337/dc06-2022).

Urgent care and emergency room visits were analyzed together because patients access the urgent care center when it is open at the community clinic and use the emergency room at the hospital when the clinic is closed. Thus, reasons for using one or the other is logistical, not medical. The urgent care/emergency room visits and hospitalizations during the DMCP year in these 331 patients were compared with the year before enrolling into the DMCP. This study received approval from the institution review board at Charles R. Drew University. Urgent care/emergency room and hospitalization data for these 331 patients were obtained from the county’s management information system. The first five discharge diagnoses and the total charges specific for each visit or hospitalization (not charges related to diabetes care per se) were recorded. Preventable diabetes-related visits and hospitalizations were defined as either metabolic (diabetic ketoacidosis, hyperglycemia, or hypoglycemia) or infection (cellulitis, foot ulcer, osteomyelitis, fungal infection, or urinary tract infection). Other possible diabetes-related diagnoses that could not be realistically affected by 1 year of appropriate diabetes care, such as angina, myocardial infarction, stroke, and non-diabetes-related causes (e.g., gynecological surgery or psychiatric visits), were excluded from the analysis. The assignment of causes for visits and hospitalizations was carried out by one of the authors (A.A.) who was blinded to when (year prior or DMCP year) the visit or hospitalization occurred. When the primary reason for the urgent care/emergency room visit or hospitalization was not clear, she obtained the chart for review.

Continuous variables were analyzed with the Student’s paired t test. Categorical variables were analyzed with the χ² test. The rates of emergency room/urgent care visits and hospitalizations were analyzed with a Z test for the difference of two Poisson rates. Significance was accepted at a 0.05 level (two tailed).

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<td>Data are years ± SD or n (%) unless otherwise indicated.</td>
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RESULTS — The baseline demographics of the 331 patients at time of entry into the DMCP are shown in Table 1. Baseline A1C levels were 8.8 ± 2.5% (SD), which fell to 7.1 ± 1.4% after 1 year in the DMCP (P < 0.001). At entry into the DMCP, 28% met the ADA A1C goal of <7.0% compared with 64% after 1 year (P < 0.002). At entry into the DMCP, 37% met the ADA LDL cholesterol goal, whereas 80% met it after 1 year (P < 0.04).

There were 95 total urgent care/emergency room visits and hospitalizations in the year before entering the DMCP compared with 52 during the DMCP year, a 45% reduction. However, there were far fewer visits and hospitalizations for preventable diabetes-related causes (Table 2). During the prior year, 14 patients made 15 emergency room/urgent care visits (11 for metabolic reasons and 4 for infections) and 5 patients had 6 hospitalizations (2 for a metabolic reason and 4 for infection). One patient had two ur-

<table>
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<th>Table 2—Preventable diabetes-related urgent care and emergency room visits and hospitalizations</th>
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DKA, diabetic ketoacidosis; UC/ER, urgent care/emergency room.
gent care/emergency room visits, one had two hospitalizations, and a third had one of each.

During the DMCP year, four different patients made five urgent care/emergency room visits (one for a metabolic reason and four for infection) and another one was hospitalized for an infection. Note that there was only 1 episode of hyperglycemia during the DMCP year resulting in an urgent care/emergency room visit compared with the year before entering the DMCP in which 11 episodes of hyperglycemia caused urgent care/emergency room visits and 2 resulted in hospitalizations (Table 2). This difference highlights the marked effect of nurse-directed care on preventable diabetes-related metabolic causes of patients interacting with the medical care system outside of regular clinic hours. The difference between the year prior and the DMCP year for all preventable diabetes-related urgent care/ emergency room visits and hospitalizations was significantly different ($P < 0.001$). Total charges specific for these urgent care/ emergency room visits and hospitalizations during the year before entering the DMCP were $129,176 compared with $24,630 during the DMCP year.

**CONCLUSIONS** — Nurse (23–34)- and pharmacist (35–37)-directed care have been shown to yield better process and surrogate outcome measures when compared with standard medical care. Only one study evaluated a clinical outcome. Fewer patients cared for by a nurse developed diabetic retinopathy than those receiving standard care (38). This is the first study to compare urgent care/ emergency room use and hospitalizations by patients with diabetes receiving nurse-directed care compared with standard care. The results convincingly demonstrate lower resource use among diabetic patients under nurse-directed care. Although there was no control group followed under standard care for 2 years to rule out the possibility that a learning curve accounted for these results, this seems highly unlikely. The average duration of diabetes was 7.6 years, surely long enough for patients to have learned how to take measures to avoid urgent care/ emergency room visits and hospitalizations for preventable diabetes-related causes if they could. More likely, one of the reasons for the success of the DMCP was the self-management skills taught to the patients by the nurse during their year under her care.

There are several limitations to this study. Although it is possible that the patients used other centers that were not part of the county system resulting in an underestimate of actual services used, this is unlikely to have influenced the results for two reasons. First, only 15% of the population cared for in this county clinic have any medical insurance, making it unlikely for them to seek care elsewhere. Second, to account for these results, seeking care outside of the system would have had to occur much more frequently during the DMCP year than in the year prior, also very unlikely. The differences between nurse-directed and standard care may be greater in this minority population when compared with other populations. Finally, total charges do not reflect actual costs of care or reimbursement; therefore the amount of cost savings cannot easily be determined from these data.

In conclusion, nurse-directed diabetes care in this minority population resulted in less use of urgent care/ emergency room centers as well as fewer hospitalizations for preventable diabetes-related conditions. Policy makers who seek to improve diabetes care and conserve resources should seriously consider adopting this approach.

**Acknowledgments** — This study was funded by the American Diabetes Association, Pfizer Health Solutions, Inc.; and Merck & Co. Dr. Davidson was supported by National Institutes of Health Grant U54-RR014616.

The authors are grateful to Robert Stevens for accessing the county computer system and identifying which patients had urgent care/ emergency room visits and hospitalizations and for providing the ICD-9 codes and the total charges for each visit and hospitalization and to Martin L. Lee, PhD, for statistical advice.

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Progression From Newly Acquired Impaired Fasting Glucose to Type 2 Diabetes

GREGORY A. NICHOLS, PHD
TERESA A. HILLIER, MD, MS
JONATHAN B. BROWN, PHD, MPP

OBJECTIVE — We sought to estimate the rate of progression from newly acquired (incident) impaired fasting glucose (IFG) to diabetes under the old and new IFG criteria and to identify predictors of progression to diabetes.

RESEARCH DESIGN AND METHODS — We identified 5,452 members of an HMO with no prior history of diabetes, with at least two elevated fasting glucose tests (100–125 mg/dl) measured between 1 January 1994 and 31 December 2003, and with a normal fasting glucose test before the two elevated tests. All data were obtained from electronic records of routine clinical care. Subjects were followed until they developed diabetes, died, left the health plan, or until 31 December 2005.

RESULTS — Overall, 8.1% of subjects whose initial abnormal fasting glucose was 100–109 mg/dl (added IFG subjects) and 24.3% of subjects whose initial abnormal fasting glucose was 110–125 mg/dl (original IFG subjects) developed diabetes (P < 0.0001). Added IFG subjects who progressed to diabetes did so within a mean of 41.4 months, a rate of 1.34% per year. Original IFG subjects converted at a rate of 5.56% per year after an average of 29.0 months. A steeper rate of increasing fasting glucose; higher BMI, blood pressure, and triglycerides; and lower HDL cholesterol predicted diabetes development.

CONCLUSIONS — To our knowledge, these are the first estimates of diabetes incidence from a clinical care setting when the date of IFG onset is approximately known under the new criterion for IFG. The older criterion was more predictive of diabetes development. Many newly identified IFG patients progress to diabetes in <3 years, which is the currently recommended screening interval.

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The American Diabetes Association (ADA) defines impaired fasting glucose (IFG) as an intermediate state of hyperglycemia in which glucose levels do not meet criteria for diabetes but are too high to be considered normal (1). Although the ADA calls IFG “pre-diabetes” (1), reported estimates of diabetes incidence are not known. Therefore, we sought to identify predictors of diabetes development. Many newly identified IFG patients progress to diabetes in <3 years, which is the currently recommended screening interval.

From the Kaiser Permanente Center for Health Research, Portland, Oregon.

Address correspondence and reprint requests to Gregory A. Nichols, PhD, Center for Health Research, 3800 N. Interstate Ave., Portland, OR 97227-1098. E-mail: greg.nichols@kpchr.org

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Abbreviations: ADA, American Diabetes Association; CM, clinical modification; FPG, fasting plasma glucose; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; KPNW, Kaiser Permanente Northwest;

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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plasma glucose (FPG) tests are routinely ordered with lipid panels. Between 1 January 1994 and 31 December 2003, a single regional laboratory analyzed 603,486 FPG tests for 2,031,093 unique individuals. Of the 113,687 patients who had at least two tests, we identified 28,335 with two or more results of at least 100 mg/dl and no evidence of diabetes (chart diagnosis of ICD-9-CM [clinical modification] codes of 250.xx, FPG >125 mg/dl, or use of an antihyperglycemic drug) before the first elevated FPG test. From these, we identified 5,452 individuals who also had an FPG test <100 mg/dl prior to their IFG-positive tests to ensure that the first elevated glucose test represented an incident value.

**Stages of impaired fasting glucose**

For this study, we divided IFG into two “stages” that correspond to the old and new ADA criteria, 100–109 mg/dl (added IFG subjects) and 110–125 mg/dl (original IFG subjects). In both stages, patients were followed from the date of their first abnormal glucose until they progressed to diabetes (n = 614, 11.3%), died (n = 349, 6.4%), left the health plan (n = 1,044, 19.1%), or until 31 December 2005 (n = 3,445, 63.2%). Added IFG subjects who later progressed to original IFG were included in analyses of both stages. The mean ± SD number of follow-up fasting glucose tests was 5.2 ± 3.8 after entering the added IFG stage and 5.7 ± 4.3 after entering the original IFG stage.

**Analytic variables**

All analyses were conducted with SAS software, version 8.2 (SAS Institute, Cary, NC). We calculated incidence of diabetes per 100 person-years. For ease of interpretability, we report the incidence rates in terms of percent per year. To identify predictors of progression to diabetes, we constructed three generalized linear regression models using person-years of follow-up as an adjustment for unequal follow-up (12): one was for all 5,452 subjects, a second was for all 4,526 added IFG subjects, and the third was for all 1,699 original IFG subjects. We also estimated a fourth model to identify predictors of progression to original IFG among the 4,526 added IFG subjects.

KPNW uses an electronic medical record that contains up to 20 physician-recorded ICD-9-CM diagnoses at each contact. From these diagnoses, we identified comorbidities present at the time of the first fasting glucose test. The specific comorbidities (ICD-9-CM codes) used were: myocardial infarction (410.xx), stroke (430.xx–432.xx, 434.xx–436.xx, and 437.1), other atherosclerotic cardiovascular disease (411.1, 411.8, 413.xx, 414.0, 414.8, 414.9, and 429.2), congestive heart failure (428.xx), and depression (296.2–296.35, 298.0, 300.4, 309.1, and 311). In constructing the multivariate models, we combined the myocardial infarction, stroke, atherosclerotic cardiovascular disease, and congestive heart failure variables into a single marker for cardiovascular disease. Depression was not significant in any model and was therefore dropped. Age was calculated as the age at the date of the first elevated glucose test. Smoking history, height, weight, and blood pressure were also obtained from the electronic medical record. Lipid values were extracted from the laboratory database. For this study, we used the mean of all lipid, blood pressure, and BMI values recorded during a stage of IFG as predictors. Before modeling, we tested the correlation of all variables to rule out multicollinearity. With the exception of age/cardiovascular disease (0.31) and female sex/HDL (0.37), all correlation coefficients were below 0.30; thus, any variable that was significant in any model was retained.

**Results**

Of the 5,452 subjects, 4,526 (83.0%) had their first abnormal FPG within the added IFG range in an average of 17.8 months after their last normal test (Fig. 1). The remaining 926 (17.0%) subjects’ first abnormal fasting glucose result fell between 110 and 125 mg/dl (original IFG) after an average of 22.5 months. Most added IFG subjects (n = 3,552, 78.5%) did not progress to either original IFG or diabetes over a mean follow-up of 73.2 months. However, 201 added IFG subjects (4.4%) progressed straight to diabetes in an average of 31.1 months. The remaining 17.1% progressed to original IFG in a mean of 29.2 months. Of these, 164 (21.2%) developed diabetes in a mean of 29.5 months. Although nearly 30% of those who did not progress to either diabetes or original IFG either died or left the health plan, mean follow-up time (62.9 months for those who died and 45.0 months for those who left the plan) was substantially longer than progression time. Similarly, 249 (26.9%) of initially original IFG subjects developed diabetes in a mean of 28.7 months. Again, mean follow-up time among those who died or left the plan before progressing was much greater than progression time (61.0 and 41.1 months, respectively).

**Characteristics of subjects by initial IFG stage**

The 83% of subjects whose initial abnormal FPG ranged from 100 to 109 mg/dl (added IFG) were 2 years older (59.7 vs. 57.9 years, P < 0.0001) and less likely to be women (48.1 vs. 53.9%, P < 0.001) than originally IFG subjects (Table 1). The mean value of the FPG test before the initial abnormal FPG did not significantly differ between added and original IFG subjects (93.8 vs. 93.5 mg/dl, P = 0.119).

**Progression to diabetes**

Overall, 8.1% of added IFG subjects and 24.3% of original IFG subjects ultimately developed diabetes (P < 0.0001) (Table 2). Added IFG subjects who progressed to diabetes did so within a mean of 41.4 months, a rate of 1.34% per year. Of the 17.1% who progressed to original IFG, 21.2% developed diabetes (3.24% per year). Among added IFG subjects who were not known to progress to original IFG, 5.4% developed diabetes (0.91% per year).

Subjects whose first elevated fasting glucose result was 110–125 mg/dl (original IFG) converted to diabetes at a rate of 5.56% per year after an average of 29.0 months. Once subjects reached original IFG, diabetes arose at approximately the same rate among subjects who did and did not pass through the added IFG stage (5.16 vs. 5.87%, P = NS), but a significantly greater proportion of those who did not have a previous added IFG measurement progressed to diabetes (26.8 vs. 21.2%, P = 0.007). Among all subjects (n = 5,452), 11.3% developed diabetes in an average of 36.3 months, an incidence rate of 1.95% per year. This represents the rate at which subjects under the new ADA definition of IFG (100–125 mg/dl) progressed to diabetes. By comparison, the total original IFG incidence rate (5.56% per year) represents the old IFG definition.

**Predictors of hyperglycemic progression**

As shown in Table 3, each additional miligram per deciliter of initial fasting glucose increased the risk of progression from added to original IFG (model A) by 8% (odds ratio 1.08 [95% CI 1.05–1.12]) and from added IFG to diabetes (model B)
Progression from normal fasting plasma glucose to stages of IFG to type 2 diabetes. Mean ± SD months from stage to stage for those who progressed are displayed along each arrow. For those who did not progress, mean ± SD months of follow-up are displayed along the arrows.

Table 1—Characteristics of study subjects by initial stage of IFG

<table>
<thead>
<tr>
<th></th>
<th>Added IFG subjects (100–109 mg/dl)</th>
<th>Original IFG subjects (110–125 mg/dl)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>4,526 (83.0)</td>
<td>926 (17.0)</td>
<td></td>
</tr>
<tr>
<td>Months of follow-up*</td>
<td>75.6 (34.1)</td>
<td>68.2 (35.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age at IFG incidence (years)</td>
<td>59.7 (11.1)</td>
<td>57.9 (11.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Female (%)</td>
<td>48.1</td>
<td>53.9</td>
<td>0.001</td>
</tr>
<tr>
<td>FPG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior to IFG incidence</td>
<td>93.8 (4.8)</td>
<td>93.5 (5.4)</td>
<td>0.119</td>
</tr>
<tr>
<td>Incident measure</td>
<td>103.5 (2.8)</td>
<td>115.4 (4.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Months between pre- and incident FPG</td>
<td>17.8 (15.7)</td>
<td>22.5 (18.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>22.1</td>
<td>24.0</td>
<td>0.206</td>
</tr>
<tr>
<td>Comorbidities (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of myocardial infarction</td>
<td>9.1</td>
<td>8.4</td>
<td>0.524</td>
</tr>
<tr>
<td>History of stroke</td>
<td>9.2</td>
<td>8.6</td>
<td>0.595</td>
</tr>
<tr>
<td>Other ASCVD</td>
<td>21.5</td>
<td>18.6</td>
<td>0.045</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>7.5</td>
<td>10.6</td>
<td>0.002</td>
</tr>
<tr>
<td>History of depression</td>
<td>24.0</td>
<td>30.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>134 (13)</td>
<td>136 (13)</td>
<td>0.017</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79 (7)</td>
<td>80 (7)</td>
<td>0.033</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.0 (6.3)</td>
<td>33.2 (7.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>51 (15)</td>
<td>48 (14)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>190 (215)</td>
<td>212 (138)</td>
<td>0.004</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>126 (30)</td>
<td>121 (31)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are means (SD) or percent. *Follow-up was terminated at the earlier stage of progression to diabetes (11.3%), at health plan termination (19.1%), at death (6.4%), or on 31 December 2005 (63.2%). ASCVD, atherosclerotic cardiovascular disease.

CONCLUSIONS — In this retrospective cohort study of real-world patients with incident IFG, we found that 8.1% who met the added portion of the ADA’s 2003 criterion for IFG (100–109 mg/dl) progressed to diabetes over a mean follow-up of 6.3 years, an annual rate of 1.34%. Among subjects with incident IFG under the old ADA definition (110–125 mg/dl), we observed an annual rate of progression to diabetes of 5.56%. This
rate of progression is lower than rates reported by all but one previous study of subjects enrolled at unknown times after IFG had already begun (3,5–8). The progression rate from the old IFG cut point that we observed is very similar to the rate reported by Meigs et al. (9), which is the only previous study that has estimated progression from the time IFG first appeared. This confirms the importance of accounting for time since IFG onset when predicting the risk of diabetes and likely explains much of the wide variation among earlier studies.

Three times the proportion of subjects with original IFG progressed to diabetes than added IFG subjects, and they did so much more rapidly, at over four times the rate. Among added IFG subjects, progression to original IFG increased the risk of ultimately developing diabetes by threefold. Once original IFG was reached, initially added IFG subjects developed diabetes at approximately the same rate as patients who started from original IFG. Only about one-third of subjects who developed diabetes did so without first passing through original IFG. Moreover, the rate of diabetes incidence among all subjects (i.e., the rate for the ADA’s new IFG definition) was 1.95% per year, which is less than half the 5.56% rate observed for the old IFG definition. All of these findings suggest that original IFG (the old ADA definition) is much more predictive of future diabetes.

How quickly fasting glucose rises from normal to impaired may also predict type 2 diabetes. Although diabetes developed approximately equally among original IFG subjects once that level was reached, subjects who first passed through added IFG spent an average of 29.2 months in that stage. Thus, a steeper trajectory of rising fasting glucose may be an important risk factor for diabetes development. If so, whether a patient exceeds any given cut point for defining IFG may be less important than the rate at which glucose is increasing. This is a new finding, which could not have been observed in previous studies that had unknown dates of IFG onset; however, it is consistent with the Mexico City Diabetes Study, which concluded that conversion to diabetes is marked by a step increase rather than gradual progressive rise in glycemia (13).

In our data, higher BMI and lower HDL cholesterol were the most highly significant nonglucose predictors of hyperglycemic progression. Higher triglycerides and systolic blood pressure were also consistently significant risks. Previous studies have shown that this constellation of risk factors plus hyperglycemia—known as the metabolic syndrome—is predictive of diabetes, probably because of the glucose component (14–17). In the context of elevated glucose, components of the metabolic syndrome appear to independently predict further hyperglycemia, but whether the syndrome predicts diabetes over and above its individual components is beyond the scope of this study.

The prevalence of diabetes markedly increases with age (1). In our population of patients with newly acquired IFG, we found that younger, not older, age predicted diabetes development. It may be that hyperglycemia developed at a younger age reflects a greater degree of insulin resistance, in which relatively small declines in β-cell function lead to a rapid rise in glucose levels (13). However, it is also possible that the presence of other risk factors caused clinicians to test glucose more frequently among younger members, increasing the chance to identify diabetes.

Our study has several noteworthy limitations. As an observational study conducted in a clinical care setting, subjects received their fasting glucose tests at irregular intervals, which likely affected the precision of our incidence estimates. Although all subjects had previously normal fasting glucose measurements, we could not determine the precise date on which they crossed an IFG threshold. In addition, by requiring our subjects to have at least two elevated glucose values, our study may have been subject to ascertainment bias: some of our subjects were likely being followed because of glucose-related risk factors. Furthermore, those at greatest diabetes risk may have been tested more frequently, thereby increasing the likelihood of detection. Therefore, our incidence estimates may be higher than would be observed in a randomly selected population but are likely representative of real-world clinical practice. An additional limitation is that 19% of our initial population left the health plan. Over an average of 6 years of follow-up, this computes to a relatively low annual drop-out rate of <4%. It cannot be determined whether these subjects would experience diabetes incidence at similar rates as those who completed follow-up. Had we excluded these subjects from analysis, our progression rates would have been considerably higher because the denominator would have been reduced while the number of subjects progressing remained the same. It is also important to note that at all stages of progression, subjects who died or left the health plan before progression were, on average, observed for substantially longer periods than the mean progression times for those who did progress to other stages.
Moreover, other than being younger, the participants who left the health plan were not statistically significantly different from those who completed follow-up on any of the predictor variables, including fasting glucose levels. We were also unable to assess several known predictors of diabetes: family history, previous gestational diabetes, race/ethnicity, and waist circumference, for example. Exclusion of these predictors from multivariate models may have affected the performance of included variables in ways we could not observe. Finally, our study was conducted in an insured primarily Caucasian (~92%) population. Whether our results generalize to other populations is an important area for future research.

The Atherosclerosis Risk in Communities Study (18) concluded that two-thirds of those classified at the lower (100–109 mg/dl) IFG cut point had either diabetes or IGT. Thus, because many patients with IFG also have IGT, interventions proven effective in IGT populations (19–22) would likely also apply to the majority of patients with IFG. However, the implementation of lifestyle interventions takes time, and the beneficial effects are not immediate. In our data, newly identified added IFG subjects who progressed to diabetes took, on average, 3 years to do so. Even among those with newly acquired original IFG, diabetes progression time averaged 2 years. However, those at greatest risk of diabetes had steeper trajectories of glucose increase, allowing less time for time-intensive interventions. Current ADA recommendations suggest screening high-risk individuals, particularly those with a BMI \( \geq 25 \) kg/m\(^2\), at 3-year intervals to detect pre-diabetes and diabetes (1). Overall, those who developed diabetes in our study did so in an average of 36.3 months, and original IFG subjects who developed diabetes did so in a mean of \(~29\) months. Thus, a 3-year screening interval could miss individuals who progress rapidly from normal to impaired glycemia to diabetes. Shortening the screening interval, especially among the obese and those with steeper glucose trajectories, would allow more time for at-risk individuals to attempt lifestyle interventions.

Acknowledgments — This study was funded by National Institute of Diabetes and Digestive and Kidney Diseases Grant 1 R21 DK053961. Parts of this study were presented in ab-

### Table 3 — Multivariate models of hyperglycemic progression

<table>
<thead>
<tr>
<th>Model</th>
<th>OR (95% CI)</th>
<th>P value</th>
<th>OR (95% CI)</th>
<th>P value</th>
<th>OR (95% CI)</th>
<th>P value</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.31</td>
<td>0.43–3.98</td>
<td>&lt;0.0001</td>
<td>-</td>
</tr>
<tr>
<td>Model B</td>
<td>1.12</td>
<td>0.88–1.42</td>
<td>0.352</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Model C</td>
<td>1.07</td>
<td>1.04–1.10</td>
<td>0.0033</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Model D</td>
<td>1.07</td>
<td>1.04–1.10</td>
<td>0.0033</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Model A**: progression from added to original IFG; **Model B**: progression from added IFG to diabetes; **Model C**: progression from original IFG to diabetes; **Model D**: progression from either stage to diabetes. BP, blood pressure; CVD, cardiovascular disease; OR, odds ratio.
References


Androgens and Diabetes in Men

Results from the Third National Health and Nutrition Examination Survey (NHANES III)

ELIZABETH SELVIN, PHD1,2
MANNING FEINLEIB, MD, MPH1, DRPH1
LEI ZHANG, SCM3
SABINE ROHRMANN, PHD, MPH4
NADER RIFAI, PHD5

William G. Nelson, MD, PhD6,7
ADRIAN DOBS, MD, MHS8
SHEHZAD BASARIA, MD8
SHERITA HILL GOLDEN, MD, MHS1,2,8
ELIZABETH A. PLATZ, ScD, MPH1,7,9

OBJECTIVE — Low levels of androgens in men may play a role in the development of diabetes; however, few studies have examined the association between androgen concentration and diabetes in the general population. The objective of this study was to test the hypothesis that low normal levels of total, free, and bioavailable testosterone are associated with prevalent diabetes in men.

RESEARCH DESIGN AND METHODS — The study sample included 1,413 adult men aged ≥20 years who participated in the morning session of the first phase of the Third National Health and Nutrition Examination Survey, a cross-sectional survey of the civilian, noninstitutionalized population of the U.S. Bioavailable and free testosterone levels were calculated from serum total testosterone, sex hormone–binding globulin, and albumin concentrations.

RESULTS — In multivariable models adjusted for age, race/ethnicity, and adiposity, men in the first tertile (lowest) of free testosterone level were four times more likely to have prevalent diabetes compared with men in the third tertile (odds ratio 4.12 [95% CI 1.25–13.55]). Similarly, men in the first tertile of bioavailable testosterone also were approximately four times as likely to have prevalent diabetes compared with men in the third tertile (odds ratio 4.12 [95% CI 1.25–13.55]). These associations persisted even after excluding men with clinically abnormal testosterone concentrations defined as total testosterone <3.25 ng/ml or free testosterone <0.07 ng/ml. No clear association was observed for total testosterone after multivariable adjustment (P for trend across tertiles = 0.27).

CONCLUSIONS — Low free and bioavailable testosterone concentrations in the normal range were associated with diabetes, independent of adiposity. These data suggest that low androgen levels may be a risk factor for diabetes in men.


From the 1Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; the 2Welch Center for Prevention, Epidemiology and Clinical Research, Johns Hopkins University, Baltimore, Maryland; the 3Dana Center for Preventive Ophthalmology, Department of Ophthalmology, Johns Hopkins School of Medicine, Baltimore, Maryland; the 4Division of Clinical Epidemiology, Deutsches Krebsforschungszentrum, Heidelberg, Germany; the 5Department of Laboratory Medicine, Brigham and Women’s Hospital, Children’s Hospital, Harvard Medical School, Boston, Massachusetts; the 6Departments of Oncology, Urology, Pharmacology, Medicine, and Pathology, Johns Hopkins University, Baltimore, Maryland; the 7Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, Maryland; the 8Division of Endocrinology and Metabolism, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland; and the 9Brady Urological Institute, Johns Hopkins Medical Institutions, Baltimore, Maryland.

Address correspondence and reprint requests to Elizabeth A. Platz, ScD, MPH, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St., E6138, Baltimore, MD 21205. E-mail: eplatz@jhsph.edu.

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Abbreviations: AAG, androstanediol glucuronide; NHANES III, Third National Health and Nutrition Examination Survey; SHBG, sex hormone–binding globulin.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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sample sizes of specific subgroups of the U.S. population, Mexican Americans, non-Hispanic blacks, and the elderly were oversampled. Subjects participated in an interview that was conducted at home, as well as an extensive physical examination. This examination was performed at a mobile examination center and included collection of a blood sample.

NHANES III was conducted in two phases (1988–1991 and 1991–1994). Unbiased national estimates of health and nutrition characteristics can be independently produced for each phase. Within each phase, subjects were randomly assigned to participate in either the morning or afternoon/evening examination session. In total, 33,944 subjects were interviewed in NHANES III, of which 30,818 had a physical examination at the medical examination center. Of 14,781 male subjects with an examination, 9,282 participated in the morning session of phase I. Morning sample participants were chosen for this hormone study to reduce extraneous variation due to diurnal production of sex hormones. Serum was still available in the main NHANES III repository for 1,637 of these men: 716 non-Hispanic white, 411 non-Hispanic black, and 413 Mexican Americans. Of 14,781 male subjects with an examination, 9,282 had a physical examination at the medical examination center or during an interview that was conducted at home, as well as an extensive physical examination. This examination was performed at a mobile examination center and included collection of a blood sample.

Table 1—Selected characteristics of the study population in men aged ≥20 years by diabetes status, U.S. 1988–1991, NHANES III

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Diabetes</th>
<th>No diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1,413</td>
<td>101</td>
<td>1,312</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.4 ± 0.8</td>
<td>41.8 ± 0.8</td>
<td>57.0 ± 2.8</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>84.2 ± 2.6</td>
<td>84.6 ± 2.5</td>
<td>74.5 ± 6.6</td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>10.3 ± 2.0</td>
<td>9.9 ± 1.9</td>
<td>19.6 ± 5.9</td>
</tr>
<tr>
<td>Mexican American</td>
<td>5.5 ± 1.5</td>
<td>5.5 ± 1.5</td>
<td>5.9 ± 2.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.4 ± 0.2</td>
<td>26.2 ± 0.2</td>
<td>29.5 ± 1.4</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.95 ± 0.003</td>
<td>0.95 ± 0.003</td>
<td>1.02 ± 0.01</td>
</tr>
</tbody>
</table>

Data are means or proportions ± SE.

Sex steroid hormones

The main hormones of interest in this study were measured total testosterone (serum), estimated bioavailable testosterone, and estimated free testosterone. Testosterone is the major male androgen, and its free circulating levels are primarily determined by sex hormone–binding globulin (SHBG). Free (unbound) testosterone accounts for a relatively small circulating concentration of total testosterone (2–3%). Bioavailable testosterone is the concentration of non–SHBG-bound testosterone and is comprised of both free and albumin-bound (20–40%) testosterone levels. Total testosterone is the combination of circulating bioavailable levels and SHBG-bound levels (considered biologically inactive). Measurements of free and bioavailable testosterone levels more accurately represent concentrations readily available to tissues and metabolic processes.

Serum testosterone, estradiol, androstanediol glucuronide (AAG), and SHBG concentrations were measured as part of the larger Hormone Demonstration Project, and all are included in the present study for comprehensiveness. Estradiol is the major estrogen in men; AAG is an indicator of the conversion of testosterone to dihydrotestosterone, the major intraprostatic androgen; and, as mentioned above, SHBG is the major carrier of testosterone and estradiol in circulation.

Blood was drawn after an overnight fast for participants in the morning sample during either an examination at the medical examination center or during an abbreviated examination at home. After centrifugation, the serum was aliquotted and stored at −70°C until spring 2005. Levels of sex steroid hormones and SHBG are stable after multiple freeze-thaw cycles (9,10). Serum concentrations of testosterone, estradiol, AAG, and SHBG were measured in the laboratory of N.R. at Children’s Hospital in Boston, Massachusetts. We used competitive electrochemiluminescence immunoassays on the 2010 Elecsys autoanalyzer (Roche Diagnostics, Indianapolis, IN) to quantify serum testosterone, estradiol, and SHBG concentrations. AAG was measured by an enzyme immunoassay (Diagnostic Systems Laboratories, Webster, TX). The participant samples were randomly ordered for testing, and the laboratory technicians were blinded to participant characteristics. The lowest detection limits of the assays were 0.02 ng/ml testosterone, 5 pg/ml estradiol, 0.33 ng/ml AAG, and 3 nmol/l SHBG. For quality control specimens included during the analyses of NHANES III specimens, the coefficient of variation percentages were as follows: testosterone 5.9 and 5.8% at 2.5 and 5.5 ng/ml, estradiol 6.5 and 6.7% at 102.7 and 474.1 pg/ml, AAG 9.5 and 5.0% at 2.9 and 10.1 ng/ml, and SHBG 5.3 and 5.9% at 5.3 and 16.6 nmol/l. Bioavailable and free testosterone levels were calculated from serum total testosterone, SHBG, and albumin concentrations (11).

The protocols for conduct of NHANES III were approved by the institutional review board of the National Center for Health Statistics, Centers for Disease Control and Prevention. Informed consent was obtained from all participants. The assay of stored serum specimens for the Hormone Demonstration Project was approved by institutional review boards at the Johns Hopkins Bloomberg School of Public Health and the National Center for Health Statistics, Centers for Disease Control and Prevention.

Statistical analysis

All statistical analyses were performed using SUDAAN, as implemented in SAS v. 8.1 (Cary, NC) software. In each analysis, we applied sampling weights to take into account the specific probabilities of selection for the individual domains that were oversampled, nonresponse, and differences between the sample and the total U.S. population. Because the serum concentrations were not normally distributed, we compared geometric means by
Androgens and diabetes in men

Table 2—Crude and adjusted geometric means (95% CI) of sex steroid hormone concentrations in men aged ≥20 years by diabetes status, U.S. 1988–1994

<table>
<thead>
<tr>
<th>Overall</th>
<th>No diabetes</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude geometric mean (95% CI) Adjusted geometric mean (95% CI)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>3.41</td>
<td>3.25</td>
</tr>
<tr>
<td>No diabetes</td>
<td>3.41</td>
<td>3.25</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3.41</td>
<td>3.25</td>
</tr>
</tbody>
</table>

Testosterone (ng/ml)

<table>
<thead>
<tr>
<th>Overall</th>
<th>5.11 (4.93–5.29)</th>
<th>5.15 (4.97–5.34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No diabetes</td>
<td>5.11 (4.93–5.29)</td>
<td>5.15 (4.97–5.34)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>5.11 (4.93–5.29)</td>
<td>5.15 (4.97–5.34)</td>
</tr>
</tbody>
</table>

Estradiol (pg/ml)

<table>
<thead>
<tr>
<th>Overall</th>
<th>12 (9.2–15.7)</th>
<th>12 (9.2–15.7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No diabetes</td>
<td>12 (9.2–15.7)</td>
<td>12 (9.2–15.7)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>12 (9.2–15.7)</td>
<td>12 (9.2–15.7)</td>
</tr>
</tbody>
</table>

SHBG (μM)

<table>
<thead>
<tr>
<th>Overall</th>
<th>4.38 (3.75–5.09)</th>
<th>4.26 (3.64–5.01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No diabetes</td>
<td>4.38 (3.75–5.09)</td>
<td>4.26 (3.64–5.01)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4.38 (3.75–5.09)</td>
<td>4.26 (3.64–5.01)</td>
</tr>
</tbody>
</table>

Diabetes status. Molar ratios of testosterone to SHBG, estradiol to SHBG, and estradiol to testosterone were calculated and analyzed in the same way.

In logistic regression models of prevalent diabetes, the highest (third) tertile of each hormone was used as the reference group and we adjusted for age, race/ethnicity, BMI, and waist-to-hip ratio, as these factors may both influence hormone concentrations and the distributions of which vary by diabetes status. In additional logistic regression models, we also examined the association of total testosterone, estradiol, and SHBG with diabetes after simultaneously adjusting for the other two hormones and the risk factors listed above. Simultaneous adjustment for testosterone, estradiol, and SHBG allowed us to observe whether an association was present with one hormone while holding constant the concentrations of the other two.

RESULTS—Table 1 shows selected (crude) characteristics of this study population of men aged ≥20 years by diabetes status. Men with diabetes were substantially older, more likely to be non-Hispanic black or Mexican American, and had higher BMI and waist-to-hip ratio, highlighting the importance of adjustment for these factors in subsequent analyses.

Table 2 displays the crude and age- and race/ethnicity-adjusted geometric means of each hormone and molar ratios by diabetes status. Total testosterone and estimated bioavailable testosterone concentrations were lower in men with diabetes compared with men without diabetes. Estradiol levels were similar in men with and without diabetes. SHBG levels appeared higher in men with diabetes but not after age and race/ethnicity adjustment. Crude estimated free testosterone was lower in men with diabetes, but this did not persist following adjustment.

The results from our adjusted logistic regression models are displayed in Tables 2 and 3. Total testosterone, estradiol, SHBG, and AAG were not significantly associated with diabetes status after multivariable adjustment (Table 3). However, as shown in Table 4, there was some evidence of an association between total testosterone and diabetes after further adjustment for estradiol and SHBG (odds ratio [OR] 1.99 [95% CI 0.76–5.19]; P value for trend = 0.014). Estimated free testosterone and bioavailable testosterone were highly inversely associated with diabetes status, even after multivariable adjustment (Table 3). Men in the lowest tertile of free testosterone level were four times more likely to have prevalent diabetes compared with men in the third tertile (4.12 [1.25–13.55]; P value for trend = 0.04). Similarly, men in the first tertile of bioavailable testosterone were also approximately four times as likely to have prevalent diabetes compared with men in the third tertile (3.93 [1.39–11.13]; P value for trend = 0.01). These associations persisted even after further adjustment for total cholesterol, triglycerides, and systolic blood pressure (analyses not shown).

We also conducted sensitivity analyses including cases of undiagnosed diabetes (diabetes defined on the basis of a fasting glucose alone; n = 58) and excluding men with low total testosterone (<3.25 ng/ml; n = 211) or low free testosterone (<0.07 ng/ml; n = 339). The results for estimated free testosterone and total testosterone were essentially unchanged after excluding men with clinically low levels: OR 3.23 (95% CI 1.18–8.86) for free testosterone and 1.03 (0.41–2.58) for total testosterone comparing men in the first tertile with the third. The results for all models also were not altered appreciably by the inclusion of undiagnosed diabetes in our case definition (analyses not shown).

CONCLUSIONS—The independent association of low free and bioavailable testosterone levels in our adjusted models suggest that testosterone insufficiency may be a risk factor for diabetes. Associations of low free and bioavailable testosterone levels with diabetes remained even after adjustment for age and known confounding factors including race/ethnicity and adiposity, as measured by BMI and waist-to-hip ratio. The association with low free testosterone persisted even after the exclusion of men with clinically low total and/or free testosterone levels, suggesting that this association was not entirely driven by hypogonadal men.

While the directionality of the associations between low androgen levels and adiposity remain unclear, our data are consistent with the hypothesis that an...
Table 3—Adjusted* OR (95% CI) of diabetes by tertiles of sex steroid hormone concentrations, NHANES III

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Q1 (lowest)</th>
<th>Q2</th>
<th>Q3 (highest)</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total testosterone (ng/mL)</td>
<td>(≤4.54, 4.55–6.27, &gt;6.27)</td>
<td>1.27 (0.61–2.65)</td>
<td>0.51 (0.15–1.72)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Estradiol (E2) (pg/mL)</td>
<td>(≤31.90, 31.91–40.26, &gt;40.26)</td>
<td>0.88 (0.36–2.18)</td>
<td>0.96 (0.36–2.57)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>(≥28.03, 28.04–43.50, &gt;43.50)</td>
<td>0.68 (0.29–1.59)</td>
<td>0.90 (0.43–1.87)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>AAG (≤0.57, 0.95–15.44, &gt;15.44 ng/mL)</td>
<td>2.10 (0.79–5.58)</td>
<td>1.69 (0.64–4.46)</td>
<td>1.00 (reference)</td>
<td>0.12</td>
</tr>
<tr>
<td>Estimated free testosterone (≤0.99, 0.10–0.14, &gt;0.14 ng/mL)</td>
<td>4.12 (1.25–13.55)</td>
<td>2.86 (0.78–10.45)</td>
<td>1.00 (reference)</td>
<td>0.04</td>
</tr>
<tr>
<td>Estimated bioavailable testosterone (≥2.11, 2.12–3.02, &gt;3.02 ng/mL)</td>
<td>3.93 (1.39–11.13)</td>
<td>3.05 (0.85–10.88)</td>
<td>1.00 (reference)</td>
<td>0.01</td>
</tr>
<tr>
<td>Estradiol:total testosterone (0.43–1.89, &gt;8.23) (≥6.24, 6.25–8.23, &gt;8.23)*</td>
<td>0.90 (0.43–2.26)</td>
<td>1.00 (reference)</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Total testosterone:SHBG (≥0.45, 0.46–0.63, &gt;0.63)</td>
<td>2.19 (0.83–5.73)</td>
<td>1.65 (0.71–3.83)</td>
<td>1.00 (reference)</td>
<td>0.11</td>
</tr>
<tr>
<td>Estradiol:SHBG (0.79–5.58) (≥2.92, 2.93–4.75, &gt;4.75)*</td>
<td>2.10 (0.79–5.58)</td>
<td>1.50 (0.61–3.65)</td>
<td>1.00 (reference)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*Adjusted for age, race/ethnicity, BMI, and waist-to-hip ratio.

drogens may directly influence glucose metabolism and the development of insulin resistance independently of the effects of adiposity. Nonetheless, our results are not as strong in magnitude as those reported in some previous studies (12), likely because we examined these associations in the general male population with “normal” range androgens. We did not observe a clear association of total testosterone concentration with diabetes. Contrary to previous studies (13–15), we did not observe significantly lower levels of SHBG in diabetic compared with nondiabetic men before or after adjustment.

The cross-sectional design is an important limitation of this study. We cannot determine the temporality of the associations observed here between androgen levels and diabetes. However, several previous prospective analyses (13,14,16,17) suggest that decreases in testosterone level may precede the development of diabetes, lending support to a temporal if not causal relation. Additionally, including individuals with undiagnosed diabetes, a population at an earlier point in the progression of diabetes, did not change our results. This is one of the largest epidemiologic studies of androgens and diabetes in men in the published literature (12); nonetheless, we were unable to explore possible effect modification and subgroup analyses due to power limitations resulting from the relatively small number of diabetic cases (n = 101) in this general population.

To our knowledge, this is the first study to examine the association between sex steroids and diabetes in a large, nationally representative male population. Strengths of the present study were the large sample and corresponding power to detect small differences, even after adjustment for relevant covariates including measures of adiposity. Furthermore, we have shown here relationships of each major sex steroid hormone with diabetes including differences by molar ratios and multivariable models, which included simultaneous adjustment for total testosterone, estradiol, and SHBG. This study also benefited from the rigorous and standardized measurement of demographic characteristics, laboratory analyses, and anthropometric measures in the NHANES III Study.

In men, serum levels of testosterone and bioavailable testosterone decline with age (18); however, the clinical consequences of this decline are largely uncharacterized. The literature investigating the association between androgen levels in men and the development of cardiovascular disease has been equivocal (19), with some evidence of a possible protective effect of higher testosterone levels (13,20–23). Diabetes is a known risk factor for the development of atherosclerosis and cardiovascular disease. Further studies are needed to understand if the elevated risk of cardiovascular disease in hypogonadal men and in men with “low normal” androgen levels seen in some studies might be wholly or partially mediated by the development of diabetes. Additional epidemiologic and etiologic studies are needed to clarify the mechanisms by which sex steroid hormones may directly contribute to diabetes and other chronic diseases.

Table 4—Adjusted* OR (95% CI) of diabetes by tertiles of sex steroid hormone concentrations after mutually adjusting the hormones, NHANES III

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Q1 (lowest)</th>
<th>Q2</th>
<th>Q3 (highest)</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total testosterone (ng/mL)</td>
<td>(≤4.54, 4.55–6.27, &gt;6.27)</td>
<td>1.99 (0.76–5.19)</td>
<td>0.64 (0.15–2.65)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Estradiol (E2) (pg/mL)</td>
<td>(≤31.90, 31.91–40.26, &gt;40.26)</td>
<td>0.71 (0.24–2.07)</td>
<td>0.99 (0.29–3.33)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>(≤28.03, 28.04–43.50, &gt;43.50)</td>
<td>0.48 (0.20–1.18)</td>
<td>0.76 (0.35–1.63)</td>
<td>1.00 (reference)</td>
</tr>
</tbody>
</table>

*Simultaneously adjusted for age, race/ethnicity, BMI, waist-to-hip ratio, and the other two hormones.
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Acknowledgments—E.S. was supported by National Heart, Lung, and Blood Institute Grant T32HL07024. This study is the third from the Hormone Demonstration Program, which is supported by the Maryland Cigarette Restitution Fund Research Grant Program at Johns Hopkins University.

References

Serum Adiponectin and Renal Dysfunction in Men With Type 2 Diabetes

Julie Lin, MD, MPH1,2
Frank B. Hu, MD, PhD1,3,4
Gary Curhan, MD, ScD1,2,4

OBJECTIVE — Inflammation is associated with both chronic kidney dysfunction and type 2 diabetes. Adiponectin, a novel circulating anti-inflammatory protein made by adipocytes, has been reported to be lower in diabetic than nondiabetic subjects. In contrast, serum levels of adiponectin are elevated in end-stage renal disease. We sought to investigate the relation between adiponectin and mild to moderate renal dysfunction in men with type 2 diabetes.

RESEARCH DESIGN AND METHODS — Multivariate logistic regression was used to evaluate the relation between serum adiponectin concentrations and the presence of renal dysfunction (estimated glomerular filtration rate [eGFR] <60 ml/min per 1.73 m²) by the four-variable Modification of Diet in Renal Disease equation) in participants with type 2 diabetes in the Health Professionals’ Follow-Up Study. A total of 733 men were included in this cross-sectional analysis.

RESULTS — Adiponectin was positively correlated with age (Spearman coefficient, r = 0.19, P < 0.001) and negatively correlated with weight (Spearman coefficient, r = −0.18, P < 0.001). Those with adiponectin in the second quartile or higher (>10 μg/ml) compared with those in the first quartile had a reduced odds for renal dysfunction (multivariate odds ratio 0.48 [95% CI 0.28–0.81]). These results were unchanged when serum lipids were included in the multivariate model.

CONCLUSIONS — We conclude that a higher serum adiponectin concentration is associated with reduced odds of moderate renal dysfunction in men with type 2 diabetes.

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Systemic inflammation has been implicated in the progression of chronic kidney disease in animal models (1,2) and in humans (3,4). As the leading cause of kidney failure in the world, type 2 diabetes has been postulated to be a generalized inflammatory condition resulting from obesity-induced dysregulation of adipocytes, which produce an excess of inflammatory cytokines (5). Scientists have speculated that this persistent inflammatory state further contributes to the development of the extensive vascular disease characteristic of diabetes.

Adiponectin, a recently discovered circulating 30-kDa protein exclusively secreted by adipocytes, is present at concentrations of 5–30 μg/ml in healthy humans (6) and is considered to be an important modulator of insulin sensitivity (7) and dyslipidemia (8). Anti-inflammatory properties also have been attributed to adiponectin, a theory supported by observations that serum concentrations of adiponectin are inversely associated with inflammatory markers such as fibrinogen, intracellular adhesion molecule-1, E-selectin, and C-reactive protein (9,10). The observation that adiponectin may be protective against vascular disease via the above mechanisms is supported by cross-sectional analyses of individuals with coronary heart disease, who have lower concentrations of adiponectin when compared with control subjects (11,12), and prospective studies revealing that higher adiponectin is associated with a decreased risk for subsequent cardiovascular disease events in nondiabetic subjects (13), type 1 diabetic subjects (14), type 2 diabetic men (15), and in end-stage renal disease patients (16). The role of adiponectin in cardiovascular disease is not definitive, however, because some studies have found no relation between adiponectin and cardiovascular disease risk (17,18).

Adiponectin appears to play an important role in the pathogenesis of type 2 diabetes. Cross-sectional studies (19–22) have demonstrated that serum concentrations of adiponectin are decreased in type 2 diabetic subjects compared with nondiabetic control subjects. Moreover, higher adiponectin levels are associated with better lipid and glycemic control in type 2 diabetic subjects (8,9). One prospective study (23) has reported that lower baseline serum adiponectin appears to be a harbinger for the development of type 2 diabetes.

In kidney disease, higher adiponectin levels are present in dialysis patients (16) but not in nondiabetic patients with predialysis chronic kidney disease (7) when compared with healthy control subjects. The association of adiponectin with kidney function in individuals with type 2 diabetes, however, is not well described. The existing literature has focused mainly on those with glucose intolerance or end-stage renal disease. Previous work (24) by our group has revealed that in male diabetic subjects, estimated glomerular filtration rate (eGFR) <60 ml/min per 1.73 m² is inversely correlated with several circulating lipid and inflammatory markers and that adiponectin is inversely correlated with dyslipidemia and inflammation (8). We therefore hypothesized that higher adiponectin might be associated with decreased odds for mild to moderate renal insufficiency in type 2 diabetes.

RESEARCH DESIGN AND METHODS — The Health Professionals’ Follow-Up Study (HPFS) was estab-
Adiponectin and renal function in diabetes

lished in 1986 when 51,529 U.S. male health professionals, aged 40–75 years at study initiation, returned a mailed questionnaire providing information about diet, lifestyle factors, and medical history (25). Participants were mailed follow-up questionnaires every 2 years to update information. In 1993–1994, blood samples were collected and frozen (−130°C) from a subset of these participants (n = 18,159) as previously described (8). Demographic and clinical characteristics at baseline were similar between men who provided blood samples and those who did not.

Diabetes was first identified by self-report on a biennial questionnaire and confirmed by a Diabetes Supplemental Questionnaire in 2000; the validity of the Diabetes Supplemental Questionnaire in confirming diabetes has been demonstrated in the HPFS cohort (26). The HPFS diabetes blood cohort consists of 1,000 men with confirmed diabetes diagnosed before study entry or newly diagnosed up through June 1998 who provided a blood sample in 1993–1994. Exclusion criteria for the current analyses were I) age of onset of diabetes ≤25 years of age (to attempt to restrict the study to type 2 diabetes)(*n = 31, 2) reported date of diabetes diagnosis after the date of blood draw (*n = 224), 3) participants who reported on the Diabetes Supplemental Questionnaire that they were on dialysis (*n = 9) or had a kidney transplant (*n = 1), 4) serum creatinine >5.0 mg/dl (*n = 1), and 5) serum creatinine ≤0.5 mg/dl (felt to be physiologically implausible) (*n = 1). After these exclusions, 733 men were available for analysis. This study was approved by the institutional review boards at the Harvard School of Public Health and the Brigham and Women’s Hospital.

Biochemical analysis
Adiponectin was measured by competitive radioimmunoassay using a commercial reagent set from Linco Research (St. Louis, MO). A previous study (27) demonstrated that adiponectin measurements are highly stable and reproducible under transport conditions and in frozen whole-blood samples. The coefficient of variation was 3.4%. A1C was measured by turbidimetric immunoassay using hemolyzed whole blood or packed red cells with a coefficient of variation of 7.5%. Plasma creatinine was measured by a modified kinetic Jaffe reaction with a coefficient of variation of 22%.

Assessment of covariates
Race and height were initially reported on the 1986 questionnaire. Other clinical and lifestyle variables (hypertension, weight, physical activity, cigarette smoking, and medication use) were derived from data from the 1994 questionnaire, which included data closest to the time of blood draw. BMI was calculated by weight in kilograms divided by the square of height in meters. A weekly metabolic equivalent (MET) score was calculated from physical activity questions. Cardiovascular disease (myocardial infarction, coronary artery bypass grafting, or angina) was confirmed by medical record review (28).

Assessment of renal function
Renal dysfunction was defined as an eGFR <60 ml/min per 1.73 m² as calculated by the four-variable MDRD (Modification of Diet in Renal Disease) equation (eGFR [ml/min per 1.73 m²] = 186 × [Cr (mg/dl)] −1.154 × [age] −0.203 × [1.21 if subject is black]) (29). We also examined estimated creatinine clearance using the Cockcroft-Gault equation as the measure of kidney function (30). Of note, because the Cockcroft-Gault results were noticeably influenced by weight, as expected, we chose to present data only with eGFR by the MDRD equation.

Statistical analysis
The Wilcoxon signed-rank test was used for comparisons of continuous variables. Spearman correlation coefficients were calculated for pairs of continuous variables. Logistic regression was used to calculate odds ratios for adiponectin levels with eGFR <60 ml/min per 1.73 m² as the outcome. Multivariate models were adjusted for age (continuous), years, hypertension (yes/no), BMI (continuous), cigarette smoking status (never, past, or current), physical activity (quartiles, METs/week), duration of type 2 diabetes (quartiles, years), measured A1C (quartiles), and cardiovascular disease (yes/no). ACE and statin use did not change point estimates when included in the model and were removed. All analyses were performed with SAS software version 8.2 (SAS Institute, Cary, NC).

RESULTS — Characteristics of the HPFS diabetes cohort are presented in Table 1. Participants had a median age of 67 years, were mostly Caucasian, and had been carrying the diagnosis of diabetes for 9 years at the time of blood draw. The majority were overweight (BMI ≥25 kg/m²), almost half were hypertensive, one-quarter had cardiovascular disease, the median serum creatinine was 1.0 mg/dl, and median eGFR was 78 ml/min per 1.73 m².

By Spearman correlation, adiponectin was positively correlated with age (r = 0.19, P < 0.001) and inversely correlated with weight (r = −0.18, P < 0.001) and BMI (r = −0.24, P < 0.001) but not with eGFR (r = 0.01, P = 0.76). There was no association between adiponectin and serum creatinine (r = −0.04, P = 0.25) or adiponectin and eGFR when considering only those with eGFR <60 ml/min per 1.73 m² (r = −0.083, P = 0.44). Adiponectin was also significantly associated with serum LDL (r = 0.10, P = 0.008), triglycerides (r = −0.33, P < 0.001), HDL (r = 0.41, P < 0.001), and with A1C (r = 0.09, P = 0.007).

Using multivariate logistic regression, men in each of the upper three quartiles of adiponectin had a reduced odds for having eGFR <60 ml/min per 1.73 m² when compared with the lowest quartile (Table 2). Because the results suggested a threshold rather than a graded association, we combined the upper three quartiles of serum adiponectin and found that the odds ratio for kidney dysfunction in these individuals was 0.48 (95% CI 0.28–0.81). These results were unchanged after we adjusted for individual lipid markers by quartiles (odds ratio for those with adiponectin in the upper three quartiles remained significant and changed by <15% in all lipid analyses) (data not shown).

CONCLUSIONS — We found that serum adiponectin was inversely associated with presence of renal dysfunction in men with type 2 diabetes, the majority of whom had well-preserved eGFR (87% had eGFR >60 ml/min per 1.73 m²). There appeared to be a threshold in that those with an adiponectin level higher than the first quartile all had similar decreased odds ratios for renal dysfunction. Because of the reported associations between dyslipidemia and adiponectin, we also adjusted for these factors in our multivariate models and found that the relation between adiponectin and renal dysfunction remained independent and unchanged.

We adjusted for several potential confounders in the relation between adi-
Adiponectin and eGFR, including age, obesity, and hypertension. Consistent with our findings that adiponectin and age were positively correlated, previous investigations have reported that plasma adiponectin is higher in elderly men and women aged >70 years when compared with younger individuals (31). The relation between adiponectin and age appears stronger in diabetic subjects (Spearman r = 0.44) than in nondiabetic subjects (Spearman r = 0.15) (32). Serum adiponectin is decreased in obesity (10,32,33), in the presence and absence of diabetes, and in hypertension (34). By adjusting for these covariates, we found that the inverse association between serum adiponectin quartiles and presence of renal dysfunction was modestly strengthened.

Table 1—Demographic and clinical characteristics of type 2 diabetic subjects in the HPFS in 1994

<table>
<thead>
<tr>
<th></th>
<th>Entire cohort</th>
<th>eGFR ≥60 ml/min per 1.73 m²</th>
<th>eGFR &lt;60 ml/min per 1.73 m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>733</td>
<td>643</td>
<td>90</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67 (47–80)</td>
<td>66 (47–80)</td>
<td>69.5 (48–80)*</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>675 (92.2)</td>
<td>594 (92.4)</td>
<td>81 (90.0)</td>
</tr>
<tr>
<td>African American</td>
<td>11 (1.5)</td>
<td>8 (1.2)</td>
<td>3 (3.3)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>399 (45.6)</td>
<td>334 (31.9)</td>
<td>65 (72.2)*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85.5 (56.8–210.9)</td>
<td>85.0 (56.8–210.9)</td>
<td>86.4 (65.9–161.8)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1 (18.3–56.5)</td>
<td>27.1 (18.3–56.5)</td>
<td>28.2 (20.8–45.7)</td>
</tr>
<tr>
<td>BMI categories (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;22.0</td>
<td>33 (4.5)</td>
<td>31 (4.8)</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td>22–24.9</td>
<td>160 (21.8)</td>
<td>143 (22.2)</td>
<td>17 (18.9)</td>
</tr>
<tr>
<td>25–27.9</td>
<td>229 (31.2)</td>
<td>204 (31.7)</td>
<td>25 (27.8)</td>
</tr>
<tr>
<td>28–29.9</td>
<td>131 (17.9)</td>
<td>108 (16.8)</td>
<td>23 (25.6)</td>
</tr>
<tr>
<td>≥30</td>
<td>180 (24.6)</td>
<td>157 (24.4)</td>
<td>23 (25.6)</td>
</tr>
<tr>
<td>Activity (METs/week)</td>
<td>20 (0–228.8)</td>
<td>20.4 (0–228.8)</td>
<td>13.0 (0–160.3)†</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>43 (5.8)</td>
<td>38 (5.9)</td>
<td>5 (4.5)</td>
</tr>
<tr>
<td>Past</td>
<td>392 (53.5)</td>
<td>351 (54.6)</td>
<td>41 (45.6)</td>
</tr>
<tr>
<td>Never</td>
<td>261 (35.7)</td>
<td>225 (35.0)</td>
<td>36 (40.0)</td>
</tr>
<tr>
<td>Missing</td>
<td>37 (5.1)</td>
<td>29 (4.5)</td>
<td>8 (8.9)</td>
</tr>
<tr>
<td>Age at diabetes diagnosis (years)</td>
<td>55 (26–78)</td>
<td>55 (26–76)</td>
<td>55.5 (32–78)</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>9 (0.1–41.1)</td>
<td>8.6 (0.1–41)</td>
<td>10.8 (0.1–39.8)‡</td>
</tr>
<tr>
<td>Measured A1C (%)</td>
<td>7.2 (4.8–15.6)</td>
<td>7.2 (5.0–15.6)</td>
<td>6.8 (4.8–10.9)†</td>
</tr>
<tr>
<td>Baseline cardiovascular disease (myocardial infarction, coronary artery bypass graft, or angina)</td>
<td>194 (26.5)</td>
<td>156 (24.3)</td>
<td>38 (42.2)*</td>
</tr>
<tr>
<td>ACE inhibitor medication use</td>
<td>60 (8.2)</td>
<td>49 (7.6)</td>
<td>11 (12.2)</td>
</tr>
<tr>
<td>Statin medication</td>
<td>48 (6.6)</td>
<td>42 (6.5)</td>
<td>6 (6.7)</td>
</tr>
<tr>
<td>Median adiponectin (µg/ml)</td>
<td>14.3 (1.4–54.8)</td>
<td>14.4 (1.4–54.8)</td>
<td>14.0 (4.4–42.0)</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.0 (0.6–2.9)</td>
<td>1.0 (0.6–1.4)</td>
<td>1.4 (1.3–2.9)</td>
</tr>
<tr>
<td>eGFR (ml/min per 1.73 m²)</td>
<td>78 (23–142)</td>
<td>81 (60–142)</td>
<td>53 (23–59)</td>
</tr>
</tbody>
</table>

Data are median (range) or n (%) unless otherwise indicated. *P < 0.001; †P < 0.01; ‡P = 0.17 compared with eGFR ≥60 ml/min per 1.73 m².

Table 2—Age-adjusted and multivariate odds ratios for adiponectin quartiles and presence of moderate renal dysfunction (eGFR <60 ml/min per 1.73 m²)

<table>
<thead>
<tr>
<th>HPFS (n = 733)</th>
<th>Age-adjusted odds ratio (95% CI)</th>
<th>Multivariate odds ratio (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR &lt;60 ml/min per 1.73 m²</td>
<td>90/733 (12)</td>
<td>Reference</td>
</tr>
<tr>
<td>Adiponectin Q1</td>
<td>29/173 (17)</td>
<td>Reference</td>
</tr>
<tr>
<td>Adiponectin Q2</td>
<td>19/184 (10)</td>
<td>0.54 (0.29–1.02)</td>
</tr>
<tr>
<td>Adiponectin Q3</td>
<td>18/188 (10)</td>
<td>0.48 (0.26–0.92)</td>
</tr>
<tr>
<td>Adiponectin Q4</td>
<td>24/188 (13)</td>
<td>0.61 (0.34–1.09)</td>
</tr>
<tr>
<td>Adiponectin &gt;Q1</td>
<td>61/561 (11)</td>
<td>0.54 (0.33–0.88)</td>
</tr>
</tbody>
</table>

*Multivariate models are adjusted for age (continuous, years), hypertension (yes/no), BMI (continuous), cigarette smoking status (never, past, or current), physical activity (quartiles, METs/week), duration of type 2 diabetes (quartiles, years), measured A1C (quartiles), and cardiovascular disease (yes/no).
Adiponectin and renal function in diabetes

peritoneal dialysis patients (35) when compared with healthy control subjects. In predialysis individuals, two cross-sectional studies (32,36) of adiponectin and renal function in type 2 diabetic subjects have reported a positive association between adiponectin and renal function, in contrast to our study. Although both studies appropriately adjusted for potential confounding by age and BMI, the study populations were very different from ours, making direct comparisons of the results difficult. The report by Looker et al. (32) included 1,069 Pima Indians in contrast to the mostly Caucasian men in our cohort. The study by Guebre-Egziabher et al. (36) consisted of only 48 patients with a mean inulin GFR of 53.5 ml/min per 1.73 m², which is much lower than the eGFR of our study cohort. We did not observe an association, however, between adiponectin and eGFR even when we restricted our analyses to those with eGFR < 60 ml/min per 1.73 m². Another study analyzed 543 type 1 diabetic subjects and found an adjusted inverse association between adiponectin and creatinine clearance estimated by the Cockcroft-Gault equation of –0.33 ml/min difference in creatinine clearance for every 1 SD increase in adiponectin levels (37). Our study included only type 2 diabetic subjects, however, and associations between renal clearance and adiponectin may not be the same in insulin-resistant states compared with insulin-deficient ones such as type 1 diabetes. Similarly, although a study of 227 nondiabetic renal patients reported a significant inverse association between adiponectin and directly measured GFR (r = −0.25, P < 0.01) (7), the association between adiponectin and renal clearance could be different in type 2 diabetic subjects because adiponectin levels have been consistently reported to be inversely associated with insulin resistance and are therefore significantly lower in type 2 diabetes.

An alternative explanation for our contrasting findings is that this may be a chance finding, especially in light of the fact that we saw no statistically significant association between adiponectin and eGFR by Spearman correlation. It should be noted, however, that we did not find this result through multiple testing but instead entered into this project with an a priori hypothesis that adiponectin and renal clearance would be directly associated because of the existing literature on inverse association between adiponectin and vascular disease.

One potential explanation for the inverse association observed is that adiponectin reduces vascular disease. Supporting evidence for the role of adiponectin in decreasing vascular endothelial dysfunction is accruing. We previously have reported that adiponectin was positively correlated with HDL cholesterol (Spearman r = 0.42, P < 0.01) and negatively correlated with triglycerides (r = −0.38, P < 0.01), apoprotein B (r = −0.19, P < 0.01), C-reactive protein (r = −0.18, P < 0.01), and fibrinogen (r = −0.18, P < 0.01), which was independent of A1C and HDL in adjusted analyses in these diabetic men (8), suggesting that dyslipidemia and inflammation might be attenuated when serum adiponectin is higher. Similar inverse associations between adiponectin and lipids and inflammatory markers have been reported for diabetic female participants in the Nurses' Health Study (9). Adjusting for quartiles of lipid biomarkers in our analyses, however, did not influence the results.

The potential benefits of increasing serum adiponectin levels on decreasing future risk for renal function decline remains to be determined. As a circulating anti-inflammatory molecule, higher serum adiponectin has been reported to be associated with a decreased risk for cardiovascular events in diverse populations including people with non-diabetic chronic kidney disease (7), diabetic subjects (15), and end-stage renal disease patients (16), and therefore, its potential role in vascular disease is intriguing. In particular, diminishing the inflammatory state of diabetes may be central in modifying risk for progressive endothelial dysfunction and renal failure. For example, adiponectin levels are increased by thiazolidinediones (38,39), medications that improve insulin sensitivity at the cellular level (22), so raising low adiponectin states is achievable if proven to be beneficial. Rosiglitazone therapy has been confirmed to increase adiponectin levels in African Americans with impaired glucose tolerance or diabetes (40), although direct evidence for the benefit of thiazolidinediones in human diabetic nephropathy is currently lacking.

Adiponectin levels can be increased through nonmedication factors as well. Low dietary glycemic load and high-fiber diets may contribute to higher adiponectin levels in diabetic subjects (15). Moderate alcohol intake also appears to increase adiponectin levels by 0.8 mg/ml (P = 0.01) for each additional drink per day in this cohort (41). Cigarette smoking is another modifiable risk factor reported to be associated with lower adiponectin (42). The effects of these lifestyle modifications and higher adiponectin levels on kidney function decline over time remain to be determined.

Limitations to our study include its cross-sectional design, which limits conclusions about mechanism or temporal relation. No information is available on albuminuria because urine has not been collected and stored in this cohort. Lastly, renal function was estimated from creatinine-based prediction equations. The relatively high coefficient of variation of plasma creatinine would presumably result in random misclassification and bias the results toward the null.

Our observation of the inverse association of adiponectin with moderate renal dysfunction in men with type 2 diabetes needs to be replicated in non-Caucasians and in women. How serum adiponectin is related to eGFR decline over time also warrants further study. Based on these initial data, we hypothesize that sustained higher serum adiponectin levels, independent of glucose and lipid control, would be associated with slower rates of eGFR loss in type 2 diabetes.

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Impact of Self-Reported Patient Characteristics Upon Assessment of Glycemic Control in the Veterans Health Administration

Miriam Maney, MA, CP IQ1
Chen-Lin Tseng, DRPH1,2
Monika M. Safford, MD3
Donald R. Miller, SC D4
Leonard M. Pogach, MD, MBA1,2

OBJECTIVE — The purpose of this article was to evaluate the impact of self-reported patient factors on quality assessment of Veterans Health Administration medical centers in achieving glycemic control.

RESEARCH DESIGN AND METHODS — We linked survey data and administrative records for veterans who self-reported diabetes on a 1999 national weighted survey. Linear regression models were used to adjust A1C levels in fiscal year 2000 for socioeconomic status (education level, employment, and concerns of having enough food), physical and mental health status, BMI, and diabetes duration. Medical centers were ranked by deciles, with and without adjustment for patient characteristics, on proportions of patients achieving A1C <7 or <8%.

RESULTS — There was substantial medical center level variation in patient characteristics of the 56,740 individuals from 105 centers, e.g., grade school education (mean 15.3% [range 2.3–32.7%]), being retired (38.3% [19.9–59.7%]) or married (65.2% [43.7–77.8%]), food insufficiency (13.9% [7.2–24.6%]), and no reported exercise (43.2% [31.1–53.6%]). The final model had an R² of 7.8%. The Spearman rank coefficient comparing the thresholds adjusted only for age and sex to the full model was 0.71 for <7% and 0.64 for <8% (P < 0.0001). After risk adjustment, 4 of the 11 best-performing centers changed at least two deciles for the <7% threshold, and 2 of 11 changed two deciles for the <8% threshold.

CONCLUSIONS — Adjustment for patient self-reported socioeconomic status and health impacts medical center rankings for glycemic control, suggesting the need for risk adjustment to assure valid inferences about quality.

Performance measurement is an integral part of health care management. Because intermediate health outcome measures are closely linked to morbidity and mortality (1), they are increasingly being used to assess the quality of care (2–4), despite concerns over possible unintended consequences of performance measurement and “pay-for-performance” (5).

From the 1Department of Veterans Affairs, New Jersey Healthcare System–Center for Healthcare Knowledge Management, East Orange, New Jersey; the 2University of Medicine and Dentistry of New Jersey–New Jersey Medical School, Newark, New Jersey; the 3Deep South Center on Effectiveness at the Birmingham VA Medical Center and the University of Alabama at Birmingham, Birmingham, Alabama; and the 4School of Public Health, Boston University, Boston, Massachusetts, and the Bedford VA Medical Center for Health Quality, Outcomes and Economic Research, Bedford, Massachusetts.

Address correspondence and reprint requests to Leonard M. Pogach, MD, MBA, VA HSR&D Center for Healthcare Knowledge Management Research, VA New Jersey Healthcare System, 385 Tremont Ave., East Orange, NJ. E-mail: len.pogach@verizon.net.

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Abbreviations: LVHS, Large Veterans Health Survey; NCQA, National Committee for Quality Assurance; VHA, Veterans Health Administration.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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to "take action" during the study period. Using a combination of survey and administrative data, we developed a regression model to adjust A1C levels. Results from this model were used to risk adjust VHA medical center level rankings based upon the proportion of individuals achieving A1C thresholds of <8 and <7%. We hypothesized that patient-level variables would vary sufficiently among VHA medical centers to result in substantial changes in rankings with and without risk adjustment.

RESEARCH DESIGN AND METHODS

Data sources and cohort identification
The Large Veterans Health Survey (LVHS), a weighted national representative survey of about 1,400,000 VHA enrollees was conducted in the summer of 1999 (13) with a response rate of 63.1% (n = 887,775). In this survey 190,374 individuals reported being told by a doctor that they had diabetes. We merged utilization and laboratory data available in VHA administrative databases for this cohort as previously described (14). Briefly, inpatient and outpatient utilization data and ICD-9 codes were obtained from the National Patient Clinical Dataset (Austin, TX), and laboratory data were obtained from the VA Healthcare Analysis Information Group.

To approximate NCQA criteria for indemnity plan member inclusion in Healthcare Employee Data Information Set reporting (15), we included only veterans who had at least two diabetes-related (250.x diagnostic code) visits with a clinician in the VHA health care system in fiscal year 1998 and/or fiscal year 1999 and excluded individuals whom we identified as being deceased before 1 October 1999, using the VHA Beneficiary Identification and Records Locator System and the Medicare Denominator File (13). We identified 132,076 subjects who met these criteria.

To minimize any impact of difference in A1C methodologies, we eliminated medical centers that used A1C methodologies in fiscal year 2000 that were not certified by the National Glycohemoglobin Standardization Program as previously described (16). Although the Health Plan Employee Data and Information Set (HEDIS) measures for glycemic control count A1C not measured as being greater than the threshold value, we also excluded individuals at remaining medical centers who did not have laboratory tests performed in the VHA both to focus our study upon the influence of patient characteristics on glycemic control and because administrative data could not capture laboratory tests performed outside the VHA and recorded in progress notes. This resulted in exclusion of 54,460 subjects.

Of the remaining 77,616 individuals, we excluded 20,876 who did not answer questions on the LVHS that were included in our analysis (Table 1). Our final study population consisted of 56,740 VHA clinical users with diabetes at 105 medical centers. We compared baseline characteristics of veterans with diabetes in our final study cohort with those who were excluded as described above. The VA New Jersey Healthcare System Institutional Review Board approved this study.

Model development
Variables used in risk adjustment. We selected LVHS variables that reflect characteristics largely outside the control of a health care plan yet are known to be associated with adherence to intensive treatment and improved outcomes (12). Demographic variables included age and sex. Health status variables included physical health and mental health component scores measured by the Veterans Short Form-36. Health behavior included smoking, alcohol use, and exercise level. Social support variables included marital status and living arrangements (living alone or not). Socioeconomic status variables included education level, employment, and extreme economic hardship.

The latter was assessed by a food sufficiency question that asks "In the past 30 days have you been concerned about having enough food for you or your family?" with yes or no responses. Other variables included duration of diabetes and height and weight (enabling calculation of BMI).

Modeling. We used general linear regression models to develop a risk adjustment model for individual A1C levels. We retained variables that were significant at the P < 0.05 level in the final model. Our comparison model included only age and sex (17) to evaluate the marginal impact of the variables of interest upon glycemic control and medical center level performance.

Medical center profiling. We evaluated the impact of risk adjustment on medical center profiling, separately for each of two A1C thresholds (<7 and <8%), by comparing ranks from before and after risk adjustment. Whereas the <7% threshold has always been considered the target goal for glycemic control, the 8% level was consistent with 1999-2000 American Diabetes Association Clinical Practice Recommendations (18) for taking action. An individual subject met adherence criteria to an individual measure if the last A1C level achieved in fiscal year 2000 was below the threshold.

To determine the ranks for unadjusted A1C values among medical centers, we first identified individuals at each medical center whose A1C values were below the unadjusted A1C threshold (the "observed"). We then used the total number with diabetes at that medical center to generate a proportion and ranked medical centers on these proportions of observed patients (19). To determine the ranks using adjusted A1C values, we used two different models: Model 1 included only age and sex as independent variables; model 2 included all the variables described above. For each of the models, we performed the following steps. First, we calculated the percentage of individuals in the entire study population (105 medical centers) with A1C values below the threshold of observed A1C. Next, we identified individuals with adjusted A1C values at or below the corresponding population-level percentage for each threshold. Of these individuals, we then counted the number of individuals at each medical center (the "expected"). This information was used to generate observed-to-expected ratios for each medical center, and medical centers were then ordered on these ratios to obtain risk-adjusted ranks. We repeated this process for both 8 and 7% thresholds of A1C. For each medical center, six unique ranks were possible: by both 7 and 8% thresholds, ranking based on unadjusted ratios and ranking based on two adjusted models with age and sex in the first and all socioeconomic self-reported variables already described.

Because the use of best and worse decile rankings is one industry standard for identifying best and worse health plans (2), we ranked VHA medical centers into deciles and used league tables to determine the degree of ranking movement for medical centers in the best and worse two deciles for both the <7 and <8% threshold after risk adjustment. The degree of movement was evaluated by shifts in decile ranks. We reported the number of medical centers that changed decile ranks and the magnitude of the change.
RESULTS — The individuals in the study sample with complete data were mostly male (mean 98%) and elderly (mean age 64.6 years). Individuals who were included in the analysis were slightly younger than those who were excluded (19.7 vs. 15.2% <55 years of age), more likely to be married (65.5 vs. 60.3%), and better educated (Table 1). There were marked differences in the socioeconomic status and health-related characteristics of the patient populations among VHA medical centers (Table 2). For example, there were substantial variations in having completed only grade school education (mean 15.3% [range 2.3–32.7%]), being retired (38.3% [19.9–59.7%]) or married (65.2% [43.7–77.8%], reporting concern about food sufficiency (13.9% [7.2–24.6%]), or reporting no exercise (43.2% [31.1–53.6%]). There was also variation in self-reported health status as assessed by the mental health component score (43.8 [38.7–48.9]) and the physical health component score (31.4 [27.6–36.4]) of the Veterans Short Form-36. Mean medical center levels of A1C varied from 7.14 to 8.58%, and the proportion of individuals achieving A1C levels <7 and <8% varied from 23 to 56% and from 44 to 79%, respectively.

Variables and interaction terms that contributed significantly to variations in A1C and were retained in the final model included age, marital status, BMI, duration of diabetes, education level, employment status, concern for food sufficiency, smoking frequency, and exercise frequency (Table 2). The $R^2$ of this model was 7.8%. Being older, being female, having a higher education level, and exercising more frequently were associated with having lower A1C levels. Longer duration of diabetes and higher BMI were associated with higher A1C levels.

Medical center rankings from the <7 and <8% thresholds were highly correlated (Spearman rank coefficient unadjusted 0.89 [P < 0.001] and adjusted 0.85 [P < 0.001]). Medical center rankings using unadjusted and adjusted values were also highly correlated, although somewhat less so (Spearman rank coefficient 0.71 [P < 0.001] for <7% and 0.64 [P < 0.0001] for <8%).

There were changes in medical center ranks when the models using all available variables were compared with the age- and sex-adjustment models. For example, the top and bottom 20% (two deciles each) had 21 medical centers ranked. For the <7% threshold (Fig. 1A), 4 medical centers that initially ranked in the best decile in the model with age and sex moved down two deciles, no longer ranked in the best decile after adjustment using the full model. On the lower end, two medical centers ranked in the worst decile when using the age- and sex-adjusted model improved by one decile as a result of using the full model. For the 8% threshold (Fig. 1B), two medical centers moved from the best decile (shifting two deciles) and three medical centers moved from the worst decile (also shifting one decile) using the full model compared with using the age- and sex-adjusted model only.

For the <7% threshold measure, we compared the means of age, sex, and self-reported variables for those facilities (n = 4) changing two deciles with those for the other facilities (n = 7). However, despite considerable differences among facilities, we were unable to demonstrate differences in the group means of any individual variable (data not shown).

CONCLUSIONS — Our study demonstrated that patient characteristics, which can influence glycemic control, are largely outside of the control of health providers or health plans, can change the identification of best- and worse-performing medical centers and thus could potentially change quality assessments by internal and external stakeholders. Self-reported socioeconomic status, health status, and health behaviors varied widely among VHA medical centers but explained only a small proportion of A1C variation. Nonetheless, when these characteristics were used to risk adjust A1C levels at <7 or <8%, about 29 and 24%, respectively, of medical centers initially identified as best and worse medical centers shifted out of these categories. Whereas <7% is considered a threshold for “excellent control” (10), an A1C threshold >8% has been proposed as a threshold by which to assess clinical inertia (20,21). Our findings demonstrate the importance of taking into account socioeconomic position factors when ranking systems of health care for public reporting on the basis of A1C levels lower than the current >9% threshold for poor glycemic control.

Our observations that patient-level factors such as fewer years of completed education, concerns over food sufficiency, less social support (unmarried or living alone), and unemployment were associated with worse glycemic control in the veteran population are consistent with the literature (12). Similarly, the observation that <10% of the variance in A1C levels could be explained by these variables is also consistent with prior studies demonstrating a poor explanatory value for individual-level variables on glycemic control (22–24). These findings extend recent findings (25) that poorer individuals enrolled in managed health care plans had slightly higher A1C values (8.1%) than those with higher incomes (7.8%). However, in contrast to our findings, there were no differences in glycemic control by education. It is important to note that none of these previous studies evaluated the impact of patient-level socioeconomic characteristics upon quality assessment of different health care plans.

It is unclear how these variables interact to impact glycemic control. For example, socioeconomic barriers could lead to belief systems or attitudes (26) that impact with provider-patient communication regarding glycemic goal setting (27). Alternatively, it may be more difficult to make healthy food choices or have an environment in which to exercise, thus impeding progress in achieving glycemic goals (12). Regardless of the mechanisms, our empirical findings indicate the difficulties in generalizing from landmark clinical trials with activated patients to real-world settings with more heterogeneous populations (28) for the purpose of public reporting and payment.

Our findings are most immediately relevant to the recent decision by NCQA to implement a public reporting measure using a <7% threshold. Although the NCQA groups health care plans into Commercial, Medicaid, and Medicare enrollment status, this level of aggregation is unlikely to be sufficient to control for differences in socioeconomic characteristics among plan enrollees. Whether or not health plans should be further stratified by rural or urban location or patient income and educational status needs to be considered. Geocoding patient addresses from enrollment data to the census block group level could be used as an alternative to individually collected data (29), although this approach is admittedly an approximation. Alternatively, there could be differential weighting of adherence to the <7 and <9% thresholds, as is done in the Bridges to Excellence Program at the physician practice level (30) to reflect the fact that “optimal” control is less under physician control than “poor” control.
### Table 1—Baseline (fiscal year 1999) patient characteristics of subjects with a standardized A1C test performed in fiscal year 2000*

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<tr>
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*A1C test performed in a VHA Medical Center using A1C methodology certified by the National Glycohemoglobin Standardization Program. MCS, mental component score; NA, not applicable; PCS, physical component score.
Table 2—Medical center variation in population socioeconomic status and health

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<th>Minimum</th>
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MCS, mental component score; PCS, physical component score.
The strengths of our study were the ability to link survey and administrative data based on a large national sample of health care systems with at least 100 individuals with diabetes per medical center (31). We were also able to ascertain that each medical center in the study performed A1C testing with a methodology certified by the National Glycohemoglobin Standardization Program. We also recognize limitations of our study. The veteran population, predominantly male, tends to be older and have more comorbid conditions than the general population. On the other hand, because veterans who use the VHA tend to be of lower socioeconomic status than the general public (32), variations in socioeconomic status may be more marked among private sector health plans. Consequently, our findings need to be examined in other populations to ensure generalizability.

In summary, the socioeconomic status and health-related characteristics of VA medical center populations had a substantial impact on medical center rankings for quality of diabetes care. Our findings suggest that if an A1C <7% measure is to be used for public reporting of plans and therefore presumably for physician accountability within plans, then adjustment for patient socioeconomic positioning status may be necessary to assure that inferences regarding plan or physician group level performance in controlling glycemia are valid and do not have an unfair impact on payment that could lead to unintended consequences such as adverse selection at the physician level (5).

Acknowledgments—This study was funded by Grant IIR 00-072-1 from the VA Health Services Research Service to L.P.

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References


Increasing Expenditure on Health Care Incurred by Diabetic Subjects in a Developing Country

A study from India

Amabdy Ramachandran, MD, PhD
Shobhana Ramachandran, PhD
Chamukuttan Snehalatha, DSC
Christina Augustine, PhD

Narayanasamy Murugesan, PhD
Vijay Viswanathan, MD, PhD
Anil Kapur, MD
Rhys Williams, MD, PhD

OBJECTIVE — This study aimed to assess the direct cost incurred by diabetic subjects who were in different income groups in urban and rural India, as well as to examine the changing trends of costs in the urban setting from 1998 to 2005.

RESEARCH DESIGN AND METHODS — A total of 556 diabetic subjects from various urban and rural regions of seven Indian states were enrolled. A brief uniform coded questionnaire (24 items) on direct cost was used.

RESULTS — Annual family income was higher in urban subjects (rupees [Rs] 100,000 or $2,273) than in the rural subjects (Rs 36,000 or $818) (P < 0.001). Total median expenditure on health care was Rs 10,000 ($227) in urban and Rs 6,260 ($142) in rural (P < 0.001) subjects. Treatment costs increased with duration of diabetes, presence of complications, hospitalization, surgery, insulin therapy, and urban setting. Lower-income groups spent a higher proportion of their income on diabetes care (urban poor 34% and rural poor 27%). After accounting for inflation, a secular increase of 113% was observed in the total expenses between 1998 and 2005 in the urban population. The highest increase in percentage of household income devoted to diabetes care was in the lowest economic group (34% of income in 1998 vs. 24.5% in 2005) (P < 0.01). There was a significant improvement in urban subjects in medical reimbursement from 2% (1998) to 21.3% (2005).

CONCLUSIONS — Urban and rural diabetic subjects spend a large percentage of income on diabetes management. The economic burden on urban families in developing countries is rising, and the total direct cost has doubled from 1998 to 2005.

FROM THE

1 Diabetes Research Centre, M.V. Hospital for Diabetes, World Health Organization Collaborating Centre for Research, Education and Training in Diabetes, Royapuram, Chennai, India; and the 2 School of Medicine, University of Wales Swansea, Swansea, U.K.

Address correspondence and reprint requests to Prof. Amabdy Ramachandran, MD, PhD, DSc, FRCP (Pitond) (Edin), Director, Diabetes Research Centre, M.V. Hospital for Diabetes, WHO Collaborating Centre for Research, Education and Training in Diabetes, 4 Main Rd, Royapuram, Chennai 600 013, India. E-mail: ramachandran@vsnl.com.

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Abbreviations: Rs, rupees.

A table elsewhere in this issue shows conventional and Systeme International (SI) units and conversion factors for many substances.

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reported annual earning. This was validated against the possession of wealth asset indexes, such as possession of one's own house, automobiles, or agricultural lands. If gross disparities were noted in the reported income, appropriate correction was made in the categorization of income. The annual income of the subjects were arbitrarily classified into four levels. These were (in rupees [Rs]) 1) <40,000 ($909), 2) 40,000–80,000 ($909–1,818), 3) >80,000–120,000 ($1,818–2,727), and 4) >120,000 ($2,727). The costs were analyzed in relation to presence and number of complications (zero, one, and two or more).

Statistical analyses
Due to the skewed distribution of the variables, the median values and ranges are reported. Median test was used for intergroup comparisons. χ² test with Yate's correction was used for comparison of proportions and for comparison of mean values. Multiple linear regression equation was computed to find the influence of complications, duration of diabetes, habitat, treatment modalities, surgery, and hospitalization on the total expenditure. The actual bill values and the subjects' reported costs were compared by Wilcoxon's matched-pairs signed-rank test. Intercooled Stata 7.0 was used for data analyses. The reported expenses for the year 2005 were corrected in real terms, accounting for inflation using gross domestic product deflator with 1993–1994 as the base year.

RESULTS — The data were collected from 556 subjects (urban = 309, rural = 247); none refused to answer any of the questions. The questionnaire results of 158 subjects were compared with the entries on the relevant institution's bills, and there were no significant differences between the reported cost and the bills. Median bill values (total cost) were Rs 9,742 ($221.4) (range 300–153,120 [$7.0–$2,065]), reported value was Rs 11,000 ($250) (1,000–88,000 [23–2,000]), and z was −1.89 (P = 0.06). Therefore, the direct cost data given by the subjects were accepted as valid.

The demographic data of the subjects presented in Table 1. Urban subjects had higher mean income and education than the rural subjects, as well as for laboratory tests and medical consultation (P < 0.001 for all comparisons). Expenses on surgery were higher in urban subjects (Rs 21,000 or $477) versus rural subjects (Rs 10,000 or $227) than the rural subjects, as well as for laboratory tests and medical consultation (P < 0.001 for all comparisons). Expenses on surgery were higher in urban subjects (Rs 21,000 or $477) versus rural subjects (Rs 10,000 or $227) than the rural subjects.

Table 1—Demographic data of diabetic subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Urban</th>
<th>Rural</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>194 (62.8)</td>
<td>147 (59.5)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>115 (37.2)</td>
<td>100 (40.5)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.2 ± 10.5</td>
<td>54.8 ± 11.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>5 (1.6)</td>
<td>17 (6.9)</td>
<td>0.003</td>
</tr>
<tr>
<td>Elementary</td>
<td>92 (30)</td>
<td>144 (58.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Higher secondary</td>
<td>72 (23.4)</td>
<td>58 (23.4)</td>
<td>0.959</td>
</tr>
<tr>
<td>Graduation</td>
<td>84 (27.4)</td>
<td>14 (5.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Post graduation</td>
<td>54 (17.6)</td>
<td>14 (5.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Employment status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not employed</td>
<td>31 (10.6)</td>
<td>30 (12.3)</td>
<td>0.511</td>
</tr>
<tr>
<td>Housewife</td>
<td>93 (31.6)</td>
<td>94 (38.6)</td>
<td>0.059</td>
</tr>
<tr>
<td>Clerical</td>
<td>8 (2.7)</td>
<td>4 (1.6)</td>
<td>0.625</td>
</tr>
<tr>
<td>Management</td>
<td>70 (23.8)</td>
<td>42 (17.2)</td>
<td>0.122</td>
</tr>
<tr>
<td>Professional</td>
<td>50 (17)</td>
<td>53 (21.7)</td>
<td>0.138</td>
</tr>
<tr>
<td>Non-professional</td>
<td>11 (3.7)</td>
<td>19 (7.8)</td>
<td>0.050</td>
</tr>
<tr>
<td>Retired</td>
<td>31 (10.6)</td>
<td>2 (0.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Income status (Rs)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40,000</td>
<td>47 (15.2)</td>
<td>124 (50.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>40,000–80,000</td>
<td>92 (29.7)</td>
<td>75 (30.3)</td>
<td>0.95</td>
</tr>
<tr>
<td>80,000–120,000</td>
<td>62 (20)</td>
<td>27 (10.9)</td>
<td>0.005</td>
</tr>
<tr>
<td>&gt;120,000</td>
<td>108 (34.9)</td>
<td>21 (8.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>10.4 ± 7.1</td>
<td>7.5 ± 5.5</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are n (%) or means ± SD. *Rs 44 = $1.00 (approximately).

Table 2—Income and treatment expenses of the diabetic subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Urban</th>
<th>Rural</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>309</td>
<td>247</td>
<td></td>
</tr>
<tr>
<td>Annual family income</td>
<td>100,000 (10,000–1,000,000)</td>
<td>36,000 (10,000–300,000)</td>
<td>0.001</td>
</tr>
<tr>
<td>Expenditure on medications</td>
<td>$2,272 (227–22,727)</td>
<td>$818 (227–6,818)</td>
<td></td>
</tr>
<tr>
<td>Laboratory tests</td>
<td>4,000 (300–70,000)</td>
<td>2,500 (100–50,000)</td>
<td>0.001</td>
</tr>
<tr>
<td>Medical consultations</td>
<td>1,500 (50–15,000)</td>
<td>500 (30–30,000)</td>
<td>0.001</td>
</tr>
<tr>
<td>Expenditure on hospitalization</td>
<td>1,000 (30–22,000)</td>
<td>600 (30–30,000)</td>
<td>0.005</td>
</tr>
<tr>
<td>Expenditure on surgery</td>
<td>10,000 (350–150,000)</td>
<td>6,000 (300–75,000)</td>
<td>0.07</td>
</tr>
<tr>
<td>Total median expenditure</td>
<td>10,000 (1,000–319,000)</td>
<td>6,260 (1,000–125,000)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are median (range) in Indian Rs unless otherwise stated. Rs 44 = $1.00 (approximately).
In the urban subjects, 56.9% had no complications, and 13.5% had two or more complications. In the rural group, 56.6% had no complications, 29.5% had one complication, and 13.7% had two or more complications.

Multivariate regression analysis showed that the expenditure increased significantly with the presence of complications ($\beta = 9,877$, $P < 0.0001$), duration of diabetes ($\beta = 801$, $P < 0.0001$), urban habitat ($\beta = 8,757$, $P < 0.0001$), insulin treatment ($\beta = 5,516$, $P < 0.0001$), surgery ($\beta = 15,787$, $P < 0.0001$), and hospitalization ($\beta = 16,548$, $P < 0.0001$). The model explained 33% of the variations.

When the present data from the urban sample were compared with the data of 1998, significant differences were noted. Annual income had increased two-fold from Rs 48,000 ($1,091) to Rs 100,000 ($2,273) ($P < 0.0001$). The families’ median expenditure on diabetes health care in 1998 was Rs 4,510 ($103), and it had more than doubled by 2005: Rs 10,000 ($227) ($P < 0.0001$). The increase was significant in all components of diabetes care ($P < 0.0001$ for all comparisons). The direct cost was computed to account for inflation, which is shown in Table 3. The corrected median expenditure of the urban sample had more than doubled from Rs 4,194 ($95) in 1998 to Rs 8,930 ($203) in 2005. Significant increases were observed in all components of the expenditure ($P < 0.0003$ in all comparisons). The largest increase was on expenses of surgery (632%), and the least was on expenditure of medications (39.5%). The annual cost of diabetes care had increased by 112.9%, while there was only a 108.0% increase in the annual income.

There was an increase in the proportion of income spent on diabetes care, between 1998 and 2005, which was statistically significant in all categories of family income except in the high-income group (Fig. 2). The largest proportional increase was seen in the lowest economic group (34.0 vs. 24.5%) ($P < 0.01$).

Medical reimbursement was obtained in 14.2% of urban, but in only 3.2% of rural, subjects. The proportion of subjects receiving reimbursement was the highest (21.3%) in the urban higher-income group.

CONCLUSIONS — The present study illustrates the increasing trend in expenditure on diabetes care in this developing country. The study sample was collected from different urban and rural regions of India to represent the national populations. Urban families spent more on diabetes than rural families, both as absolute values and as proportions of family income. This was due to the higher expenditure on medical consultations, laboratory investigations, and medications and may be partly attributed to the differences in the availability of these more expensive treatments in urban areas. However, it cannot be inferred from these results alone whether this availability leads to better quality of health care.

Table 3—Comparison of treatment expenses of the urban sample of diabetic subjects between 1998 and 2005 after accounting for inflation

<table>
<thead>
<tr>
<th>Variables</th>
<th>1998 (Rs)</th>
<th>2005 (Rs)</th>
<th>P value</th>
<th>Increases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expenditure on medications</td>
<td>2,560</td>
<td>3,571</td>
<td>&lt;0.0001</td>
<td>39.5</td>
</tr>
<tr>
<td>Laboratory tests</td>
<td>307</td>
<td>1,339</td>
<td>&lt;0.0001</td>
<td>336.2</td>
</tr>
<tr>
<td>Medical consultations</td>
<td>299</td>
<td>893</td>
<td>&lt;0.0001</td>
<td>198.7</td>
</tr>
<tr>
<td>Expenditure on hospitalization</td>
<td>4,267</td>
<td>8,929</td>
<td>0.0002</td>
<td>109.3</td>
</tr>
<tr>
<td>Expenditure on surgery</td>
<td>2,560</td>
<td>18,750</td>
<td>0.0003</td>
<td>632.4</td>
</tr>
<tr>
<td>Median expenditure</td>
<td>4,194 (871–64,182)</td>
<td>8,930 (893–284,821)</td>
<td>&lt;0.0001</td>
<td>112.9</td>
</tr>
</tbody>
</table>

Data are median or median (range) in Indian Rs unless otherwise indicated. Rs 44 = $1.00 (approximately).
Studies on the outcome of care in urban and rural settings are needed, which account for the variations in confounding factors such as diet, physical activity, and the influence of comorbidity.

Awareness of diabetes is likely to be better in urban than in rural subjects due to higher literacy levels in the former. Awareness of diabetes is the subject of an on-going study being carried out under the Diabetes Action Now program (10). Moreover, both diabetic subjects and many medical practitioners lack awareness of the need for constant disease monitoring and consistent glycemic control, and this may also differ in urban and rural settings (11). The apparent contradiction of lower-income groups spending a higher proportion of their income on diabetes care among both the populations, but rural residents spending a lower proportion of their income on diabetes care than urban residents, may be explained by the combination of greater availability and awareness of diabetes care among urban poor.

The proportion of annual income spent on health care by the urban poor has increased from 24.5% in 1998 to 34.0% in 2005. This is an important observation, as subjects with limited financial resources continue to spend a major proportion of their income on diabetes management. This scenario has been found to be similar even in the developed countries (12). In India, the expenditure on health care is borne mainly from self-earned resources. Only 6.4% of the urban low-income group received medical reimbursement, whereas this was 21.3% in the high-income group. In urban settings, the concepts of health insurance and medical claim policies seem better understood and are utilized by the high-income group. Even the urban low-income group prefers treatment from private practitioners or health centers rather than government hospitals (5). Although private health care facilities are sought after, it is likely that many patients may cross over to public health care facilities. We lack data on this aspect.

The cost of diabetes care in urban areas showed a twofold increase from 1998 to 2005, due to significantly increased expenditure on diabetes medications, laboratory tests, medical consultations, hospitalizations, and surgical procedures. Presence of complications, duration of diabetes, need for surgery, and hospitalization increased the expenditure considerably. Similar observations were made in the study done in 1998 (7), which showed that diabetic subjects with foot infections had costs threefold higher than subjects with no complications, and costs increased further when hospitalization was required. According to Björk (13), three times the health care resources were being spent on diabetes complications than on diabetes control. Jonsson et al. (14) made an important observation that young adults diagnosed with diabetes between ages 15 and 34 years spent larger amounts on diabetes care, which decreased in subsequent years. A second phase of high cost might result with the onset of complications.

The direct cost of diabetes care in India has been reported by others (8,9,11). There was one report (11) on the indirect cost from this part of the world, being Rs 12,756 ($290). The methodological difficulties of estimating indirect costs, particularly in developing countries, are many. The present study did not attempt to principally estimate indirect cost because the main foci of this study were the comparisons of direct costs and comparisons between income groups, urban and rural settings, and between 1998 and 2005.

The major limitations of the study were as follows: there could have been some underreporting of family income, which would have caused an overestimation of the percentage of income spent on health care. This was most likely to have occurred in the high-income group. However, similar bias is likely to have existed even in the previous study. Therefore, the comparisons of the two datasets are likely to be valid. Secondly, we did not have data on a comparison population showing the expenses on general health care. The major objective of the study was to note the cost of treating a chronic disease, which would be higher than that of general health care, and also to see whether it is increased with time.

The present study indicated that the economic burden of diabetes care on families in developing countries is rising rapidly, even after accounting for the inflation. Further studies of these costs in India and other developing countries might address in more detail factors such as the duration of diabetes, diabetes treatments, the extent of glycemic and other metabolic control, the presence and severity of diabetes complications, and important comorbidities, which influence personal and family costs. Such studies have been published on developed countries (e.g., Brandle et al. (15)) with methods that could be adapted to developing countries. They have demonstrated the substantial impact that several of these factors have on diabetes costs.

Acknowledgments — We acknowledge the participation of the World Diabetes Foundation trainee doctors and patients in the study. We thank the staff of the Department of Education and Psychosocial Research who facilitated the study and the secretarial service of Ms. Bobby Alex.

References

Figure 2—Change in the proportion of income spent on diabetes by different income groups between the 1998 and 2005 in the urban population. The x-axis shows the income group, and y-axis shows the percentages of income spent. The low-income group showed the highest increase (34% in 2005 vs. 24.5% in 1998, P < 0.01). a, $x^2 = -7.25$, P = 0.007; b, $x^2 = -16.94$, P < 0.0001; c, $x^2 = -10.34$, P < 0.001; d, $x^2 = -3.01$, P = 0.08.
Cost of diabetes in India

The BIGTT Test

A novel test for simultaneous measurement of pancreatic β-cell function, insulin sensitivity, and glucose tolerance

RESEARCH DESIGN AND METHODS — For our purpose, data from an oral glucose tolerance test (OGTT) (18 samples during 240 min) and a tolbutamide-modified intravenous glucose tolerance test (IVGTT) (33 samples during 180 min) from 258 individuals with fasting plasma glucose <7 mmol/l and 2-h plasma glucose <7.8 mmol/l were used for model development and internal validation. Data from an additional 28 individuals were used for external validation. Bergman’s minimal model was used to calculate $S_I$ and the trapezoidal method was used to calculate AIR$_{0–n}$ min. Multiple linear regression was applied to derive predictive equations of log($S_I$) and log(AIR$_{0–n}$ min) using data on sex, BMI, plasma glucose, and serum insulin levels obtained during the OGTT.

RESULTS — We demonstrate that it is possible to obtain estimates of $S_I$ (BIGTT-$S_I$) and AIR (BIGTT-AIR) that are highly correlated to IVGTT-derived values of $S_I$ ($R^2 = 0.77$) and AIR ($R^2 = 0.54$). In the two validation datasets we obtained similar results.

CONCLUSIONS — Data from OGTTs can provide accurate measures of insulin sensitivity and β-cell function, which can be used in large scale metabolic, genetic, and epidemiological studies.

Both methods are considered to provide exact and, among individuals with normal glucose tolerance, comparable measurements of insulin sensitivity ($S_I$). However, because these methods are time consuming and expensive and cannot be performed on the same day as an oral glucose tolerance test (OGTT), neither is suitable for large-scale studies.

In the present study, we examine nondiabetic individuals without impaired glucose tolerance (IGT) to derive equations that are more accurate than those currently available for the $S_I$ and the AIR from an OGTT (1,6–12). We have used detailed information from both plasma glucose and serum insulin levels during an extended and frequently sampled OGTT combined with information on anthropometric measures and sex to generate equations and data from a frequently sampled IVGTT performed in the same individuals as reference. The approach is empirical and data driven, with multiple regression statistics being applied to the physiological data obtained.

RESEARCH DESIGN AND METHODS

Individuals for model development and internal validation

OGTT data from 258 individuals with fasting plasma glucose <7.0 mmol/l and 2-h plasma glucose <7.8 mmol/l (nondiabetic without IGT) were used to develop (75% of the data) and validate (25% of the data) models. All participants were from 1 of 60 families as described previously (13). The performance of the model was further examined in 28 individuals with IGT identified in the same 60 families. Before participation in the study, all individuals provided informed consent. The study was approved by the Ethical Committee of Copenhagen and was in accordance with the principles of the Declaration of Helsinki.

OGTT

All individuals underwent a standardized and extended 75-g frequently sampled OGTT. After a 12-h overnight fast, venous blood samples were drawn in triplicate at
The BIGTT test

All individuals underwent a 33-point tolbutamide-modified, frequently sampled IVGTT (13) within 1 week after the OGTT examination except for a few individuals who underwent an IVGTT within 4 weeks. The $S_I$ was calculated using the Bergman MINMOD computer program (15). The glucose-induced serum AIR$_{\text{insulin} 0-8\text{ min}}$ was calculated as the incremental areas under the curves from 0 to 8 min.

Individuals for external validation

Twenty-eight nondiabetic individuals without IGT, randomly chosen from participants in a population-based study at the Research Centre for Prevention and Health (RCPH) of 695 individuals born in 1936 (16), were examined by both an OGTT and an IVGTT. An extended 75-g OGTT was performed after a 10-h overnight fast. Blood samples were drawn in duplicate between 7:45 and 10:00 a.m. before the oral glucose load, i.e., at −5 and 0 min and at 15, 30, 45, 60, 90, 120, and 180 min after the glucose load. Plasma glucose levels were analyzed at the RCPH using a glucose oxidase method (GranuTest; Merck, Darmstadt, Germany). Serum insulin was determined by enzyme-linked immunosorbent assay with a narrow specificity excluding des(31,32)-proinsulin and intact proinsulin (DAKO Diagnostics, Ely, U.K.) (14).

IVGTT

An extended 75-g OGTT and an IVGTT. An extended 75-g OGTT was performed after a 10-h overnight fast. Blood samples were drawn in duplicate between 7:45 and 10:00 a.m. before the oral glucose load, i.e., at −5 and 0 min and at 15, 30, 45, 60, 90, 120, and 180 min after the glucose load. Plasma glucose levels were analyzed at the RCPH using a glucose oxidase method (GranuTest; Merck, Darmstadt, Germany). Serum insulin was determined by enzyme-linked immunosorbent assay with a narrow specificity excluding des(31,32)-proinsulin and intact proinsulin (DAKO Diagnostics, Ely, U.K.) (14).

Anthropometric measurements

Body weight (with light clothing) was measured to the nearest 0.1 kg and height (without shoes) was estimated to the nearest 0.5 cm. BMI was calculated as weight in kilograms divided by the square of height in meters. Waist circumference (to the nearest centimeter, individuals without clothes) was measured midway between the lowest rib and the iliac crest on standing subjects. Hip circumference (to the nearest centimeter with individuals in underwear) was measured over the widest part of the gluteal region. Fat mass was measured using a bioelectrical impedance method (18).

Statistical methods

An empirical data-driven approach was chosen, using multiple linear regression to derive predictive equations of $\log(S_I)$ and $\log(AIR)$, i.e., the natural logarithm. Demographic data and glucose and insulin concentrations during the OGTT were used as independent variables in the analysis. To adjust for within-family correlation, a random family factor was added to the models. An a priori analysis strategy was formulated to ensure efficient exploitation of the data. The models were based on untransformed and transformed glucose and insulin results (square and reciprocal transformation) and standardized areas under the concentration curves for plasma glucose and serum insulin, defined as the area under the curve, above baseline from time 0 to time $t$ minutes divided by $t$. The demographic factors included age, sex, height, body weight, waist circumference, hip circumference, fat mass, BMI, waist-to-hip ratio, and fat mass-to-body weight ratio.

Of the total data, ~75% were randomized (stratified according to age and missing data status) to an estimation sample used for predicting $\log(S_I)$ and $\log(AIR)$. The remaining 25% of the data were considered as an internal validation sample for assessment of the accuracy of the predictive equations, i.e., a cross validation. Furthermore, external validation was provided by the application of the predictive models to the data from the RCPH.

A combination of backwards elimination and forward selection was chosen in the modeling phase to handle the large number of variables in each model. A model was initially based on plasma glucose and serum insulin levels at 0, 30, 60, 90, 120, and 180 min as regression variables to describe the established indexes of insulin sensitivity and $\beta$-cell function. Depending on which of these variables was statistically significant at a 10% significance level, values at adjacent time points were added sequentially to the model. We have thus used plasma glucose and serum insulin responses from several time points during the OGTT to generate the optimal equations for OGTT-derived indexes of insulin sensitivity (BIGTT-$S_{I(0-30-120)}$ and BIGTT-AIR$_{0-30-120}$) and $\beta$-cell function (BIGTT-AIR$_{0-180}$). Also, we generated and tested simple indexes of $S_I$ and AIR using time points that are routinely measured in most metabolic, genetic, and epidemiological studies, i.e., at 0, 30, and 120 min (BIGTT-$S_{I(0-30-120)}$ and BIGTT-AIR$_{0-30-120}$, and 0, 60, and 120 min (BIGTT-$S_{I(0-60-120)}$ and BIGTT-AIR$_{0-60-120}$) respectively.

Evaluations and comparisons between models were based on the residual SD and the correlation coefficient ($R^2$) obtained from the regressions. $R^2$ describes the degree of variation explained by the model relative to the total variation in the population subjected to analysis. Because $R^2$ is population dependent, it can only be used for comparisons of models within a dataset, not between datasets. However, the SD is comparable between models applied to various datasets, as the SD on the log scale corresponds approximately to the coefficient of variation of the predicted $S_I$ and AIR on the original scale.

Calculations of insulin sensitivity and acute insulin response using previously published models

Formulas from previously published models were applied to the present datasets to compare the accuracy of the different models (Table 3). As previously described models were not based on log-transformed measures of insulin sensitivity and $\beta$-cell function, the accuracy has been assessed after performance of an adjustment based on a regression analysis of the log-transformed result of each formula, measuring concordance with the $\log(S_I)$ and $\log(AIR)$ estimating an intercept based on the estimation dataset. Thus, the risk of performing an unfair comparison with the BIGTT models is eliminated, because additional corrections to the data are obtained by the adjustment.

RESULTS

Characteristics of participants are given in Table 1. Table 2 shows the predictive equations that were generated for calculations of BIGTT-$S_I$ and BIGTT-AIR. Estimates of $S_I$ (BIGTT-$S_{I(0-30-120)}$ and AIR (BIGTT-AIR$_{0-30-120}$) were highly correlated to IVGTT-derived values of $S_I$ ($R^2 = 0.77$) and AIR ($R^2 = 0.54$) (Table 3).

Each of the OGTT-derived estimates of $S_I$ was compared to estimates of $S_I$ obtained in the internal and external validation dataset as well as to estimates of insulin sensitivity using the fasting insulin level, the homeostasis model assessment (HOMA), and four other previously published models (Table 3). On the basis of the SD of the estimates, both the full model (BIGTT-$S_{I(0-30-120)}$ and the two more simple models (BIGTT-$S_{II(0-30-120-180)}$ and BIGTT-$S_{I(0-60-120)}$) for estimation of $S_I$ had an ~30% higher accuracy than two
widely used simple indexes of insulin sensitivity, i.e., fasting insulin and HOMA (Table 3). The OGTT-derived estimates of $S_i$ were almost similar in the two validation datasets (Table 3). The relationship between $S_i$ obtained from the IVGTT and BIGTT-$S_{i0-30-120}$ is presented in Fig. 1A, which shows a high degree of correlation at all levels of insulin sensitivity. A similar correlation at all levels of insulin sensitivity is seen by applying the optimal model (BIGTT-$S_{i\text{full}}$) as well as the BIGTT-$S_{i0-30-120}$ (data not shown).

The SD and $R^2$ for various indexes of $\beta$-cell function are also shown in Table 3. The novel BIGTT-AIR methods are about 20–30% more accurate than three currently used models for estimation of $\beta$-cell function, and the estimates were very similar in the two validation datasets (Table 3). The relationship between $\beta$-cell function estimated by the IVGTT and by the BIGTT-AIR$_{0-60-120}$ is shown in Fig. 1B.

For subjects with IGT, the BIGTT models for the estimation of indexes of insulin sensitivity and $\beta$-cell function were less accurate than when they were applied to data from nondiabetic individuals without IGT (see Table 1 of the online appendix available at http://dx.doi.org/10.2337/dc06-1240). This was a general observation for all applied models. However, the Matsuda, Cederholm, and Belli’s indexes did seem to capture the insulin sensitivity for subjects with IGT in a more satisfactory manner than the BIGTT model. Regarding the indexes of $\beta$-cell function, the BIGTT model was more accurate than the alternative models (see Table 1 of the online appendix).

**Conclusions** — We present novel and validated models for calculation of $S_i$ and AIR with data available from an OGTT. The models are developed for accurate measurement of $S_i$ and AIR in nondiabetic individuals without IGT. The plasma glucose level obtained at 120 min after the ingestion of the glucose load identifies individuals for whom the method is applicable and at the same time identifies individuals having IGT or overt diabetes. Patients with type 2 diabetes and IGT are characterized by various degrees of abnormalities in both insulin action and insulin secretion (19), and it is therefore unlikely that models for mea-

### Table 1—Clinical and biochemical data for 258 nondiabetic individuals without IGT used for model development and internal validation and for 28 nondiabetic individuals without IGT for external validation

<table>
<thead>
<tr>
<th></th>
<th>Model development and internal validation</th>
<th>External validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (men/women)</td>
<td>110/148</td>
<td>16/12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42 ± 12</td>
<td>61 ± 0.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0 ± 4.5</td>
<td>25.1 ± 2.6</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t = 0$ min</td>
<td>5.1 ± 0.7</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>$t = 30$ min</td>
<td>8.1 ± 1.5</td>
<td>7.8 ± 1.4</td>
</tr>
<tr>
<td>$t = 60$ min</td>
<td>7.9 ± 2.0</td>
<td>7.5 ± 2.0</td>
</tr>
<tr>
<td>$t = 120$ min</td>
<td>5.7 ± 1.2</td>
<td>5.0 ± 1.3</td>
</tr>
<tr>
<td>Serum insulin (pmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t = 0$ min</td>
<td>40 ± 26</td>
<td>32 ± 13</td>
</tr>
<tr>
<td>$t = 30$ min</td>
<td>316 ± 198</td>
<td>228 ± 125</td>
</tr>
<tr>
<td>$t = 60$ min</td>
<td>371 ± 232</td>
<td>260 ± 132</td>
</tr>
<tr>
<td>$t = 120$ min</td>
<td>214 ± 182</td>
<td>164 ± 121</td>
</tr>
<tr>
<td>$S_i [10^{-5} \cdot (\text{min} \cdot \text{pmol/l})^{-1}]$</td>
<td>10.6 ± 6.2</td>
<td>12.7 ± 6.9</td>
</tr>
<tr>
<td>AIR$_{0-8}$ min (min · pmol/l)</td>
<td>2,478 ± 1,762</td>
<td>1,523 ± 925</td>
</tr>
</tbody>
</table>

*Values are means ± SD.*

### Table 2—OGTT-derived models for calculations of $S_i$ and AIR in nondiabetic individuals without IGT

<table>
<thead>
<tr>
<th>Name of model</th>
<th>Blood sampling time points for measurements of plasma glucose and serum insulin during an OGTT</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIGTT-$S_{i0-30-120}$</td>
<td>0, 30, and 120 min</td>
<td>$\exp(4.90 - (0.00402 \cdot I_0) - (0.000556 \cdot I_{30}) - (0.00127 \cdot I_{60}) - (0.152 \cdot G_{60}) - (0.00871 \cdot G_{90}) - (0.0373 \cdot G_{120}) - (0.145 \cdot sex) - (0.0376 \cdot BMI))$</td>
</tr>
<tr>
<td>BIGTT-$S_{i0-60-120}$</td>
<td>0, 60, and 120 min</td>
<td>$\exp(4.62 - (0.00385 \cdot I_0) - (0.000917 \cdot I_{30}) - (0.000760 \cdot I_{60}) - (0.0551 \cdot G_{60}) - (0.0178 \cdot G_{90}) - (0.0524 \cdot G_{120}) - (0.144 \cdot sex) - (0.0380 \cdot BMI))$</td>
</tr>
<tr>
<td>BIGTT-$S_{i\text{full}}$</td>
<td>0, 30, 60, 105, 180, and 240 min</td>
<td>$\exp(4.39 - (0.000287 \cdot I_0) - (0.000424 \cdot I_{30}) - (0.000848 \cdot I_{60}) - (0.0309 \cdot I_{90}) + (0.00144 \cdot I_{120}) - (0.000282 \cdot I_{150}) - (0.161 \cdot G_{60}) - (0.0357 \cdot G_{90}) - (0.0130 \cdot G_{120}) - (0.0416 \cdot G_{150}) - (0.106 \cdot G_{180}) + (0.169 \cdot G_{210}) - (0.177 \cdot sex) - (0.031 \cdot BMI))$</td>
</tr>
<tr>
<td>BIGTT-AIR$_{0-30-120}$</td>
<td>0, 30, and 120 min</td>
<td>$\exp(8.20 + (0.00178 \cdot I_0) + (0.00168 \cdot I_{30}) - (0.00383 \cdot I_{60}) + (0.314 \cdot G_{60}) - (0.109 \cdot G_{90}) + (0.0781 \cdot G_{120}) + (0.180 \cdot sex) + (0.032 \cdot BMI))$</td>
</tr>
<tr>
<td>BIGTT-AIR$_{0-60-120}$</td>
<td>0, 60, and 120 min</td>
<td>$\exp(8.19 + (0.00339 \cdot I_0) + (0.00152 \cdot I_{30}) - (0.000959 \cdot I_{60}) - (0.389 \cdot G_{60}) + (0.142 \cdot G_{90}) + (0.164 \cdot G_{120}) + (0.250 \cdot sex) + (0.038 \cdot BMI))$</td>
</tr>
<tr>
<td>BIGTT-AIR$_{\text{full}}$</td>
<td>0, 10, 50, and 140 min</td>
<td>$\exp(7.91 + (0.000896 \cdot I_0) + (0.00163 \cdot I_{30}) + (0.00027 \cdot I_{60}) - (0.000966 \cdot I_{90}) - (0.0323 \cdot G_{60}) - (0.0377 \cdot G_{90}) - (0.0985 \cdot G_{120}) + (0.143 \cdot G_{150}) + (0.289 \cdot sex) + (0.036 \cdot BMI))$</td>
</tr>
</tbody>
</table>

Sex (female = 0, male = 1); $I_i$ is serum insulin (picomoles per liter); $G_i$ is plasma glucose (millimoles per liter) at time $t$ min; $\exp[ ]$ denotes the exponential function; 0 min is calculated as the mean of values obtained at −10, −5, and 0 min.
In the present study we have chosen to use insulin sensitivity estimates from a frequently sampled IVGTT as reference for the BIGTT-S$_I$ models. Most other published OGTT-derived models for estimation of insulin sensitivity are developed on the basis of data from euglycemic-hyperinsulinemic clamp studies. We believe, however, that our models provide an estimate of insulin sensitivity that could be compared with clamp data, as previous reports have shown a strong positive correlation between $S_I$ obtained from the frequently sampled IVGTT and $S_I$ obtained from the euglycemic-hyperinsulinemic clamp in normoglycemic individuals (5,21).

The models derived from OGTT data for measurement of $\beta$-cell function were developed with the AIR$_{0-8}$ min response from the IVGTT, which is the gold standard, as reference. The models are highly accurate compared with other available methods using OGTT data for calculation of insulin secretion (Table 3) (9,12,22–26). Both our models and other models for measurement of $\beta$-cell function were less accurate when applied to individuals with IGT, but the BIGTT model was still more accurate than the alternative models (see Table 1 of the online appendix). Adding information on sex, height, and weight to previously used indexes did not improve their accuracy.

In contrast to anthropometric variables, which are reproducible among laboratories, assay characteristics for measurement of serum insulin such as linearity, recovery, accuracy, precision, and cross-reactivity to proinsulin and its primary conversion intermediates vary among laboratories (27). In the present model we have used an insulin assay with no cross-reactivity to proinsulin and its primary conversion intermediates (14). Whether our model is valid when serum insulin levels are analyzed by other assays remains to be studied.

In summary, we have developed and validated novel models for assessment of OGTT-derived estimates of $S_I$ and AIR. Information on sex and BMI combined with analysis of plasma glucose and serum insulin levels in the fasting state and up to eight time points during 4 h (full models) provides indexes for sex and BMI that are highly correlated to indexes obtained from a frequently sampled IVGTT. Furthermore, we have validated more simple OGTT-based models that can be used to predict estimates of $S_I$ and AIR, incorporating information on sex, BMI, and sex and BMI. The models are accurate compared with clamp data, as previous reports have shown a strong positive correlation between $S_I$ obtained from the frequently sampled IVGTT and $S_I$ obtained from the euglycemic-hyperinsulinemic clamp in normoglycemic individuals (5,21).

The BIGTT test

Table 3—SD and $R^2$ for various indices of the $S_I$ compared with the $S_I$ as estimated from an IVGTT and indices of $\beta$-cell function compared with AIR as calculated from an IVGTT

<table>
<thead>
<tr>
<th>Model</th>
<th>Estimation dataset</th>
<th>Internal validation dataset</th>
<th>External validation dataset</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_I$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIGTT-S$<em>{I</em>{full}}$</td>
<td>0.30 (0.77)</td>
<td>0.36</td>
<td>ND</td>
</tr>
<tr>
<td>BIGTT-S$<em>{I</em>{0-30-120}}$</td>
<td>0.36 (0.69)</td>
<td>0.38</td>
<td>0.38</td>
</tr>
<tr>
<td>BIGTT-S$<em>{I</em>{0-60-120}}$</td>
<td>0.34 (0.71)</td>
<td>0.35</td>
<td>0.39</td>
</tr>
<tr>
<td>Fasting serum insulin</td>
<td>0.46 (0.49)</td>
<td>0.53</td>
<td>0.45</td>
</tr>
<tr>
<td>HOMA</td>
<td>0.45 (0.50)</td>
<td>0.54</td>
<td>0.45</td>
</tr>
<tr>
<td>Matsuda</td>
<td>0.38 (0.65)</td>
<td>0.41</td>
<td>0.43</td>
</tr>
<tr>
<td>Cederholm</td>
<td>0.47 (0.45)</td>
<td>0.49</td>
<td>0.47</td>
</tr>
<tr>
<td>Belfiori</td>
<td>0.41 (0.51)</td>
<td>0.41</td>
<td>0.41</td>
</tr>
<tr>
<td>Stumvoll</td>
<td>0.45 (0.51)</td>
<td>0.57</td>
<td>0.42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Indices of $\beta$-cell function</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BIGTT-AIR$<em>{I</em>{full}}$</td>
<td>0.42 (0.54)</td>
</tr>
<tr>
<td>BIGTT-AIR$<em>{I</em>{0-30-120}}$</td>
<td>0.46 (0.47)</td>
</tr>
<tr>
<td>BIGTT-AIR$<em>{I</em>{0-60-120}}$</td>
<td>0.45 (0.47)</td>
</tr>
<tr>
<td>Modified Kadowaki model</td>
<td>0.59 (0.14)</td>
</tr>
<tr>
<td>HOMA</td>
<td>0.54 (0.25)</td>
</tr>
<tr>
<td>Stumvoll</td>
<td>0.55 (0.27)</td>
</tr>
</tbody>
</table>

Data are SD ($R^2$) or SD. In the various formulas below, plasma glucose is entered as millimoles per liter and serum insulin as picomoles per liter, unless otherwise stated. $G$ and $I$, denote plasma glucose and serum insulin concentrations, respectively, at a given time (in minutes) after ingestion of the oral load. $G_{mean}$ and $I_{mean}$ are the means of plasma glucose and serum insulin concentrations during the sampling periods in question. Models of insulin sensitivity are as follows. HOMA model: Only fasting values of plasma glucose and serum insulin are used (9): $I/G(22.5-10^{-6}c)$. Matsuda model: Plasma glucose and serum insulin levels are measured at $t = -30$, $-15$, $0$, $30$, $60$, $90$, and $120$ min during the OGTT. Values of plasma glucose and serum insulin are entered in the formula as milligrams per deciliter and milliunits per liter, respectively (8): $10000/\sqrt{\text{square root of (}G_0 \cdot I_0 \cdot G_{mean} \cdot I_{mean})}$. In the present study plasma glucose and serum insulin levels measured at $t = -10$ and $t = -5$ were used instead of $t = -30$ and $t = -15$, respectively. Cederholm model: Plasma glucose and serum insulin levels are measured at $t = 0$, $30$, $60$, and $120$ min during the OGTT (11): $\{75/120 + (G_0 - G_{120})/1.15 + 180 \cdot 0.19 \cdot \text{body weight} / (120 + \log(I_{mean}) + G_{mean}) \}$. Belfiori model: Plasma glucose and serum insulin levels are measured at $t = 0$, $15$, $30$, and $120$ min during the OGTT (7): $\{2(\text{mean-person} / \text{mean-population}) \cdot (G_{mean-person} / G_{mean-population}) + 1 \}$. Stumvoll model: Plasma glucose and serum insulin levels are measured at $t = 0$, $90$, and $120$ min during the OGTT (10): $18.8 - 0.271 \cdot \text{BMI} - 0.0052 \cdot I_{120} - 0.27 \cdot G_{30}$. Models of $\beta$-cell function are as follows. HOMA model: Only fasting values of plasma glucose and serum insulin are used (9): $I/G(30 - 3.5)$. Modified Kadowaki model: Plasma glucose and serum insulin levels are measured at $t = 0$ and 30 min (12,22): $(I_{30} - I_0) / G_{30}$. Stumvoll model: Plasma glucose and serum insulin levels are measured at $t = 0$ and 30 min during the OGTT (10): $1.283 + 1.829 \cdot I_{30} - 138.7 \cdot G_{30} + 3.772 \cdot G_{0}$, ND, not determined.

measurements of insulin sensitivity and $\beta$-cell function derived from an OGTT could be accurate throughout the whole spectrum of glucose tolerance (20). As expected, our model did not work as well in individuals with IGT (see Table 1 of the online appendix). If detailed information on $\beta$-cell function and/or insulin sensitivity is essential in these individuals, other methods have to be used in addition to the OGTT. All three BIGTT-S$_I$ models were highly positively correlated ($R^2 = 0.69-0.77$) to the $S_I$ derived from an IVGTT. This was the case for both the estimation dataset and the validation datasets. As correlation coefficients should not be used for comparisons between studies, we also report the residual SD of log($S_I$) for the various models derived from the present dataset. We find that all of our three models provide more accurate estimates of insulin sensitivity than various previously published methods (7–12). The higher accuracy of the present models might be due to 1) the fact that the models were developed using detailed data from a relatively large study population ($n = 194$), 2) only data from nondiabetic individuals without IGT were used for model development to avoid the confounding effect of overt hyperglycemia on estimates of insulin secretion and action, and 3) the fact that information on sex and BMI were included. It is noteworthy that for log($S_I$) the estimated parameters for sex and BMI show a high degree of consistency among the different models, indicating that adjustment for these factors is indeed relevant. However, adding information about sex and BMI to previously used indexes did not improve their accuracy.
and measurement of plasma glucose and serum insulin levels at 0, 30, 60, and 120 min. The models are designed for non-diabetic individuals without IGT; glucose tolerance is also being estimated from the OGTT. Because of the simplicity of these models, they can be implemented in large scale metabolic, genetic, and epidemiological studies.

**Acknowledgments** — This study was supported by grants from the Danish Medical Research Council, the European Union (BMH-CT98-3084 and QLK-CT-200-01038), the Velux Foundation, the Danish Diabetes Association, and The Danish Heart Foundation. We thank the participants who took part in the studies and the staff at the Steno Diabetes Center and Research Centre for Prevention and Health.

**References**


6. Hollenbeck CB, Chen N, Chen YD,
The BIGTT test


The Loss of Postprandial Glycemic Control Precedes Stepwise Deterioration of Fasting With Worsening Diabetes

LOUIS MONNIER, MD1
CLAUD COLETT, PHD2
GARETH J. DUNSEATH, MPHIL3
DAVID R. OWENS, MD3

OBJECTIVE — The aim of the study was to determine whether the loss of fasting and postprandial glycemic control occurs in parallel or sequentially in the evolution of type 2 diabetes.

RESEARCH DESIGN AND METHODS — In 130 type 2 diabetic patients, 24-h glucose profiles were obtained using a continuous glucose monitoring system. The individuals with type 2 diabetes were divided into five groups according to A1C levels: 1 (≤6.5%, n = 30), 2 (6.5–6.9%, n = 17), 3 (7–7.9%, n = 32), 4 (8–8.9%, n = 25), and 5 (≥9%, n = 26). The glucose profiles between the groups were compared. The overall glucose concentrations for the diurnal, nocturnal, and morning periods, which represent the postprandial, fasting, and the dawn phenomenon states, respectively, were also compared.

RESULTS — Glucose concentrations increased steadily from group 1 to 5 in a stepwise manner. The initial differences in mean glucose concentrations reaching statistical significance occurred 1) between groups 1 and 2 (6.4 vs. 7.7 mmol/l, P = 0.0004) for daytime postprandial periods, followed by differences; 2) between groups 2 and 3 (7.9 vs. 9.3 mmol/l, P = 0.0003) for the morning periods (dawn phenomenon); and finally 3) between groups 3 and 4 (6.3 vs. 8.4 mmol/l, P < 0.0001) for nocturnal fasting periods.

CONCLUSIONS — The deterioration of glucose homeostasis in individuals with type 2 diabetes progresses from postprandial to fasting hyperglycemia following a three-step process. The first step related to the three diurnal postmeal periods considered as a whole, the second step occurred during the morning period, and the third and final step corresponded to sustained hyperglycemia over the nocturnal fasting periods. Such a description of the key stages in the evolution of type 2 diabetes may be of interest for implementing antidiabetes treatment.

Diabetes Care 30:263–269, 2007

The steady decline in the quality of glucose homeostasis (1) as observed in type 2 diabetes results from an increasing defect (2) in both insulin sensitivity and secretion (3). The data from the UK Prospective Diabetes Study indicate that the gradual increase in both A1C levels and fasting glucose concentrations is mainly due to a relentless linear deterioration in β-cell function from the time of diagnosis. In contrast, the years that precede the development of type 2 diabetes are characterized by a progressive decline in both insulin action and defects in the early phase of the insulin secretion (4, 5). Such abnormalities lead to a progressive transition from normal glucose tolerance to impaired glucose tolerance and finally to frank type 2 diabetes. As impaired glucose tolerance is acknowledged as a prediabetic stage, it has been postulated that losses of postprandial glucose control occur before deterioration in fasting glucose concentration (4, 6, 7). In a previous study (8), we have demonstrated that postprandial glucose increments are predominant contributors to the overall hyperglycemia in patients with an A1C <7.3%, while fasting increments represent the major contributor to worsening diabetic control. These results, along with the findings of others (9), indicate that postprandial glucose deteriorates before fasting glucose. However, the exact sequence of events in the deterioration of glycemic status is not completely understood. It remains unclear whether the loss in glucose control during fasting or postprandial periods occurs in parallel or sequentially. Furthermore, it is known that postmeal glucose excursions after breakfast, lunch, and dinner are not equally affected and may deteriorate at different rates over the time course of the disease, which may also differ across different population groups. To gain further insight into these questions, which are of practical importance for tailoring the introduction of available antidiabetes treatments, we used the CGMS data (10) in 130 patients with type 2 diabetes. The glucose profiles obtained during this investigation were further analyzed after the patients had been stratified into 5 groups according to A1C levels.

RESEARCH DESIGN AND METHODS — A total of 130 individuals with type 2 diabetes (100 men and 30 women) were entered consecutively into the study with an A1C ranging from 5.2 to 12.5%. Diabetes duration from diagnosis ranged from newly diagnosed to 36 years (means ± SE duration, 7.0 ± 0.8 years). None were on insulin therapy, with treatment consisting of diet alone or diet plus different individual or combinations of oral antidiabetes drugs (Table 1). Exclusion criteria included patients who had experienced an acute intercurrent illness or who had been treated with steroids during the preceeding 3-month period. Also, all individuals treated with α-glucosidase inhibitors or glinides were excluded to avoid any bias in the interpretation of postprandial glucose excursions. The study was conducted only after the patients had given their oral informed consent in accordance with the

From the 1Department of Metabolic Diseases, Lapeyronie Hospital, Montpellier, France; the 2Laboratory of Human Nutrition and Atherogenesis, University Institute of Clinical Research, Montpellier, France; and the 3Diabetes Research Unit, Academic Centre, Llandough Hospital, Penarth, Cardiff, U.K.
Address correspondence and reprint requests to Professor Louis Monnier, Department of Metabolic Diseases, Lapeyronie Hospital, 34295 Montpellier Cedex 5, France. E-mail: l-monnier@chu-montpellier.fr.
Received for publication 31 July 2006 and accepted in revised form 30 October 2006.
Abbreviations: CGMS, continuous glucose monitoring system; FPG, fasting plasma glucose.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.
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Stepwise glycemic deterioration in diabetes

Table 1—Clinical and laboratory data

<table>
<thead>
<tr>
<th>Groups</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Entire group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1C (%)</td>
<td>&lt; 6.5</td>
<td>6.5–6.9</td>
<td>7–7.9</td>
<td>8–8.9</td>
<td>≥ 9</td>
<td></td>
</tr>
<tr>
<td>Patients tested (n)</td>
<td>30</td>
<td>17</td>
<td>32</td>
<td>25</td>
<td>26</td>
<td>130</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.7 ± 1.9</td>
<td>57.9 ± 1.7</td>
<td>59.6 ± 1.8</td>
<td>59.3 ± 2.2</td>
<td>61.4 ± 1.8</td>
<td>59.9 ± 0.8</td>
</tr>
<tr>
<td>M/F ratio</td>
<td>28/2</td>
<td>15/2</td>
<td>25/7</td>
<td>18/7</td>
<td>14/12</td>
<td>100/30</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.0 ± 1.1</td>
<td>29.8 ± 1.5</td>
<td>28.6 ± 0.7</td>
<td>26.3 ± 0.9</td>
<td>32.1 ± 1.0</td>
<td>29.6 ± 0.5</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>135 ± 3</td>
<td>141 ± 5</td>
<td>137 ± 3</td>
<td>133 ± 4</td>
<td>138 ± 3</td>
<td>137 ± 2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>83 ± 2</td>
<td>82 ± 3</td>
<td>79 ± 2</td>
<td>77 ± 2</td>
<td>78 ± 3</td>
<td>80 ± 1</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>0.7 ± 0.3</td>
<td>4.4 ± 2.3</td>
<td>8.4 ± 1.4</td>
<td>10.0 ± 2.2</td>
<td>11.5 ± 1.7</td>
<td>7.0 ± 0.8</td>
</tr>
<tr>
<td>Dietary intakes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal/day)</td>
<td>2,252 ± 94</td>
<td>2,299 ± 111</td>
<td>2,018 ± 71</td>
<td>2,041 ± 66</td>
<td>1,795 ± 80</td>
<td>2,069 ± 40</td>
</tr>
<tr>
<td>Carbohydrates (g/day)</td>
<td>263 ± 13</td>
<td>273 ± 15</td>
<td>235 ± 10</td>
<td>242 ± 8</td>
<td>205 ± 10</td>
<td>242 ± 5</td>
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<tr>
<td>Fats (g/day)</td>
<td>88 ± 4</td>
<td>89 ± 4</td>
<td>78 ± 3</td>
<td>79 ± 3</td>
<td>69 ± 3</td>
<td>80 ± 2</td>
</tr>
<tr>
<td>Proteins (g/day)</td>
<td>103 ± 3</td>
<td>101 ± 5</td>
<td>93 ± 3</td>
<td>90 ± 4</td>
<td>84 ± 3</td>
<td>94 ± 2</td>
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<td>Glycemic index values</td>
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<tr>
<td>Breakfast</td>
<td>79 ± 0</td>
<td>78 ± 0</td>
<td>79 ± 0</td>
<td>79 ± 0</td>
<td>80 ± 1</td>
<td>79 ± 0</td>
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<td>Lunch</td>
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<td>72 ± 1</td>
<td>74 ± 1</td>
<td>73 ± 1</td>
<td>75 ± 1</td>
<td>74 ± 0</td>
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<tr>
<td>Dinner</td>
<td>70 ± 0</td>
<td>71 ± 1</td>
<td>71 ± 1</td>
<td>71 ± 1</td>
<td>73 ± 1</td>
<td>71 ± 0</td>
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<tr>
<td>Timing of meals</td>
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<tr>
<td>Breakfast</td>
<td>7.48 ± 0.12</td>
<td>8.19 ± 0.26</td>
<td>8.00 ± 0.09</td>
<td>8.08 ± 0.11</td>
<td>8.17 ± 0.13</td>
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<td>13.22 ± 0.16</td>
<td>12.37 ± 0.07</td>
<td>12.58 ± 0.09</td>
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<td>12.58 ± 0.05</td>
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<tr>
<td>Dinner</td>
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<td>(sulfonylurea and metformin)</td>
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<td>7</td>
<td>45</td>
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<tr>
<td>A1C (%)</td>
<td>5.9 ± 0.1</td>
<td>6.8 ± 0.0</td>
<td>7.4 ± 0.1</td>
<td>8.4 ± 0.1</td>
<td>10.1 ± 0.2</td>
<td>7.7 ± 0.1</td>
</tr>
</tbody>
</table>

Data are means ± SE or n.

European directives that require no approval from an ethics committee for the study design as described herein. The inclusion criteria required that the dietary and/or the drug regimens had been kept constant for at least 3 months before the study.

Protocol of the study

The subcutaneous interstitial glucose was monitored on an ambulatory basis over a period of 3 consecutive days by using the second-generation Minimed CGMS (CGMS; Northridge, CA) (10). The implantation of the sensor occurred at the outpatient clinics of either the Department of Metabolism (Montpellier, France) for 72 patients or the Diabetes Research Unit (Cardiff, U.K.) for 58 patients. The sensor was inserted on day 0 and removed on day 3 at midmorning. The data were downloaded on a computer for evaluation of the glucose profiles to include estimation of glucose variations limited to data collected on days 1 and 2. The patients were instructed to maintain their usual diet and treatment and to not modify their meal timing over the 3-day period of continuous monitoring. The newly diagnosed diabetic patients were maintained on their usual diet. In the other patients, isocaloric and moderately hypocaloric diets were prescribed at for at least 3 months before the investigation, according to whether the BMI was < 25 or ≥ 25 kg/m². The caloric intakes estimated from Schofield’s formula (11) were multiplied by a coefficient of 1.55, corresponding to the sedentary physical activity as observed in most of our patients (12). The proportions of calories provided from carbohydrates was set at 45 and 50% in patients with BMI < 25 and ≥ 25 kg/m², respectively. The calories contributed by fats was ~35% regardless of the patient’s weight, with proteins providing 20 and 15% in those with a BMI < 25 or ≥ 25 kg/m², respectively. The content of food was determined by a diettian at the end of the 3-day study periods. All patients who differed by >10% from the theoretical energy and carbohydrate intakes were excluded from the study. The glycemic index values for the different meals were calculated using the method described by Wolever (13). The mean energy, the nutrient content, the mean glycemic index values, and the mean timings of meals are indicated in Table 1. The characteristic glucose pattern of each individual was calculated by averaging the profiles obtained on study days 1 and 2. On day 0, after an overnight fast, venous blood samples were drawn into tubes containing EDTA for A1C determination.

Laboratory measurements

A1C measurements were made by using either a high-performance liquid chromatography assay (Menarini Diagnostics, Florence, Italy in Montpellier) (14) or
TOSOH HLC-723 G7 analyzer in Cardiff, U.K. (15). For both methods, the intra- and interassay coefficient of variations (CVs) were <2% with a nondiabetic reference range of 3.7–5.1% A1C. Both assay methods were certified as being in conformity with the Diabetes Control and Complications Trial standards (16), which recommend CVs <5% and ideally <3%. The two assays were compared in 44 patients and found to be highly correlated ($R^2 = 0.98$); the relationship was described by an identity line: $Y = -0.133 + 1.049 X$, with $Y$ and $X$ corresponding to the Menarini and TOSOH assays, respectively.

**Analysis of the CGMS data**

The subjects were divided into five groups defined according to the A1C concentration: group 1, A1C <6.5% ($n = 30$); group 2, A1C 6.5–6.9% ($n = 17$); group 3, A1C 7–7.9% ($n = 32$); group 4, A1C 8–8.9% ($n = 25$); and group 5, A1C ≥9% ($n = 26$). The rationale for selection of the different groups was based on the fact that A1C goals for patients have been set at <6.5% by the International Diabetes Federation (17) and at 7% by the American Diabetes Association (18), respectively, and up until 2002, 8% was the A1C threshold value selected by the American Diabetes Association for additional therapeutic intervention (19). In the patients of groups 1 and 2, a subanalysis of the CGMS data was done after excluding those patients who were on pharmacological treatment to evaluate the changes in glycomic profiles in patients treated with diet alone (i.e., 29 and 11 patients in subgroups 1 and 2, respectively).

Mean glucose concentrations in each group were calculated by taking two time points as reference. Midnight (i.e., 0000 h) was taken as the start of the nocturnal fasting period while immediate prebreakfast times were considered as time 0 of the daytime postprandial period. Because the study was conducted in real-life conditions, meal times differed between both study days and patients. Therefore, glucose sensor values of each participant were read for each recording on days 1 and 2 at prebreakfast times, after satisfactory concordance ($r > 0.8$) had been validated between the glucose values calculated by the sensor and those obtained from capillary blood measurements. The mean of the two values as obtained were further averaged in the 130 patients to determine the mean prebreakfast time point that was set at 0805 h following this calculation. Considering that the postprandial state was defined as a 3- to 4-h period following ingestion of a meal (20) and after having calculated that the average timings of the two other meals, i.e., lunch and dinner, were found at 1258 h and 1931 h, respectively, in the population considered as a whole, we have estimated that the period of daytime starting with breakfast (i.e., prebreakfast time) and ending 3–4 h after dinner covered the entire postprandial period of the day, while the remaining period beginning at midnight and ending at prebreakfast time corresponds to the nocturnal fasting period. In addition to the daytime postprandial and nocturnal fasting periods, we defined a morning period starting 1 h before breakfast and ending 3 h later, corresponding to the “dawn” or “extended dawn” phenomenon, which appears to play a crucial role in determining the diabetenic control of many patients with type 2 diabetes (21,22). The glucose values obtained from the CGMS data were averaged for the three periods as described above.

**Statistical analysis**

Results are given as means ± SE or as geometric mean and 95% CI according to whether the variables were normally distributed. Log-transformed glucose concentrations over the three study periods were tested for normal distribution by using the test statistic for the Kolmogorov-Smirnov goodness-of-fit test for continuous data (23). Comparisons between mean glucose values in the different groups of patients were made using one-way ANOVA followed by Bonferroni-Dunn post hoc testing. Statistical comparisons were considered significant when $P$ values were ≤0.05/n ($n =$ number of comparisons). Analyses were performed with the STATVIEW statistical package, version 5 for Macintosh (SAS Institute, Cary, NC).

**RESULTS** — The main clinical and laboratory data are included in Table 1. The known duration of diabetes (years from diagnosis) progressively increased from group 1 to group 5. The average values of the 24-h glucose profiles in the five groups of individuals with type 2 diabetes are represented in Fig. 1. The results show a progressive deterioration of the glycemic profiles from group 1 to 5 associated with increasing levels of A1C. This progressive deterioration from group 1 to 5 was statistically and quantitatively analyzed step by step (Fig. 2). The first statistical significant change in mean glucose levels was for the daytime postprandial period (Fig. 2A), followed by the morning period (Fig. 2B), and finally the nocturnal fasting period (Fig. 2C). The results indicate that statistical changes occurred in a stepwise manner.

**Comparison of daytime postprandial periods**

As indicated in Fig. 2A, the mean glucose concentrations for the daytime postprandial periods increased steadily from group 1 to 5. The first significant difference was observed between group 1 (A1C <6.5%) and group 2 (A1C between 6.5 and 6.9%), i.e., 6.4 mmol/l (95% CI 6.1–6.7) vs. 7.7 mmol/l (7.0–8.4), respectively ($P = 0.0004$) (Table 2).

**Comparison of morning periods (dawn phenomenon)**

As shown in Fig. 2B, the mean glucose concentrations for the morning periods were not statistically different between groups 1 and 2. The first difference occurred only when group 2 (A1C between 6.5 and 6.9%) and group 3 (A1C between 7 and 7.9%) were compared: 7.5 mmol/l (95% CI 6.8–8.3) vs. 9.3 mmol/l (8.7–10.0) ($P = 0.0003$), even though the mean glucose concentrations for the morning period continued to increase with worsening A1C as seen in both groups 4 and 5, i.e., when A1C values were >8 and 9%, respectively (Table 2).

**Comparison of nocturnal fasting periods**

As indicated in Fig. 2C, the mean glucose concentrations for the nocturnal fasting periods remained at near-normal levels as long as A1C levels were <8%. The first difference was noted when group 3 (A1C between 7 and 7.9%) and group 4 (A1C between 8 and 8.9%) were compared (6.3 mmol/l [95% CI 5.7–6.9] vs. 8.4 mmol/l [7.7–9.3]; $P < 0.0001$), even though further deterioration occurred in group 5 (Table 2).

**Comparison of glycemic profiles in the patients of subgroups 1 and 2 who were treated with diet alone**

Mean glucose concentrations over nocturnal periods were similar in both subgroups, while the mean glucose concentrations over postprandial periods of daytime were higher in subgroup 2 (7.9 mmol/l [95% CI 7.2–8.9], $n = 29$) than in
subgroup 1 (6.3 mmol/l [6.1–6.7], n = 11, P = 0.0002).

CONCLUSIONS — The results from this study indicate that a gradual loss in daytime postmeal glycemic control precedes a stepwise deterioration in nocturnal fasting periods with worsening diabetes. The morning periods (dawn phenomenon) are interposed as an intermediary step between the nocturnal and diurnal periods. Furthermore, these results demonstrate that the nocturnal fasting glycemic control remains essentially unchanged as long as the A1C levels remained <8%. In contrast, the glycemic control during the postprandial periods was subject to early deterioration occurring as A1C levels exceeded 6.5%. These data indicate that in those patients who fail to achieve A1C <7% but who have near-normal FPG concentrations, the failure in optimizing glycemic control is mostly due to a residual and persistent elevation in postprandial glucose following the three main meals of the day. Therefore, our own results and those of Woerle et al. (26) suggest strongly that treat-to-target treatment strategies should be aimed at normalizing FPG values and then reducing postprandial glucose concentrations. In the present study, patients who had an A1C <6.5% exhibited near-normal fasting glucose values and maintained their mean postprandial glucose <7.8 mmol/l. In addition, the subanalysis of the CGMS in subgroups 1 and 2 after excluding the patients who were pharmacologically treated is of particular interest since this analysis confirmed that in patients on diet alone, the first deterioration of the glycemic profile occurred during the postprandial period. As the glucose profiles of these patients were not dependent on the therapeutic choice made by their physicians for selecting the pharmacological treatment, one can consider that the observed deterioration is a reflection of the natural course of worsening diabetes. This observation is reinforced by the fact that the carbohydrate intakes were similar in these two early subgroups. While these results suggest that excessive postprandial glucose increments should be managed earlier in the course of the disease to achieve A1C <6.5%, amelioration of postprandial excursions cannot be assumed to delay the progression of the disease (28).

Another point concerns the second step in the deterioration of the glycemic profiles, i.e., the significant increase in glucose concentrations as observed during the morning periods in group 3 due to an overproduction of glucose by the liver.

Figure 1 — Twenty-four hour recordings from the CGMS in the five groups of patients with type 2 diabetes. Curve 1 (blue): A1C <6.5%; curve 2 (red): 6.5% to <7%; curve 3 (green): 7% to <8%; curve 4 (orange): 8% to 9%; curve 5 (purple): 9%.
These metabolic disturbances, better known as the so-called “dawn phenomenon” (21), start at the end of the overnight fast, i.e., at prebreakfast time, but have a prolonged deleterious effect on glucose concentrations over the entire postbreakfast period. The combined effects of the dawn phenomenon and of glucose derived from the intestinal hydrolysis of breakfast carbohydrates can result in an “extended dawn phenomenon” characterized by sustained blood glucose excursions during the morning period. Our results seem to indicate that the dawn and extended dawn phenomena remained relatively controlled as long as A1C remains <7%. In contrast, in patients exhibiting A1C ≥7%, the metabolic abnormalities that characterize the dawn and extended dawn phenomena are no longer normalized by dietary measures and/or by treatments with oral antidiabetes drugs. In such situations, i.e., in patients with A1C between 7 and 7.9%, it remains to be seen whether treatments with once-daily injection using small doses of rapid insulin analogs or inhaled insulin at prebreakfast time should or should not be initiated to limit postbreakfast blood glucose excursions during the morning period, with the aim of achieving an A1C <6.5%, the latest target defined in the International Diabetes Federation’s Global Guideline for Type 2 Diabetes (17). Our results might also be important for defining more precisely the time for introduction for the recently available glucagon-like peptide 1 analogs, which exert a glucose-dependent insulinotropic action (29,30). According to our results, these treatments might be particularly useful for patients with A1C levels between 7 and 8% to reduce the postbreakfast glucose excursions that are particularly marked at this stage of the disease.

Finally, in those patients in whom the A1C level was ≥8%, the therapeutic prerequisite is first to reduce the nocturnal hyperglycemia by using long-acting insulin analogs given before dinner or at bedtime. According to the treat-to-target concept (31), the insulin replacement therapy should target both A1C and FPG to achieve A1C ≤7% and FPG ≤5.6 mmol/l. However, even when the patients meet these objectives, it is not necessary that optimal glycemic control has been achieved. The present data indicate that one or several major glucose excursions may still occur daily in these patients and
suggest that adding prandial insulin before such meals may further improve glycemic control.

In conclusion, the monitoring of glucose patterns over 24 h indicates that the deterioration of glucose homeostasis can be approximated to a three-step process. The first step corresponds to a loss of postprandial glycemic control. The second step is characterized by a deterioration of the glycemic control during the prebreakfast and postbreakfast periods that correspond to the dawn and extended dawn phenomena (21). The final step in the deterioration of the diabetic control is represented by a sustained hyperglycemia over the nocturnal period resulting in fasting hyperglycemia. These observations should be of great interest for guiding the choice of antidiabetes therapeutic strategies during the evolution of type 2 diabetes in a attempt to achieve near normoglycemia.

### References


### Stepwise glycemic deterioration in diabetes

**Table 2—Data of Fig. 2**

<table>
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<th>4</th>
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<td>A1C (%)</td>
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<td>7–7.9</td>
<td>8–8.9</td>
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<td>Patients tested</td>
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<td></td>
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<td>Over daytime postmeal period*</td>
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<td>9.5 (8.7–10.3)†</td>
<td>11.4 (10.5–12.3)</td>
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<tr>
<td>Over morning periods‡</td>
<td>7.0 (6.7–7.5)</td>
<td>7.5 (6.8–8.3)†</td>
<td>9.3 (8.7–10.0)†</td>
<td>10.2 (9.5–10.9)†</td>
<td>12.2 (11.1–13.4)</td>
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<tr>
<td>Over nocturnal fasting periods§</td>
<td>5.6 (5.3–5.9)</td>
<td>6.0 (5.4–6.7)</td>
<td>6.3 (5.7–6.9)†</td>
<td>8.4 (7.7–9.3)†</td>
<td>10.3 (9.3–11.3)</td>
</tr>
</tbody>
</table>

Data are geometric mean (95% CI) or n. *Data from breakfast to midnight. †Mean glucose concentration of the group significantly different from the following. ‡Data from midnight to breakfast.


Probe-to-Bone Test for Diagnosing Diabetic Foot Osteomyelitis

Reliable or relic?

Lawrence A. Lavery1
David G. Armstrong2
Edgar J.G. Peters3
Benjamin A. Lipsky4

OBJECTIVE — We sought to assess the accuracy of the probe-to-bone (PTB) test in diagnosing foot osteomyelitis in a cohort of diabetic patients with bone culture proven disease.

RESEARCH DESIGN AND METHODS — In this 2-year longitudinal cohort study, we enrolled 1,666 consecutive diabetic individuals who underwent an initial standardized detailed foot assessment, followed by examinations at regular intervals. Patients were instructed to immediately come to the foot clinic if they developed a lower-extremity complication. For all patients with a lower-extremity wound, we compared the results of the PTB test with those of a culture of the affected bone. We called PTB positive if the bone or joint was palpable and defined osteomyelitis as a positive bone culture.

RESULTS — Over a mean of 27.2 months of follow-up, 247 patients developed a foot wound and 191 developed 199 foot infections. Osteomyelitis was found in 30 patients: 12% of those with a foot wound and 20% in those with a foot infection. When all wounds were considered, the PTB test was highly sensitive (0.87) and specific (0.91); the positive predictive value was only 0.57, but the negative predictive value was 0.98.

CONCLUSIONS — The PTB test, when used in a population of diabetic patients with a foot wound among whom the prevalence of osteomyelitis was 12%, had a relatively low positive predictive value, but a negative test may exclude the diagnosis.

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Individuals with diabetes have an ~25% lifetime risk of developing a foot complication (1), the most common of which is skin ulceration. Over half of these foot wounds may eventually become infected, which greatly increases the risk of lower-extremity amputation (2–5). While most diabetic foot infections involve only the soft tissue, bone involvement occurs in 20–66% of cases (6–8). Furthermore, foot infections complicated by osteomyelitis generally have a worse outcome and often require surgical resection and prolonged antibiotic therapy (6,7).

While diagnosing osteomyelitis is important, it is unfortunately also difficult. Clinical and laboratory signs and symptoms are generally unhelpful (6,7). Bone infection may not show up on plain radiographs in the first 2 weeks, and any X-ray abnormalities detected may be caused by the neuropathic bone disorders that frequently occur in diabetes. More accurate imaging studies, such as radionuclide scans or magnetic resonance imaging, are expensive and not universally available (9–21). In 1995, Grayson et al. (22) described a clinical technique they used in diabetic patients with a foot infection consisting of exploring the wound for palpable bone with a sterile blunt metal probe. Their most important finding was that the probe-to-bone (PTB) test had a positive predictive value of 89%, leading them to conclude that a positive test usually made imaging studies for diagnosing osteomyelitis unnecessary (22). Since then, many have considered a positive PTB sufficient evidence for osteomyelitis. In the study by Grayson et al., however, the prevalence of osteomyelitis in their population with “severe limb-threatening infections” was 66%. Furthermore, the investigators did not obtain a bone specimen for analysis, the criterion standard for the diagnosis, from all patients and used histopathological rather than microbiological confirmation to diagnose osteomyelitis. To assess the value of the PTB test in an unselected population of individuals with diabetes, we conducted the test as part of a prospective cohort study of foot complications in diabetic patients and confirmed the presence of osteomyelitis by bone culture.

RESEARCH DESIGN AND METHODS — As part of a diabetes disease management program to study and prevent lower-extremity complications and in cooperation with two large primary care physician groups in south Texas, we prospectively enrolled 1,666 patients in an observational trial over an 8-month period. As part of a systematic screening program, we documented each patient’s medical history for all potential foot complications and screened them for established risk factors (23). Patients were then seen at regular intervals (i.e., every 2–12 months, depending on their foot risk classification) for routine foot care and repeat evaluations (24). In addition, all patients were instructed to immediately return to the foot clinic if they developed any foot complication. We followed the patients for an average of 27.2 months (range 4–32) and tracked all pertinent clinical outcomes, verifying all hospital
admissions and lower-extremity amputations with claims data. The disease management program's foot clinic was the primary source for foot care, as well as for referral and consultation for diabetes-related lower-extremity complications. This project was approved by our institutional review board.

We defined a foot wound as a full thickness lesion involving any portion of the foot or ankle (25–27). We excluded wounds characterized as blisters, minor lacerations, or abrasions (n = 16). We defined a wound infection clinically, by criteria consistent with the International Working Group guidelines (28), i.e., the presence of wound purulence or at least two signs or symptoms of local inflammation or systemic symptoms of infection with no other apparent cause. We evaluated all wounds to determine the extent of soft tissue involved and for any evidence of bone infection (osteomyelitis) (6,29,30). As part of this evaluation, each patient underwent the PTB test, conducted by one of two experienced podiatrists using a sterile probe to gently explore the wound. We defined a positive test as palpating a hard or gritty substance that was presumed to be bone or joint space. Each patient with a clinically infected wound also underwent a series of plain radiographs and had additional imaging studies as indicated. If, based on the clinical examination (other than the PTB test) and imaging studies, we thought bone infection was possible, the patient underwent bone biopsy. Using aseptic techniques, we obtained specimens for culture, either in the clinic or operating room, following standard surgical skin preparation with betadine. We obtained bone specimens by needle aspirate, curet-

tage, or rongeur at the time of debridement or through sites that were noncontiguous with the wound. Specimens were transferred to a sterile container or transport tube with culture media and quickly transported to the clinical microbiology laboratory. We used the results of bone culture to determine the presence or absence of bone infection. A positive culture was defined as growth of any organism from the bone specimen. Although our data forms did not specifically record information on antimicrobial treatment in all cases, most patients presented with an acute wound and were not receiving any antibiotic therapy. We followed all patients with a foot wound until it either healed or required surgical intervention.

To assess the value of PTB in diagnosing osteomyelitis, we calculated the sensitivity, specificity, and positive and negative predictive values of the test using the results of the bone culture as the criterion standard. We calculated statistical values using SPSS version 11.0 for Macintosh (SPSS, Chicago, IL) and Diagnostic and Agreement Statistics DAG Software (Mental Health Research Institute, Parkville, Victoria, Australia).

**RESULTS** — The demographic and clinical characteristics of the patients we enrolled are shown in Table 1. Over a mean of 27.2 months of follow-up, 247 (14.8%) of the 1,666 enrolled patients developed a foot wound and 151 (9.1%) developed 199 foot infections. One patient with cellulitis did not have a wound, precluding conducting the PTB test. All of the patients with osteomyelitis presented with signs and symptoms of a soft tissue foot infection. Bone infection was documented in 30 patients, representing 20% of the 150 infected patients and 12% of all 247 with a foot wound. The PTB test was performed in all of the 247 patients with a wound; it was positive in 46 (18.6%), 26 (56.5%) of whom had osteomyelitis. The test was positive in 26 (86.7%) of the 30 with culture-proven bone infection, as well as in 20 (9.2%) of the 217 without osteomyelitis. Among the 150 patients with a clinically infected wound, the test was positive in 46 (30.7%). There were no complications attributable to the PTB test.

The values for sensitivity, specificity, and positive and negative predictive values of the PTB test for all patients with a foot wound and for the patients with a clinically infected foot wound are shown in Table 2. The sensitivity was 87% for both groups (i.e., all wounds and infected wounds), while the specificity was 91% for all wounds and 87% for infected wounds. The negative predictive value was extremely high (96–98%), but the positive predictive value was only 57–62%. The positive likelihood ratio was 9.4 for all wounds and 6.5 for infected wounds, similar to the values for the negative likelihood ratios for both populations.

**CONCLUSIONS** — Osteomyelitis of the foot in individuals with diabetes is often difficult to diagnose. Bone biopsy is considered the criterion standard for the diagnosis. While histopathological definitions may be useful for diagnosing osteomyelitis, most prefer microbiological methods (6,31). Many clinicians (and patients) are hesitant to undertake this invasive and rather expensive procedure. Thus, clinicians have sought clinical evi-
PTB test for diabetic foot osteomyelitis

Table 2—Statistical analysis of the PTB test for diagnosing osteomyelitis in all foot wounds and in clinically infected wounds

<table>
<thead>
<tr>
<th>Statistic</th>
<th>All wounds value (n = 247)</th>
<th>Infected wounds value (n = 150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.87 (0.71–0.96)</td>
<td>0.87 (0.69–0.96)</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.91 (0.89–0.92)</td>
<td>0.87 (0.79–0.92)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>0.57 (0.46–0.62)</td>
<td>0.62 (0.46–0.76)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>0.98 (0.96–0.99)</td>
<td>0.92 (0.91–0.99)</td>
</tr>
<tr>
<td>Positive likelihood ratio</td>
<td>9.40 (6.05–14.61)</td>
<td>6.50 (4.03–10.48)</td>
</tr>
<tr>
<td>Negative likelihood ratio</td>
<td>6.81 (2.73–16.97)</td>
<td>6.50 (2.60–16.23)</td>
</tr>
</tbody>
</table>

Data in parentheses are 95% CI.

dence to help them determine what patients were likely to have diabetic foot osteomyelitis. Unfortunately, local inflammatory signs and symptoms may be blunted because of diabetes-related vascular insufficiency, peripheral neuropathy (32), and leukocyte dysfunction (33). While clinical findings (34) or elevations in hematological inflammatory markers (e.g., white blood cell count, erythrocyte sedimentation rate [35], or C-reactive protein [36]) may be helpful (37,38), these are not sufficiently accurate for diagnosis (3,4,35,39–45). Furthermore, evaluating published reports of the sensitivity, specificity, and predictive value of various diagnostic methods is complicated by inconsistent operational definitions and outcome measures, as well as the variability in the prevalence of osteomyelitis in the populations studied (46). It is not surprising, therefore, that the clinical assessment for diagnosing osteomyelitis has a reported sensitivity ranging from 0 to 54% (9,20,47,48). Various imaging studies, especially magnetic resonance, certainly enhance the accuracy of diagnosing osteomyelitis, but these are expensive, time-consuming, and not universally available (49,50). Thus, clinicians have sought a simple inexpensive bedside test to help determine which patients should undergo more extensive evaluations.

Since its introduction, the PTB technique has been widely used for evaluating diabetic patients with a foot wound. Pulpation of bone with a metal probe is a simple bedside procedure predicated on the concept that if the probe can reach the bone, so can infectious bacteria. In the report by Grayson et al. (51) on 76 hospitalized patients enrolled in a diabetic foot infection antibiotic trial, 66% were found to have osteomyelitis, defined by histology on bone biopsy (in most subjects) and by surgical exploration or radiological imaging (in the rest). They calculated that the PTB test had a sensitivity of 66%, specificity of 85%, positive predictive value of 89%, and a negative predictive value of 56% (22). Our study evaluated more than three times as many patients with a foot wound and more than twice as many with a foot infection. Unlike in the study by Grayson et al., our patients were identified (and largely treated) in an outpatient setting. Furthermore, in all of our patients, osteomyelitis was defined exclusively by a positive bone culture. We found very little difference in positive and negative predictive values when we compared PTB results in all patients who had a wound with the subset who had clinical signs of infection. In our patient population, the PTB had high sensitivity and specificity, but because of the lower prevalence of osteomyelitis, our positive predictive value was only 57–62%. Thus, a positive PTB only slightly increased the probability of osteomyelitis over tossing a coin. The negative predictive value, however, was considerably higher, at 96–98%. A negative test, therefore, argues strongly against the diagnosis of osteomyelitis. These results confirm the importance of disease prevalence in assessing any test for making the diagnosis of diabetic foot osteomyelitis (46).

At least three factors may have contributed to the apparent disparity in outcomes between our study and that of Grayson et al. (51). First, the lower positive predictive value in our population may be attributable to their lower prevalence of osteomyelitis (20 vs. 66%) (46). Second, all of the patients in the Grayson et al. study required hospitalization for severe foot infections, which required parental antibiotics. Our study population was derived from patients who mostly presented in a clinic setting, and only 61% of patients with a foot wound had evidence of infection. Third, when bone biopsy was performed by Grayson et al., they histologically defined osteomyelitis (in 46 of 50 cases by the presence of inflammatory cells, fibrosis, necrosis, and reactive bone), while we defined it microbiologically (by a positive culture of a bone specimen). Because most of our patients presented with an acute foot wound, we believe that few were receiving antibiotic therapy, enhancing the value of a microbiologically based diagnosis. Thus, it is possible that they missed cases of osteomyelitis that did not have histological changes (false negatives) or that we included cases that represented microbial contamination (false positives) of the bone specimen. Our patient population is probably more representative of those in a typical clinical practice where the PTB would be most commonly used.

Our study had several potential limitations. First, we did not perform histological examination of the bone specimens to compare against the culture results. Rather, we elected to use a positive bone culture as our criterion standard. We did so because it is often difficult to obtain an adequate core of bone from the small bones of the feet (especially toes) to allow histopathological analysis and because the criteria for histologically diagnosing osteomyelitis are not well-defined. Furthermore, because we believe that most of our patients were not receiving antibiotic therapy at the time the bone biopsy was taken and they underwent careful wound cleansing and debridement before the procedure, we thought that the risk of false negative or positive results was low. Additionally, for samples collected in this study and in our greater clinical experience, readings of histological specimens often refer to signs of inflammation or inflammatory cells but do not specifically describe osteomyelitis. Second, we did not conduct a bone biopsy on patients with a foot wound in whom there was no suspicion of bone involvement. While work in this area suggests that bone biopsy is both safe (52,53) and helpful (49), we believed it would be unethical to do this procedure on patients with no suspicion of osteomyelitis. As previously stated, none of the patients who did not undergo a biopsy were later found to have developed osteomyelitis. Because the average follow-up for patients in this population was 27 months and our group was the sole source of diabetic foot referral, it was unlikely that we missed any cases of bone infection. Third, the PTB was con-
ducted by one of two podiatrists, but we did not test the interrater reliability.

We were only able to find two other studies in the literature of the PTB test in patients with a diabetic foot wound. In a recent brief report, Shone et al. (54) described 81 patients with 104 foot wounds on whom they did the PTB test. They did not diagnose osteomyelitis by bone biopsy but rather clinically (mostly by physical examination and plain X-rays, with bone histology in a minority). Their patients included both those in whom the diagnosis had already been made and those in whom it was made later. Interestingly, their results were similar to ours, i.e., PTB had a positive predictive value of 53% and a negative predictive value of 85%. They diagnosed osteomyelitis in 19 (24%) of their patients, a prevalence similar to that in our study (20%). Balsells et al. (55) performed a PTB test in a series of 33 episodes of foot ulceration (on 28 diabetic patients) that required the patient to be hospitalized. Among the 21 who had osteomyelitis (defined by either positive nuclear medicine scans or characteristic X-ray changes associated with a foot ulcer), only 7 (33%) had a positive PTB. Unfortunately, they reported no data on the results of PTB in the 12 patients who did not have bone infection, limiting the ability to evaluate the test’s accuracy in this study.

If we are to use the PTB test in clinical practice, we must understand both its value and limitations. Unfortunately, some have inappropriately generalized the results from the study by Grayson et al. to all foot (and even other) wounds in various clinical settings (56, 57). We have also observed clinicians using devices and methods for the test that are quite different from those described in the original study. Furthermore, there are no data on the interrater or intrarater reliability of the test. Perhaps most importantly, clinicians must realize that the prior prevalence of osteomyelitis greatly affects the usefulness of the PTB test. In a population with “limb-threatening” infections and a high prevalence of osteomyelitis, a positive PTB is probably quite helpful in diagnosing bone infection. In more typical clinical settings, however, this is less likely to be true, and the PTB test is a better tool to exclude osteomyelitis. We need further studies on this test to answer the remaining questions and to help understand its value in different settings.

References
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Functional Vascular Endothelial Growth Factor –634G>C SNP Is Associated With Proliferative Diabetic Retinopathy

A case-control study in a Brazilian population of European ancestry

Flavia I.V. Errera, PhD1,2
Luís Henrique Canani, MD, PhD3
Maria Elisabeth R. Silva, MD, PhD4
Erika Yeh1
Walter Takahashi, MD, PhD5
Katia G. Santos, PhD6
Katia E.P. Souto, PhD7
Balduíno Tschiedel, MD, PhD7
Israel Roisenberg, MD, PhD6
Jorge Luis Gross, MD, PhD3
Maria Rita Passos-Bueno, PhD1

OBJECTIVE — The purpose of this study was to evaluate the effect of the single nucleotide polymorphism (SNP) –634G>C at the 5′ regulatory region of the vascular endothelial growth factor (VEGF) in the risk of proliferative diabetic retinopathy (PDR) in the Brazilian population of European ancestry with type 2 diabetes.

RESEARCH DESIGN AND METHODS — A case-control study was conducted in 501 type 2 diabetic patients of European ancestry. Patients underwent a standardized clinical, ophthalmological, and laboratory evaluation. Of these, 167 patients had PDR (case patients), and 334 were considered as control subjects (patients without PDR) for PDR. A reference population (110 individuals of European ancestry) was also evaluated.

RESULTS — No evidence of association between –634G>C/VEGF and the presence of diabetic retinopathy or type 2 diabetes was observed (P > 0.05). However, CC homozygous for the SNP –634G>C was significantly more frequent in patients with PDR (37 of 167; 22.2%) than in the corresponding control group (40 of 334; 12%) in accordance with a recessive model (P = 0.003). This effect was further observed when creatinine, BMI, sex, duration of type 2 diabetes, HDL cholesterol, and systolic blood pressure were taken into account (odds ratio 1.9 [95% CI 1.01–3.79], P = 0.04). However, CC homozygous for the SNP –634G>C showed a decreased risk of PDR (odds ratio 0.7 [95% CI 0.46–1.09], P = 0.05). However, CC homozygous for the SNP –634G>C did not show a significant association with the severity of diabetic retinopathy.

CONCLUSIONS — The presence of the allele –634C/VEGF in homozygosity is an independent risk factor for the development of PDR in type 2 diabetic patients of European ancestry.

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D iabetic retinopathy is a common microvascular complication in patients with diabetes, constituting a major cause of blindness in this group. Although the risk of development of this complication increases with poor glycemic control, there are several indications suggesting that the occurrence or progression of diabetic retinopathy also depends on genetic factors (1–3).

Proliferative diabetic retinopathy (PDR), characterized by increased vascular permeability, tissue ischemia, and neovascularization, affects 10–20% of diabetic patients (4). This process depends on the local production of angiogenic factors and components of the extracellular matrix, which will be substrates for endothelial migration. Vascular endothelial growth factor (VEGF), a potent activator of angiogenesis, enhances collateral vessel formation and increases the permeability of the microvasculature (5,6). VEGF expression is induced by high glucose levels and hypoxia and plays an important role in normal and abnormal angiogenesis (7–9). Its levels have been found to be markedly increased in the vitreous and aqueous fluids in the eyes of patients with PDR (10–12).

Several polymorphisms at the VEGF 5′ regulatory region have been characterized and evaluated as risk alleles for the susceptibility or progression of both diabetic retinopathy and diabetic nephropathy through case-control studies (13–16). The single nucleotide polymorphism (SNP) –634G>C was found to be associated with nonproliferative diabetic retinopathy (NPDR) and diabetic macular edema in type 2 diabetic patients (13,16). Ray et al. (15) detected an association between the SNP –634G>C and PDR in diabetic patients. Therefore, association of the SNP –634G>C still remains controversial.

These data thus suggest that polymorphisms located at the 5′ regulatory region of VEGF might represent at-risk alleles to diabetic retinopathy or to its progression. Considering the importance of validating
VEGF SNP and PDR

this hypothesis, this work was undertaken to evaluate the effect of the SNP −634G>C/VEGF in the risk of diabetic retinopathy in Brazilian patients of European ancestry with type 2 diabetes, a nation-wide representative sample of our population. The allele −634C/VEGF, with a frequency of at least 10% in Asian and European populations (13–17), is associated with increased VEGF transcription and translation (16–18).

RESEARCH DESIGN AND METHODS — A total of 501 type 2 diabetic patients were included. Of these, 359 patients belonged to a cohort being followed at the Federal University of Rio Grande do Sul, and a detailed description can be found elsewhere (19). Briefly, patients with type 2 diabetes were identified from a multicenter study that started recruiting patients in southern Brazil in 2002. The aim of that project was to study risk factors for chronic complications of diabetes. It included four centers located at general hospitals in the state of Rio Grande do Sul, namely Grupo Hospitalar Nossa Senhora da Conceição, Hospital Vicente de Paula, Hospital Universitário de Rio Grande, and Hospital de Clínicas de Porto Alegre. The remaining 142 patients were ascertained at Hospital das Clínicas, University of São Paulo Medical School, São Paulo. All of these 501 patients were of European ancestry (defined as descendants of Portuguese, Spanish, Italians, and Germans). The ethnic groups were defined on the basis of self-classification and subjective classification (skin color, nose and lip shapes, hair texture, and information about family ancestry). Those who defined themselves as having mixed or other ancestry were not included. The study was approved by the ethics committees of the Institute of Biosciences, University of São Paulo, the Hospital das Clínicas, University of São Paulo, and the Federal University of Rio Grande do Sul. Blood was drawn only after the informed consent was obtained.

Patients underwent a standardized evaluation consisting of a questionnaire, physical examination, and laboratory tests. Diagnosis of type 2 diabetes was based on the guidelines of the report of the Expert Committee of the American Diabetes Association (20). Weight, without shoes and in light outdoor clothes, and height were measured, and BMI was calculated as weight in kilograms divided by the square of height in meters. Hypertension was defined as blood pressure ≥140/90 mmHg or use of antihypertensive medication (21).

Diabetic retinopathy Fundus examination was performed in all patients by a trained ophthalmologist using direct and indirect ophthalmoscopy through dilated pupils. Retinopathy was classified as absent, nonproliferative (microaneurysms, hemorrhage, and hard exudates), or proliferative (newly formed blood vessels and/or growth of fibrous tissue into the vitreous cavity). Patients with panophotocoagulation were classified as presenting PDR. The severity of diabetic retinopathy was graded on the basis of the worst eye. In two patients in whom the presence of media opacities due to vitreous hemorrhage (one patient) and cataract (one patient) prevented funduscopy in one eye, the contralateral eye was used to classify diabetic retinopathy. No patient was excluded as a result of unreadable funduscopy tests in both eyes. The diagnosis of PDR on the basis of funduscopy performed by an ophthalmologist was used to classify the patients. For a subset of 240 patients, selected for reasons of convenience, stereoscopic color fundus photographs of seven standard fields (22) were obtained to analyze the agreement between the classification of retinopathy using this method and ophthalmoscopy performed by the physicians. Initially, two ophthalmologists, who were unaware of the patients’ clinical data, classified the fundus photographs independently according to the criteria of the American Academy of Ophthalmology (AAO) (23). The agreement of diabetic retinopathy classification performed by ophthalmoscopy and stereoscopic fundus photographs was then analyzed to validate the ophthalmoscopy procedure used to classify studied patients. The χ² coefficient was used to assess the agreement between diabetic retinopathy classification by different ophthalmologists and by different methods (stereoscopic fundus photographs and ophthalmoscopy) and to evaluate the agreement of diabetic retinopathy classifications performed by the same ophthalmologist on two separate occasions in the subset patients. The agreement of diabetic retinopathy classification by stereoscopic fundus photographs performed by the different ophthalmologists was 93.3% (χ² = 0.774; P < 0.001) when they used the simplified diabetic retinopathy classification (presence or absence of PDR) and 88.8% (χ² = 0.771; P < 0.001) when they used the AAO classification (23). Moreover, the agreement of diabetic retinopathy classification performed by ophthalmoscopy and by stereoscopic fundus photographs was 95.1% (χ² = 0.735; P < 0.001) for the simplified classification and 84.3% (χ² = 0.698; P < 0.001) for the AAO classification (23).

Considering that the allele −634C/VEGF is associated with increased expression levels of VEGF and possibly associated with PDR, we would expect an increased frequency of this allele among patients with PDR. Therefore, patients with PDR were considered case patients and patients without PDR with at least 10 years of disease were considered control subjects. This strategy was used because epidemiological and familial studies suggested that PDR has a genetic background. This approach was also used by other authors (24–26).

Reference population As a reference group for the allele frequencies of the specific polymorphism, 110 healthy blood donors of European ancestry who did not have type 2 diabetes or a family history of the disease (mean ± SD age 52.00 ± 17.06 years) were included. The first consecutive samples from the DNA bank of blood donors that fulfilled these criteria were included.

Laboratory analysis Glucose was determined by a glucose oxidase method, creatinine was determined by the Jaffe reaction, A1C was determined by an ion-exchange high-performance liquid chromatography procedure (reference range 2.7–4.3%; Merck-Hitachi L-9100 glycated hemoglobin analyzer; Merck, Darmstadt, Germany), and triglyceride and cholesterol levels were determined by enzymatic methods. Albuminuria was measured by immuno-turbidimetry (Sera-Pak immuno microalbuminuria; Bayer, Tarrytown, NY) (mean intra- and interassay coefficients of variance 4.5 and 7.6%, respectively). Total cholesterol, HDL cholesterol, and triglycerides were measured by standard enzymatic methods.

Analysis of the SNP VEGF −634G>C Genomic DNA was extracted from peripheral blood using standard protocols (27) and was PCR amplified using primers and conditions as reported previously (13). The SNP VEGF-634G>C was detected using single nucleotide primer ex-
tension (primer: 5’ GTCACTCACTTTCG CCCTGTC 3’) and MegaBACE (single nucleotide primer extension; Amersham Biosciences, Piscataway, NJ).

Statistics
Continuous clinical data were compared by unpaired Student’s t tests. Allele frequencies were determined by gene counting, and departures from Hardy-Weinberg equilibrium were verified using a χ² test or Fisher’s exact test. The level of significance adopted was P < 0.05. Logistic regression analysis was performed to assess the independent role of the VEGF genotype and other variables, including BMI, systolic blood pressure, diastolic blood pressure, A1C, duration of diabetes, and the presence of microalbuminuria. Statistical analyses were done with SSPS, version 10.0.

RESULTS

Characterization of the patient sample
The main clinical features of the patients are depicted in Table 1. The mean duration of diabetes was 13.78 ± 7.78 years. Case patients (PDR) differed from control subjects (NPDR and nondiabetic retinopathy) for diabetes duration, sex, BMI, serum HDL cholesterol and serum creatinine levels, and systolic blood pressure.

SNP −634G>C/VEGF
Genotypic distribution for the SNP −634G>C was in Hardy-Weinberg equilibrium in all groups (P > 0.05). The genotypic and allelic frequencies did not differ statistically between the reference population group (44 GG [40%], 57 GC [51.8%], and 9 CC [8.2%]): −634C = 0.66 and −634G = 0.34) and patients with type 2 diabetes (P = 0.13), suggesting that this SNP is not influenced by the presence of type 2 diabetes. LDL cholesterol (P = 0.63), HDL cholesterol (P = 0.71), total cholesterol (P = 0.11), creatinine serum levels (P = 0.95), diabetic nephropathy (P = 0.43), BMI (P = 0.08), systolic blood pressure (P = 0.63), and diastolic blood pressure (P = 0.73) in type 2 diabetic patients are not associated with the GG, GC, or CC genotypes of the SNP −634G>C/VEGF.

SNP −634G>C/VEGF and diabetic retinopathy
For the case-control analysis, 167 patients with type 2 diabetes and PDR were considered as case patients and 334 patients with NPDR (n = 55) or without diabetic retinopathy (n = 279) were considered as control subjects. The CC genotype was more frequent in case patients than in control subjects of the European ancestry group (χ² = 9.27; P = 0.01) (Table 1). Assuming a dominant model (CC + CG vs. GG), the −634C allele was not associated with PDR (P = 0.12; P = 0.36). However, in a recessive model (CC vs. GC + GG), the CC genotype was significantly more frequent in case patients (37 CC [22.2%], 130 CG + GG [77.8%]) than in control subjects (40 CC [12.0%], 294 CG + GG [88.0%]) and was significantly associated with PDR (P = 0.003), with an odds ratio (OR) of 1.85 (95% CI 1.2–2.8). Logistic regression including diabetes duration, systolic arterial blood pressure, serum HDL cholesterol and creatinine levels, BMI, and sex showed that the CC genotype was independently associated with PDR (P = 0.04, OR adjusted 1.96 [1.01–3.79]). These results suggest that the CC genotype is associated with the severity but not with the occurrence of retinopathy.

CONCLUSIONS — In the present article we have evaluated whether there is an association between the SNP −634G>C/VEGF and PDR in Brazilian type 2 diabetic patients of European ancestry. Our data suggest that the −634C allele when in homozygosis increases by 1.9 times the chance of an individual of European ancestry developing the proliferative form of diabetic retinopathy. This association was also observed after we controlled for other possible risk factors: serum creatinine, BMI, sex, duration of diabetes, HDL cholesterol, and systolic blood pressure (P = 0.04; OR adjusted 1.96 [95% CI 1.01–3.79]). Therefore, our results suggest that the −634CC genotype is an independent risk factor for the development diabetic retinopathy in patients of European ancestry.

The SNP −634G>C is in linkage disequilibrium with the SNPs −460C>T, −2.578C>A, and −1.154G>A, which have been studied in type 2 diabetes and diabetic retinopathy (13,15,16). Awata et al. (13) observed an association between the SNP −634G>C and susceptibility for diabetic retinopathy but not with progression of diabetic retinopathy in Japanese patients, in opposition to our results. Ray et al. (15) did not find an association.

Table 1—Clinical characteristics of type 2 diabetic patients of European ancestry according to diabetic retinopathy classification

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>Case</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male:female)</td>
<td>201:133</td>
<td>67:100</td>
<td></td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>12.81 ± 7.48</td>
<td>15.22 ± 8.39</td>
<td>0.001</td>
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<tr>
<td>Age (years)</td>
<td>48.51 ± 10.05</td>
<td>45.17 ± 11.08</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.05 ± 5.26</td>
<td>27.79 ± 4.60</td>
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</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>132.77 ± 44.08</td>
<td>141.45 ± 49.52</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>45.30 ± 12.03</td>
<td>42.03 ± 10.44</td>
<td>0.008</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>210.63 ± 49.69</td>
<td>212.18 ± 48.35</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.11 (0.7–1.4)</td>
<td>2.27 (1.8–3.3)</td>
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</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>189.03 ± 131.7</td>
<td>194.62 ± 124.30</td>
<td></td>
</tr>
<tr>
<td>A1C (%)</td>
<td>7.23 ± 2.26</td>
<td>7.92 ± 2.60</td>
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</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>141.84 ± 22.76</td>
<td>149.12 ± 23.10</td>
<td>0.007</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>86.18 ± 14.45</td>
<td>86.02 ± 12.89</td>
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</tr>
<tr>
<td>GG/GC/CC genotypes</td>
<td>41.6/46.4/12</td>
<td>34.1/43.7/22.2</td>
<td>0.01</td>
</tr>
<tr>
<td>G/C alleles</td>
<td>0.65/0.35</td>
<td>0.56/0.44</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Data are means ± SD, median (range), or percent.
between the SNP $-634G>C$ and diabetic retinopathy or PDR, but they found an association between the SNP $-460C>T$/VEGF and diabetic retinopathy in a group comprising both of type 2 and type 1 diabetic patients. These findings suggest that the SNPs at the 5' regulatory region of VEGF are involved with diabetic retinopathy or its progression, and their involvement might vary among populations. It is worth noting that linkage disequilibrium at the 5' region is not complete, and, therefore, discordant results may reflect haplotype differences among distinct populations. Alternatively, these differences may be related to small sample size and diabetes heterogeneity: Awata et al. (13) included 70 patients with PDR and 80 with NPDR, whereas our study included 207 patients with PDR and 74 with NPDR. On the other hand, Ray et al. (15) included 69 patients with PDR and 198 without PDR with type 1 or type 2 diabetes, in contrast to our sample and that of Awata et al. (13) who included only type 2 diabetic patients. We observed that the age of onset of the disease in patients with PDR was earlier than in the respective control subjects, whereas the duration of the disease was significantly longer in case patients compared with control subjects (Table 1). These data, together with those from the literature (13,15), suggest that the PDR group is indeed etiologically distinct.

Genetic association of an isolated SNP with a given phenotype is inherently weak, and other SNPs in linkage disequilibrium with the one under study could also be responsible for the result obtained. Our findings should be interpreted very cautiously. However, functional studies have shown that the $-634C$ allele is associated with increased transcriptional levels of VEGF, both in vitro and in vivo (16). This allele is also related to a higher activity at ribosome sites (17,28,29), suggesting that it is associated with developmental severity of diabetic retinopathy in South Indian type 2 diabetic patients. 


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References


Total and High-Molecular Weight Adiponectin in Relation to Metabolic Variables at Baseline and in Response to an Exercise Treatment Program

Comparative evaluation of three assays

Matthias Büher, MD1
Aoife M. Brennan, MD2
Theodorus Kelesidis, MD2
Jürgen Kratzsch, PhD3
Mathias Fasshauer, MD1
Susan Kralisch, PhD1
Catherine J. Williams, MPH2
Christos S. Mantzoros, MD2

OBJECTIVE — Adiponectin, an adipocyte-secreted hormone, circulates in the serum in several multimeric forms. Compared with total adiponectin, high–molecular weight (HMW) adiponectin has been suggested to be a better predictor of metabolic parameters and insulin sensitivity in humans. Our objective was to compare total adiponectin with HMW adiponectin as predictors of metabolic variables and insulin sensitivity at both baseline and after an exercise intervention.

RESEARCH DESIGN AND METHODS — We obtained blood samples from 60 men and women with normal glucose tolerance (n = 20), impaired glucose tolerance (IGT) (n = 20), or type 2 diabetes (n = 20) at baseline and after 4 weeks of training to measure metabolic variables. Using commercially available assays, we measured plasma total adiponectin using LINCO, Mediagnost, and ALPCO assays and HMW adiponectin using an ALPCO assay.

RESULTS — HMW adiponectin and total adiponectin (ALPCO) had similar ability to predict the presence of insulin resistance. Total adiponectin, as measured by radioimmunoassay (LINCO) and enzyme-linked immunosorbent assay (ELISA) (Mediagnost), correlated most strongly with measures of insulin sensitivity (P < 0.01) and lipid profile (P < 0.01) at baseline, showed greater improvements of adiponectin levels (P < 0.001), was more closely associated with improvements of lipid measures with exercise training (P < 0.01), and more accurately predicted insulin resistance and IGT in comparison with total adiponectin or HMW measured with the ALPCO ELISA.

CONCLUSIONS — These results do not support the superiority of HMW over total adiponectin (measured using currently available assays) in assessing metabolic variables at baseline or in response to physical training. Moreover, there are significant differences in the ability of commercially available assays for total adiponectin to predict metabolic variables.


Adiponectin is an adipocyte-secreted hormone that has been proposed to play a central role in metabolism in humans (1–3). Cross-sectional studies have linked decreased adiponectin levels with several metabolic traits, including insulin resistance, dyslipidemia, and the metabolic syndrome (2,4,5). In addition, low adiponectin levels have been shown to predict future development of diabetes (6), cardiovascular disease (7), and obesity-associated malignancies (8,9) in observational studies.

Adiponectin has been shown to circulate in serum in several multimeric forms, and these different forms have been postulated to have differing biologic activity (10,11). High–molecular weight (HMW) adiponectin recently has been proposed to be the biologically active form of the hormone, and, thus, it has been hypothesized that HMW adiponectin may better predict metabolic parameters than total adiponectin (12). Several studies (12–13) have reported an association between HMW adiponectin and insulin sensitivity, but whether this relationship is stronger than the well-documented associations of total adiponectin with insulin sensitivity and metabolic variables remains to be determined.

We previously have shown that total adiponectin levels in serum and expression of adiponectin receptors in skeletal muscle are correlated with insulin resistance, lipid levels, and obesity in Caucasian men and women (5). Moreover, we have demonstrated that 4 weeks of exercise resulted in an increase in total adiponectin and an increase in the expression of adiponectin receptors in skeletal muscle from subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 diabetes (5). However, the effect of exercise on HMW adiponectin has not been conclusively demonstrated. Bobbert et al. (14) have reported that total adiponectin increases with exercise in 17 obese men and...
women and showed that exercise resulted in a relative increase in the HMW form (assessed by Western blotting), but these changes did not correlate with improved insulin sensitivity.

Progress in the study of HMW adiponectin has been impaired by lack of a commercially available assay to measure individual multimers of adiponectin with high sensitivity and accuracy in large numbers of samples. A novel enzyme-linked immunosorbent assay (ELISA) system for the selective measurement of human adiponectin multimers recently has been described (16), and, using this method, Hara et al. (12) have reported that HMW adiponectin has better predictive power to detect the presence of insulin resistance and the metabolic syndrome than total adiponectin measured using the same ELISA.

To study whether measuring HMW adiponectin provides a better predictive value than total adiponectin in assessing metabolic variables, and to identify the assay method that correlates most closely with insulin sensitivity and best predicts improvements in metabolic factors at baseline and after exercise training, we measured serum total adiponectin using two different commercially available assay methods: ADIPOQ (LINCO), ADIPOQ (Mediagnost), and ADIPOQ (ALPCO), as well as HMW adiponectin before and after an exercise intervention program in 60 men and women.

**Metabolic assessment**

Metabolic and anthropometric assessment was performed as previously described (5). Insulin sensitivity was assessed in all subjects at baseline and after 4 weeks of training using the euglycemic-hyperinsulinemic clamp method. Briefly, after an overnight fast intravenous catheters were inserted into antecubital veins in both arms. One was used for the infusion of insulin and glucose; the other was used for the frequent sampling. After a priming dose of 1.2 mmol/m2 insulin, the infusion with insulin was started with a constant infusion rate of 0.28 mmol/m2 body surface per min and continued for 120 min. After 3 min, the variable 20% glucose infusion rate was added. The glucose infusion rate was adjusted during the clamp to maintain the blood glucose at 5.0 mmol/l. Bedside glucose measurements were performed every 5 min.

**Adiponectin assays**

Serum adiponectin levels were measured using radioimmunoassay (LINCO Research, St. Charles, MO) (ADIPOQ) with a sensitivity of 1 ng/ml and an intra-assay coefficient of variation (CV) of 6.6% and also using ELISA (Mediagnost, Reutlingen, Germany) (ADIPOQ), as previously described (18). In addition, serum levels of total adiponectin, as well as HMW adiponectin, were determined using a novel ELISA (ALPCO Diagnostics, Salem, NH) (ADIPOQ). The sensitivity of this assay was 0.04 ng/ml. The recovery rate was 99−103% for total adiponectin and 97−105% for HMW adiponectin. The effect of serial dilutions has been tested on human serum samples, and linearity and specificity of the assay has been documented (16). Total and HMW adiponectin values per subject time point were obtained together in the same assay, and the ratio of HMW to total adiponectin per subject time point was calculated by dividing the respective values. All respective samples before and after exercise were measured together in the same assay.

In the current study, 10 different serum samples were used as internal controls to estimate precision for both HMW and total adiponectin. Concentrations of adiponectin, ranging from 2.02 to 11.54 μg/ml, were consecutively measured four times. For total adiponectin, the intra-assay CV was 5.3% (range 2.8−8.4) and the average interassay CV was 7.4% (3.2−9.3). The same human serum samples also were analyzed after treatment with proteinase K (used to digest adiponectin multimers), and precision was estimated at different concentrations of HMW adiponectin (0.4−6.6 μg/ml). For HMW adiponectin, the intra-assay CV was 6.8% (range 5.4−8.4) and the interassay CV was 8.7% (7.2−11.5). Based on our serum samples, the intra-assay CV was 4.2% and the interassay CV was 5.6% for total adiponectin, while for HMW adiponectin the intra-assay CV was 6.4% and the interassay CV was 7.9%. The CV of the assay was also evaluated in heparin-treated plasma samples and was 9.1% for total adiponectin and 7.9% for HMW adiponectin based on 16 pairs of internal controls. To our knowledge, this is the first study to evaluate the precision of this assay for both serum and plasma based on a large number of samples (16).

**Statistical analysis**

Comparisons of descriptive characteristics, expressed as means ± SD, were conducted using one-way ANOVA with Bonferroni-corrected post hoc tests and were repeated using nonparametric Kruskal-Wallis. Nonparametric Spearman correlation coefficients were calculated among baseline measures of study variables, HMW, and total adiponectin, as well as between changes in total and HMW adiponectin with changes in measures of insulin sensitivity. Comparisons of baseline and after training measures were made using both paired t tests and Wilcoxon’s rank-sum tests among all subjects and then stratified by sex and glucose tolerance group (NGT, IGT, or type 2 diabetes). A level of α = 0.05 was used to determine statistical significance. Statistical analyses were performed using SPSS version 8 (SPSS, Chicago, IL).

**RESULTS**

Participants with IGT and type 2 diabetes were significantly older and had higher BMI, waist-to-hip ratio, percentage body fat, fasting plasma glucose, 2-h OGTT glucose, fasting plasma insulin, fasting leptin, and total and LDL cholesterol compared with subjects with NGT (Table 1). ADIPOQ and ADIPOQ, total adiponectin measures were strongly correlated to each other (r = 0.96) and inversely associated with baseline anthropometric variables (BMI, waist-to-hip ratio, and percentage fat mass), as well as baseline parameters of insulin resistance (fasting plasma glucose, 2-h OGTT glucose, and fasting plasma insulin and positively associated with whole blood glucose uptake) (Table 2). Moderately strong associations were also appar-
ent with baseline lipid profile, with negative correlations of total adiponectin with total and LDL cholesterol and positive associations with HDL. The novel ELISA, ADIPOA, produced values of total and HMW adiponectin that were highly correlated (r = 0.96) but did not show strong associations with baseline body composition, measures of insulin sensitivity, or lipid profile. HMW and total adiponectin as quantified by ADIPOA showed weak associations with ADIPO and ADIPO (Table 2). Strength of associations between ADIPO and ADIPO total adiponectin with anthropometric measures, parameters of insulin sensitivity, and lipid profile ranged from ±0.32 to 0.61, whereas the correlations of these variables were much weaker with total and HMW adiponectin as measured by ADIPO (±0.03 to 0.27).

With respect to identifying insulin resistance, ADIPO and ADIPO better predicted these conditions than ADIPO total or HMW (Fig. 1). The area under the curve (AUC) for insulin resistance, ADIPO and ADIPO, respectively, and each had sig-

Table 1—Baseline characteristics by glucose tolerance group

<table>
<thead>
<tr>
<th></th>
<th>NGT</th>
<th>IGT</th>
<th>Type 2 diabetes</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>0.77</td>
</tr>
<tr>
<td>Men/women</td>
<td>9/11</td>
<td>9/11</td>
<td>11/9</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.8±2.5</td>
<td>56.0±2.6*</td>
<td>53.1±1.5*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>69.6±3.2</td>
<td>87.6±3.7†</td>
<td>94.7±4.4*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3±0.3</td>
<td>29.8±0.9*</td>
<td>31.4±0.7*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.84±0.02</td>
<td>1.21±0.04*</td>
<td>1.28±0.03*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>24.5±0.7</td>
<td>34.9±1.9*</td>
<td>38.2±1.8*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tobacco use (n)</td>
<td>1 (5)</td>
<td>4 (20)</td>
<td>8 (40)‡</td>
<td>0.03</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.2±0.1</td>
<td>5.7±0.1†</td>
<td>6.2±0.1†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2-h OGTT glucose (mmol/l)</td>
<td>6.0±0.2</td>
<td>9.4±0.2*</td>
<td>13.1±0.3*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/l)</td>
<td>66.8±8</td>
<td>695±110*</td>
<td>319±47‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Whole-blood glucose uptake (µmol·kg⁻¹·min⁻¹)</td>
<td>75.9±3.8</td>
<td>18.7±9.0*</td>
<td>21.5±9.2*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting leptin (pmol/l)</td>
<td>0.41±0.04</td>
<td>0.54±0.06</td>
<td>0.56±0.06</td>
<td>0.11</td>
</tr>
<tr>
<td>Fasting leptin (pmol/l)</td>
<td>2.8±0.7</td>
<td>20.7±3.0†</td>
<td>31.9±3.5*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>6.1±0.8</td>
<td>42.2±7.2*</td>
<td>53.2±5.3*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>4.6±0.11</td>
<td>5.34±0.12†</td>
<td>5.60±0.16*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.6±0.07</td>
<td>1.21±0.04*</td>
<td>1.11±0.04*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total LDL (mmol/l)</td>
<td>2.34±0.10</td>
<td>3.22±0.12*</td>
<td>3.30±0.19*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.03±0.06</td>
<td>2.01±0.10</td>
<td>2.11±0.07</td>
<td>0.63</td>
</tr>
<tr>
<td>ADIPO (µg/ml)</td>
<td>8.95±0.55</td>
<td>3.38±0.26*</td>
<td>3.48±0.42*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADIPO (µg/ml)</td>
<td>8.81±3.43</td>
<td>3.51±1.47*</td>
<td>3.82±2.16*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADIPO (µg/ml)</td>
<td>6.72±0.65</td>
<td>6.25±0.68</td>
<td>5.15±0.90</td>
<td>0.32</td>
</tr>
<tr>
<td>HMW adiponectin (µg/ml)</td>
<td>2.88±0.32</td>
<td>2.80±0.44</td>
<td>2.42±0.63</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Data are means ± SD. *P < 0.001 for Bonferroni-corrected comparison with the group with NGT. †P < 0.01 for Bonferroni-corrected comparison with the group with NGT. ‡P < 0.05 for Bonferroni-corrected comparison with the group with NGT.

Table 2—Spearman correlation matrix of study variables with adiponectin measures

<table>
<thead>
<tr>
<th></th>
<th>Total ADIPO</th>
<th>Total ADIPO</th>
<th>Total ADIPO</th>
<th>HMW ADIPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADIPO</td>
<td>0.96*</td>
<td>0.25</td>
<td>0.20</td>
<td>0.03</td>
</tr>
<tr>
<td>ADIPO</td>
<td>0.25</td>
<td>0.25</td>
<td>0.20</td>
<td>0.03</td>
</tr>
<tr>
<td>HMW adiponectin</td>
<td>0.20</td>
<td>0.20</td>
<td>0.96*</td>
<td>0.03</td>
</tr>
<tr>
<td>Age</td>
<td>−0.39*</td>
<td>−0.32*</td>
<td>−0.14</td>
<td>−0.12</td>
</tr>
<tr>
<td>BMI</td>
<td>−0.53*</td>
<td>−0.42*</td>
<td>−0.16</td>
<td>−0.12</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>−0.51*</td>
<td>−0.41*</td>
<td>−0.25</td>
<td>−0.21</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>−0.53*</td>
<td>−0.44*</td>
<td>−0.24</td>
<td>−0.21</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>−0.52*</td>
<td>−0.44*</td>
<td>−0.21</td>
<td>−0.16</td>
</tr>
<tr>
<td>2-h OGTT glucose</td>
<td>−0.60*</td>
<td>−0.53*</td>
<td>−0.27*</td>
<td>−0.23</td>
</tr>
<tr>
<td>Fasting plasma insulin</td>
<td>−0.49*</td>
<td>−0.42*</td>
<td>−0.09</td>
<td>−0.04</td>
</tr>
<tr>
<td>Whole-blood glucose uptake</td>
<td>0.61*</td>
<td>0.54*</td>
<td>0.14</td>
<td>0.13</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>−0.58*</td>
<td>−0.50*</td>
<td>−0.22</td>
<td>−0.20</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.47*</td>
<td>0.42*</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>−0.50*</td>
<td>−0.40*</td>
<td>−0.07</td>
<td>−0.03</td>
</tr>
</tbody>
</table>

*P < 0.01.
significantly better predictive performance over ADIPOₐ (P = 0.0001 for comparison with ADIPOₐ and P = 0.002 with ADIPO₇), with an AUC of 0.65 (0.51–0.79; P = 0.10), and HMW adiponectin (P = 0.0001 for comparison with ADIPO₇ and P = 0.001 with ADIPO₇), with an AUC of 0.63 (P = 0.20). Similar results were observed when defining insulin resistance by homeostasis model assessment ≥ 2.5 (ADIPO₇ AUC = 0.96 [95% CI 0.91–1.02]; ADIPO₇ AUC = 0.92 [0.83–1.00]; ADIPOₐ AUC = 0.65 [0.51–0.79], and HMW 0.628 [0.48–0.77]) with predictive values consistent with those reported recently (12). Analysis using the ratio of HMW to total adiponectin did not materially change the results (data not shown). Similarly, AUC indicated greater ability to predict IGT of ADIPO₇ (AUC = 0.95; P < 0.001) and ADIPO₇ (AUC = 0.90; P < 0.001) than ADIPOₐ for measuring total (AUC = 0.65; P = 0.05) and HMW adiponectin (AUC = 0.63; P = 0.10).

The ADIPO₇ and ADIPO₇ techniques showed a more highly significant effect of exercise training on total adiponectin in all subjects than that seen on total or HMW adiponectin using ADIPOₐ (Table 3). No significant change in total adiponectin was observed among subjects with NGT using ADIPO₇ or ADIPO₇, but substantial increases in adiponectin were detected in participants with IGT or type 2 diabetes. In contrast, ADIPOₐ showed a similar magnitude of effect of training in subjects with NGT, IGT, and type 2 diabetes, and the change in total adiponectin

Table 3—Measures of adiponectin by sex and glucose tolerance group

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Posttraining</th>
<th>Absolute change</th>
<th>% change</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All subjects (n = 60)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADIPO₇</td>
<td>5.2 ± 3.1</td>
<td>6.9 ± 2.8</td>
<td>1.7 ± 2.3</td>
<td>32.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADIPO₇</td>
<td>5.4 ± 3.5</td>
<td>7.1 ± 3.3</td>
<td>1.8 ± 2.9</td>
<td>33.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADIPO₇</td>
<td>6.0 ± 3.4</td>
<td>7.7 ± 3.4</td>
<td>1.7 ± 4.5</td>
<td>28.3</td>
<td>0.004</td>
</tr>
<tr>
<td>HMW adiponectin</td>
<td>2.7 ± 2.1</td>
<td>3.8 ± 2.1</td>
<td>1.1 ± 3.0</td>
<td>40.7</td>
<td>0.008</td>
</tr>
<tr>
<td><strong>Glucose tolerance group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects with NGT (n = 20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADIPO₇</td>
<td>8.7 ± 2.5</td>
<td>8.6 ± 2.7</td>
<td>-0.2 ± 1.7</td>
<td>-2.3</td>
<td>0.97</td>
</tr>
<tr>
<td>ADIPO₇</td>
<td>8.8 ± 3.4</td>
<td>8.8 ± 3.7</td>
<td>0.0 ± 3.2</td>
<td>0.0</td>
<td>0.97</td>
</tr>
<tr>
<td>ADIPO₇</td>
<td>6.7 ± 2.9</td>
<td>8.7 ± 3.9</td>
<td>1.9 ± 3.9</td>
<td>28.4</td>
<td>0.04</td>
</tr>
<tr>
<td>HMW adiponectin</td>
<td>2.9 ± 1.4</td>
<td>4.1 ± 2.2</td>
<td>1.2 ± 2.1</td>
<td>41.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Subjects with IGT (n = 20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADIPO₇</td>
<td>3.4 ± 1.2</td>
<td>5.8 ± 2.2</td>
<td>2.4 ± 1.8</td>
<td>70.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADIPO₇</td>
<td>3.5 ± 1.5</td>
<td>6.0 ± 2.4</td>
<td>2.5 ± 2.1</td>
<td>71.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADIPO₇</td>
<td>6.3 ± 3.0</td>
<td>7.7 ± 3.5</td>
<td>1.5 ± 4.6</td>
<td>23.8393</td>
<td>0.17</td>
</tr>
<tr>
<td>HMW adiponectin</td>
<td>2.8 ± 1.9</td>
<td>3.9 ± 2.4</td>
<td>1.1 ± 3.3</td>
<td>39.3</td>
<td>0.14</td>
</tr>
<tr>
<td>Type 2 diabetic subjects (n = 20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADIPO₇</td>
<td>3.5 ± 1.9</td>
<td>6.2 ± 2.5</td>
<td>2.7 ± 2.2</td>
<td>77.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADIPO₇</td>
<td>3.8 ± 2.2</td>
<td>6.6 ± 2.9</td>
<td>2.8 ± 2.6</td>
<td>73.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADIPO₇</td>
<td>5.1 ± 4.0</td>
<td>6.8 ± 2.8</td>
<td>1.7 ± 5.0</td>
<td>33.3</td>
<td>0.15</td>
</tr>
<tr>
<td>HMW adiponectin</td>
<td>2.4 ± 2.8</td>
<td>3.3 ± 1.7</td>
<td>0.8 ± 3.5</td>
<td>33.3</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Data are means ± SD. *P value from paired t test. Nonparametric Wilcoxon signed-rank test produced comparable results.
Total and HMW adiponectin in exercise

was less pronounced and did not achieve statistical significance in the IGT and type 2 diabetic groups. Stratifying by sex showed a similar pattern of more highly significant results using ADIPO \(_\text{Q}\) and ADIPO \(_\text{M}\) methods than ADIPO \(_\Lambda\), with a slightly stronger effect of training on adiponectin in men than women (data not shown). Additionally, changes with exercise correlated more strongly with changes in total adiponectin as measured by ADIPO \(_\text{Q}\) and ADIPO \(_\text{M}\) than by total or HMW for free fatty acids (\(r = 0.38, P < 0.01\) and \(r = 0.30, P = 0.02\) vs. \(r = 0.03, P = 0.85\) and \(r = 0.03, P = 0.81\), for ADIPO \(_\text{Q}\), ADIPO \(_\text{M}\), ADIPO \(_\Lambda\), and HMW, respectively), total cholesterol (\(r = -0.34, P < 0.01\) and \(r = -0.28, P = 0.03\) vs. \(r = 0.17, P = 0.20\) and \(r = 0.18, P = 0.16\), respectively), triglycerides (\(r = -0.34, P < 0.01\) and \(r = -0.20, P = 0.13\) vs. \(r = 0.11, P = 0.41\) and \(r = 0.09, P = 0.48\), respectively), and insulin (\(r = -0.20, P = 0.12\) and \(r = -0.18, P = 0.18\) vs. \(r = 0.06, P = 0.67\) and \(r = 0.03, P = 0.82\), respectively). Similarly, the magnitudes of correlations of change in 2-h OGTT glucose and whole-blood glucose uptake were greater with change in ADIPO \(_\text{Q}\) and ADIPO \(_\text{M}\) than ADIPO \(_\Lambda\) or HMW adiponectin; however, all associations failed to reach statistical significance (not shown). Change in HDL with training was not significantly associated with changes in total adiponectin or HMW as measured by any assays. Finally, given our SD of 0.3, we had 80% power to detect a difference in AUC of 0.15 between different adiponectin assays.

**CONCLUSIONS** — We confirm herein that total adiponectin as measured by ADIPO \(_\text{Q}\) and ADIPO \(_\text{M}\) is significantly correlated with insulin sensitivity and metabolic variables. Additionally, total adiponectin as measured by ADIPO \(_\text{Q}\) and ADIPO \(_\text{M}\) is clearly superior to either HMW or total adiponectin as measured by ADIPO \(_\Lambda\) at identifying the presence of insulin resistance and predicting levels of metabolic variables. Adiponectin levels increase with exercise and significantly correlate with improvements in lipid profile when total adiponectin is measured by ADIPO \(_\text{Q}\) and ADIPO \(_\text{M}\) but not with total adiponectin or HMW adiponectin measured by ADIPO \(_\Lambda\). Finally, we did not detect any clinically significant difference between HMW adiponectin and total adiponectin, when both are measured by ADIPO \(_\Lambda\), at predicting insulin sensitivity and metabolic variables. The associations between metabolic variables and HMW adiponectin, when measured by techniques other than the assays used herein, remains to be seen.

Previous studies (10,13,14,19) investigating the differential associations of multimeric forms of adiponectin have used either Western blot technique or, subsequently, a recently described ELISA technique to quantify the proportion of total adiponectin circulating in the HMW form (12). A recent article (12) reporting that the HMW adiponectin–to–total adiponectin ratio is slightly superior to total adiponectin (AUC 0.713 vs. 0.615) at predicting the presence of insulin resistance and the metabolic syndrome used the same ELISA technique used herein. We replicated herein the predictive value of these measurements, as expressed by almost identical receiving-operating characteristic curves in this and the previous study (12). While we report that HMW and total adiponectin, as measured by the recently reported ALPCO ELISA (16), were not different in predicting the presence of insulin resistance or IGT, we also report that the other two total adiponectin assays evaluated herein are significantly better in this respect. This study was adequately powered to detect a 0.15 difference in AUC, which is clinically important.

We also report herein that both total and HMW adiponectin increase with exercise but that correlations with metabolic parameters are indistinguishable when total adiponectin and HMW adiponectin are considered (as measured using a commercially available ELISA from the same source). Our findings are in agreement with those reported by Bobbert et al. (14), who found that both HMW and total adiponectin increased to a similar degree with exercise and weight loss in 17 obese men and women, but there was no correlation between adiponectin (HMW or total) and insulin sensitivity either before or after weight loss in this study. HDL was the only metabolic variable correlated with total or HMW adiponectin in the previous study (14). The small study group, which included subjects with NGT, IGT, and type 2 diabetes and/or the interassay variability as well as the relative insensitivity of the Western methodology compared with radioimmunoassay or ELISA may have accounted for these prior findings (14). We have used a more sensitive and precise methodology, i.e., ELISA, to quantitate HMW adiponectin, and our sample size was 3.5 times larger.

Importantly, both total adiponectin and HMW adiponectin showed similar correlation with insulin sensitivity and metabolic variables, although HMW adiponectin (measured using the current commercially available ELISA) was not significantly associated with any study variable in the current study. The magnitude of the correlation coefficients was similar to those previously reported using a larger sample size (16), however, supporting the validity of our measurements.

Similar to changes in total adiponectin, the process regulating the production of adiponectin oligomers is incompletely understood in humans. Posttranslational modification of lysine residues within the collagenous domain of the molecule appears to be involved and adiponectin glycosylation is reduced in individuals with diabetes, explaining the low HMW adiponectin observed in this population (11). Several studies where HMW adiponectin is measured by Western blot have reported a selective reduction in HMW adiponectin in subjects with type 2 diabetes (10,11,20) and a preferential elevation following treatment with the insulin sensitizer rosiglitazone (19). Two human mutations that impair the formation of HMW adiponectin have been described in individuals with type 2 diabetes, suggesting, albeit not proving, a possible role for HMW adiponectin in the pathogenesis of diabetes (10).

In the initial description of the ELISA system for selective measurement of adiponectin multimers, Ebinuma et al. (16) compared Western blotting to their selective ELISA. They found a strong correlation between multimer ELISA and densitometry results for HMW adiponectin using samples from 16 healthy volunteers, but the assay has not been tested in individuals with IGT and diabetes, in whom low HMW adiponectin levels have been described. It is also important to note that the precision of the total and selective ELISAs in the previous study are based on measurement of two human samples only (16). We expand herein by providing more extensive evaluation, and although we duplicate associations with total adiponectin, we report that the specific assay used may not be as sensitive as other commercially available total adiponectin assays, possibly due to differences in the antibody used.

Strengths of our study include the larger study size compared with the previous study (14) and the detailed measurement of body composition, lipids,
and parameters of insulin sensitivity before and after a 4-week exercise intervention, allowing for assessment of associations both at baseline and of changes in study variables with exercise. While it is possible that laboratory measurement error may have played a role in our finding of lack of superiority of HMW adiponectin relative to total adiponectin in predicting insulin resistance, the consistency of the magnitudes of correlations from our study with the previous studies using ADIPOX (13), ADIPOX (21), and ADIPOX (12) indicate that it is more likely that our results are due to the relatively poor performance of the ADIPOX and HMW adiponectin assay. Further studies, using additional methods to measure HMW adiponectin, are needed to draw firm conclusions regarding the biological activity of HMW adiponectin.

In summary, using a recently developed, commercially available ELISA technique, we failed to demonstrate a stronger association of insulin sensitivity and metabolic variables with HMW adiponectin versus total adiponectin measured by existing assays in individuals with NGT or IGT or type 2 diabetes. This data does not support the superiority of HMW adiponectin, as measured by the novel ELISA, over total adiponectin in assessing insulin sensitivity and changes with physical training. Moreover, we document significant differences in the ability of total adiponectin to predict the presence of insulin resistance when measured using different commercially available assays.

Acknowledgments—This study was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG), Clinical Research Group “Atherosclerosis” KFO 152 (project BL 833/1-1) (to M.B.). C.S.M. is supported by a Bessel Award of the Humboldt Foundation and National Institute of Diabetes and Digestive and Kidney Diseases R01-58785.

References
Ethnicity, Insulin Resistance, and Inflammatory Adipokines in Women at High and Low Risk for Vascular Disease

Josef V. Silha, MD, PhD
B.L. Grégoire Nyomba, MD, PhD
William D. Leslie, MD, FRCPC
Liam J. Murphy, MB, FRACP

OBJECTIVE — We sought to compare the relationship between body composition, insulin resistance, and inflammatory adipokines in Aboriginal Canadian women, who are at high risk of vascular disease, with white women.

RESEARCH DESIGN AND METHODS — A subgroup of the First Nations Bone Health Study population, consisting of 131 Aboriginal women and 132 matched white women, was utilized. Body composition was determined by whole-body dual X-ray absorptiometry, and blood analyses were measured after an overnight fast.

RESULTS — After excluding individuals with diabetes, A1C, BMI, percent trunk fat, and homeostasis model assessment of insulin resistance (HOMA-IR) were greater in First Nation women compared with white women, whereas adiponectin, retinol binding protein (RBP)4, and insulin-like growth factor binding protein-1 (IGFBP-1) were lower. First Nation women had more trunk fat for any given level of total fat than white women. There were no differences in resistin, leptin, tumor necrosis factor (TNF)-α, or C-reactive protein (CRP) levels between First Nation and white women. Insulin resistance correlated with leptin and inversely with adiponectin levels in both First Nation and white women. There were weak correlations between insulin resistance and TNF-α, interleukin-6, and CRP, but these were not significant after correction for body fat. No correlation was found between RBP4 and insulin resistance. ANCOVA revealed a higher HOMA-IR adjusted for total body fat in First Nation women than in white women (P = 0.015) but not HOMA-IR adjusted for trunk fat (P > 0.2).

CONCLUSIONS — First Nation women are more insulin resistant than white women, and this is explained by trunk fat but not total fat. Despite the increased insulin resistance, inflammatory adipokines are not significantly increased in First Nation women compared with white women.


An increased prevalence of vascular disease in insulin-resistant states such as pre-diabetes, type 2 diabetes, and the metabolic syndrome has been long recognized (1). There is considerable debate whether insulin resistance is the primary event in atherosclerosis, with consequent activation of proinflammatory signaling pathways, or, alternatively, whether low-grade inflammation and subsequent insulin resistance accounts for the association of diabetes and cardiovascular disease (2).

Aboriginal Canadian populations, which include First Nation, Metis, and Inuit individuals (3), have increased prevalence of atherosclerosis and cardiovascular and peripheral vascular disease (4,5). First Nations are Aboriginal individuals signatory to treaties and/or recognized by the Canadian Federal Government as a fiduciary responsibility and represent the large majority of Aboriginal individuals living in Canada (3). While type 2 diabetes is more prevalent among Canadian men than women in the general population, the reverse is true for the First Nation population (6,7). In the First Nation population, obesity is more prevalent among men than women, but the prevalence of metabolic syndrome and type 2 diabetes appears to be greater for women than men, suggesting that First Nation women may be more insulin resistant than their male counterparts (6,8). Studies in Canadian Aboriginal populations have found elevated adipocytokines such as tumor necrosis factor (TNF)-α, C-reactive protein (CRP), and leptin (9,10), whereas adiponectin levels were found to be reduced (11). In some of these studies, percent body fat determined by electric impedance was reported to be elevated. However, there have been no reports of a systematic comparison of body composition and insulin resistance in women from ethnic groups at high risk of vascular disease with the general female population.

In this study, we have compared body composition, insulin resistance, and adipokines in a large cohort of First Nation women with an age-matched cohort of white women from the general Canadian population.

RESEARCH DESIGN AND METHODS — The study population was based on the urban participants from a population-based, cross-sectional survey of osteoporosis: the First Nations Bone Health Study. The design and recruitment of this study is described in detail elsewhere (12). All subjects completed an entrance questionnaire that included information about health status and medications. We excluded individuals with either a history of diabetes or a fasting plasma glucose level > 6.9 mmol/l. In addition, we excluded individuals with an elevated A1C (>6.0%) to minimize the number of subjects with normal fasting plasma glucose (but impaired glucose tol-
Table 1—Comparison of demographic, anthropomorphic, and biochemical parameters

<table>
<thead>
<tr>
<th></th>
<th>White women</th>
<th>First Nation women</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>132</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.6 ± 14.1</td>
<td>41.1 ± 10.2</td>
<td>0.005</td>
</tr>
<tr>
<td>Pre-/transitional/postmenopausal</td>
<td>67/36/29</td>
<td>77/44/10</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.9 ± 16.3</td>
<td>77.8 ± 16.4</td>
<td>0.040</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.52</td>
<td>29.20 ± 5.87</td>
<td>0.011</td>
</tr>
<tr>
<td>Total fat (kg)</td>
<td>26.78 ± 10.63</td>
<td>29.37 ± 9.74</td>
<td>0.022</td>
</tr>
<tr>
<td>Total lean mass (kg)</td>
<td>42.59</td>
<td>43.72 ± 7.05</td>
<td></td>
</tr>
<tr>
<td>Trunk fat (kg)</td>
<td>12.90</td>
<td>15.63 ± 5.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trunk fat (%)</td>
<td>16.69 ± 4.68</td>
<td>19.53 ± 4.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>5.03 ± 0.41</td>
<td>5.16 ± 0.53</td>
<td></td>
</tr>
<tr>
<td>A1C (%)</td>
<td>5.50 ± 0.29</td>
<td>5.58 ± 0.29</td>
<td>0.013</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>49.65 (31.66)</td>
<td>59.0 (48.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.54 (1.07)</td>
<td>1.93 (1.66)</td>
<td>0.002</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>16.74 ± 6.36</td>
<td>16.81 ± 6.03</td>
<td></td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>17.67 ± 9.26</td>
<td>14.95 ± 8.37</td>
<td>0.012</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>33.93 ± 25.90</td>
<td>33.77 ± 24.21</td>
<td></td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>5.45 ± 1.25</td>
<td>5.60 ± 1.33</td>
<td></td>
</tr>
<tr>
<td>IL-6 (ng/ml)</td>
<td>1.18 (1.08)</td>
<td>1.51 (1.43)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (µg/ml)</td>
<td>2.05 (3.09)</td>
<td>2.37 (3.49)</td>
<td></td>
</tr>
<tr>
<td>RBP4 (ng/ml)</td>
<td>47.59 (20.88)</td>
<td>44.24 (15.31)</td>
<td>0.008</td>
</tr>
<tr>
<td>IGFBP-1 (ng/ml)</td>
<td>12.45 (19.55)</td>
<td>11.92 (10.33)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Data are means ± SD or median (interquartile range).

Assays and measurements

Dual-energy X-ray absorptiometry measurements. Body composition (lean, fat, and bone mass) was derived from whole-body dual-energy X-ray absorptiometry (Hologic QDR-4500; Hologic, Waltham, MA). A single trained operator was used to perform all dual-energy X-ray absorptiometry scans. Fat mass and lean tissue mass parameters were analyzed using the manufacturer’s software. Trunk fat, defined as the absolute amount of fat in the trunk region, including thorax, abdomen, and pelvis, was calculated.

Glucose and insulin assays and homeostasis model assessment of insulin resistance calculations. Blood samples were obtained after an overnight fast, separated, and stored in aliquots at −70°C until analysis. Blood glucose was measured with a glucose oxidase method (Yellow Springs). Insulin was measured using a two-site chemiluminescent immunometric assay (Immulite insulin; Diagnostic Products Corporation), which has 8.5% cross-reactivity with proinsulin. All samples were assayed in a single run with reagent pooled from several kits. Insulin resistance was calculated using homeostasis model assessment of insulin resistance (HOMA-IR (13)). Insulin-like growth factor binding protein-1 (IGFBP-1) was measured with reagents from Diagnostic Systems Laboratories (Webster, TX).

Adipokines and inflammatory markers. Total adiponectin was measured by radioimmunoassay using reagents from Linco Research (St. Charles, MO), whereas resistin and leptin were measured by enzyme-linked immunosassay kits obtained from Biovendor Laboratory Medicine (Brno, Czech Republic). The specificity, sensitivity, and coefficient of variation of these assays in our laboratory have been previously reported (14). TNF-α and interleukin (IL)-6 were measured by a Quantikine HS assay from R&D Systems (Minneapolis, MN). The sensitivity of the assay was 0.12 and 0.039 pg/ml, and the within-assay coefficients of variation were 5% and 7%, respectively. Serum retinol binding protein (RBP4) was measured with an enzyme-linked immunosorbent assay kit from Alpco Diagnostics (Windham, NH). The sensitivity and interassay coefficients of variation were 100 pg/ml and 5%, respectively. C-reactive protein (CRP) was measured with a high-sensitivity assay kit from BioQuatt (San Diego CA). The sensitivity and coefficients of variation of the assay were 0.001 pg/ml and 4%, respectively.

Statistical analysis

Normally distributed data are expressed as means ± SD. Measurements that were non–normally distributed (such as HOMA-IR) were reported as median (quartile range). Group differences in continuous measurements were identified with the Wilcoxon’s rank-sum test. χ² analysis was used to compare the frequency of various conditions in the two populations. The relationship between two variables was assessed with Spearman’s rank correlation coefficient. Partial correlations were computed on ranks after controlling for ethnicity, trunk fat, and total fat (15). ANCOVA was used to compare covariate relationships between the two populations (regression line intercepts and parallelism). Non–normally distributed variables were log transformed to obtain normal distributions before ANCOVA. Statistical analysis was performed using SPSS 11.0 for Windows software.

RESULTS

Differences in demographic, anthropomorphic, and biochemical parameters

Weight, BMI, total fat, trunk fat, A1C, fasting insulin, IL-6, and HOMA-IR were significantly greater in First Nation women compared with white women, while adiponectin, RBP4, and IGFBP-1 were significantly lower in First Nation women compared with white women (52.5 ± 5.9 vs. 46.9 ± 5.7%, P < 0.001). In both groups, there was a significant relationship between trunk fat and total fat. First Nation women had a significantly greater increment in trunk fat for each additional kilogram of total fat (ANCOVA P = 0.028 for comparison of regression slopes) and sig-
Insulin resistance and inflammatory markers

Table 2—Spearman correlation coefficients between HOMA-IR and other parameters in non-diabetic populations

<table>
<thead>
<tr>
<th></th>
<th>White women</th>
<th>First Nation women</th>
<th>All women</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.569*</td>
<td>0.660*</td>
<td>0.634*</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>-0.585**</td>
<td>-0.554**</td>
<td>-0.592**</td>
</tr>
<tr>
<td>Total fat</td>
<td>0.576*</td>
<td>0.623**</td>
<td>0.607**</td>
</tr>
<tr>
<td>Trunk fat</td>
<td>0.603*</td>
<td>0.697*</td>
<td>0.668*</td>
</tr>
<tr>
<td>Resistin</td>
<td>0.008</td>
<td>0.180‡</td>
<td>0.097</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-0.415**‡</td>
<td>-0.451**‡</td>
<td>-0.451**</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.525*</td>
<td>0.672**</td>
<td>0.595**</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.042</td>
<td>0.186‡</td>
<td>0.107</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.2728</td>
<td>0.373*</td>
<td>0.347*</td>
</tr>
<tr>
<td>CRP</td>
<td>0.2508</td>
<td>0.390*</td>
<td>0.324*</td>
</tr>
<tr>
<td>RBP4</td>
<td>0.088</td>
<td>0.005</td>
<td>0.008</td>
</tr>
</tbody>
</table>

*P < 0.005, ‡P < 0.05, §P < 0.01. †Significant after controlling for trunk fat.

Determinants of insulin resistance
HOMA-IR was strongly correlated with BMI, total body fat, and trunk fat in both groups and when the population was considered as a whole (Table 2). The strongest correlation was observed with trunk fat. ANCOVA regression analysis for HOMA-IR and trunk fat for the two populations showed no significant ethnicity effect. While regression analysis for HOMA-IR and total fat indicated that there was no difference in the regression slopes, the intercept was significantly higher in First Nation compared with white women (Fig. 1). When total fat was replaced by trunk fat in the ANCOVA model, no ethnicity difference was seen (P = 0.19 for comparison of regression slopes; P > 0.2 for comparison of intercepts).

A significant inverse correlation was observed between IGFBP-1 and HOMA-IR and between adiponectin and HOMA-IR, while leptin showed a strong positive correlation in all groups. Interestingly, even after correction for trunk fat, the relationships between HOMA-IR and both adiponectin and leptin remained significant (in all comparisons except for HOMA-IR versus leptin in the white women). Resistin, TNF-α, and CRP showed a weak, but significant, positive correlation with HOMA-IR, but none of these correlations remained significant after correction for trunk fat. RBP4 did not correlate with HOMA-IR in either group or the entire study population.

The contribution of adipose tissue to adipokine levels
The correlations between fat mass and adipokines are shown in Table 3. RBP4 levels did not correlate with either total or trunk fat in both groups. The intercepts were significantly different. That is, for any given amount of total fat, First Nations had a higher HOMA-IR than white women (Fig. 1). When total fat was replaced by trunk fat in the ANCOVA model, no ethnicity difference was seen (P = 0.19 for comparison of regression slopes; P > 0.2 for comparison of intercepts).

Figure 1—The relationship between insulin resistance and total fat in First Nation (FN) and white women. ANCOVA was used to examine differences in the slope and intercept of the lines of best fit. The slopes did not differ significantly, whereas the intercepts were significantly different. The predicted regression lines based on the ANCOVA model are shown.

CONCLUSIONS—Several epidemiological studies (4–8,16) have reported a higher prevalence of diabetes, insulin resistance, and cardiovascular disease in various ethnic groups including Native Americans and Canadians. Some studies (9,10) have reported increased levels of inflammatory markers and their association with insulin resistance or percent body fat in Aboriginal versus white populations. However, there are no reports that specifically address markers of insulin resistance and inflammatory markers in Aboriginal women compared with white women.
First Nation women had significantly greater total fat mass than white women and a greater proportion of their adipose mass as trunk fat. Differences in trunk fat explained the difference in insulin resistance observed between First Nation and white women. While there was no significant difference in the slope of the relationship between log HOMA-IR and total body fat in the two groups of women, First Nation women had a significantly higher intercept, indicating that at any given level of total body fat, First Nation women were more significantly insulin resistant than white women.

Consistent with increased insulin resistance, First Nation women had lower adiponectin and IGFBP-1 but greater IL-6 levels than white women. However, the majority of inflammatory markers such as resistin, TNF-α, or CRP were not significantly increased in First Nation women and would not explain the difference in insulin sensitivity between the two study groups.

Although we recognize that dysglycemia and type 2 diabetes represent a continuum and that excluding subjects with type 2 diabetes might have biased our results against finding differences between the First Nation and control women, we chose to concentrate our analysis of insulin resistance on subjects who did not have self-reported diabetes or biochemical evidence of type 2 diabetes because the validity of the HOMA-IR determinations in type 2 diabetes patients is questionable (17,18).

In both First Nation and white women, as in other reports (19,20), leptin levels directly correlated with insulin resistance and body fat. However, despite significantly greater amounts of body fat in First Nation compared with white women, leptin levels were not significantly increased. This may reflect the relatively lower expression of leptin in visceral fat compared with subcutaneous fat (21) and the tendency for First Nation women to have a significantly larger proportion of their fat as trunk fat.

In this study, as previously observed (14), the adipokine that correlated best with insulin resistance was leptin. The magnitude of the correlation coefficient for HOMA-IR and leptin was comparable with HOMA-IR and IGFBP-1, a sensitive marker of insulin resistance (22), and was significantly higher than that observed for the relationship between HOMA-IR and adiponectin. While there was no difference between resistin levels in First Nation and white women, resistin levels did significantly correlate with both total fat and trunk fat in First Nation women but not in white women consistent with previous reports (14,23). In First Nation women alone, there was a weak positive correlation between HOMA-IR and resistin levels. This correlation was not seen in the white women and was lost when data were controlled for trunk fat, suggesting that this association was due to the correlation of both HOMA-IR and resistin levels with trunk fat in First Nation women. In contrast to the rodent, where resistin appears to be important in modulating hepatic insulin sensitivity (24,25), the contribution of resistin to insulin resistance in human subjects appears to be relatively minor. While a few studies have reported a weak positive association between insulin resistance and resistin levels (14,26,27), the majority of studies have not (23,28–33). Resistin levels in human subjects are thought to correlate more closely with inflammation than with insulin resistance (28,30,34).

RBP4 has recently been proposed to be an adipocytokine (35,36) and was elevated in insulin-resistant mice (36). These authors also found that plasma RBP4 levels determined by Western blot were elevated in a small sample of obese diabetic and nondiabetic subjects compared with lean subjects, but there was no difference between obese nondiabetic and obese diabetic subjects despite the latter being more insulin resistant (36). Other studies found RBP4 levels to correlate with poor metabolic control in both type 2 (37) and type 1 diabetic patients (38). These observations suggest that elevated RBP4 concentrations result from hyperglycemia, rather than insulin resistance, which is consistent with the lack of correlation between RBP4 levels and either fat mass or HOMA-IR in the present study.

TNF-α levels have been reported to be significantly elevated in insulin-resistant type 2 diabetic subjects (39,40). Although First Nation and control women had similar levels of TNF-α, a weak correlation was found between TNF-α and HOMA-IR but only in the First Nation individuals.

First Nation women had elevated IL-6 levels, but they did not have significantly higher CRP or TNF-α levels. This contrasts with previous reports where CRP levels have been found to be elevated in diabetic and insulin-resistant individuals (9,10,39,41). However, in the Diabetes Heart Study, there was no difference in CRP levels between type 2 diabetic subjects and their unaffected siblings (42), although a significant relationship was found between BMI and CRP in the same study as in the data reported here. Although First Nation women had elevated IL-6 levels, the correlation of IL-6 with HOMA-IR became insignificant after correction for trunk fat. This is consistent with the concept that truncal obesity precedes inflammation in the development of insulin resistance (43).

Significant limitations to this study include the cross-sectional design and

### Table 3—Spearman correlation coefficients between adipose tissue and adipokine levels in nondiabetic populations with and without correction for insulin resistance

<table>
<thead>
<tr>
<th></th>
<th>White women</th>
<th>First Nation women</th>
<th>All women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total fat</td>
<td>Trunk fat</td>
<td>Total fat</td>
</tr>
<tr>
<td>Resistin</td>
<td>0.162</td>
<td>0.146</td>
<td>0.203†‡</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-0.366***</td>
<td>-0.406***</td>
<td>-0.423†‡</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.781***</td>
<td>0.743***</td>
<td>0.782***</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.128</td>
<td>0.124</td>
<td>0.139§</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.528***</td>
<td>0.574***</td>
<td>0.513***</td>
</tr>
<tr>
<td>CRP</td>
<td>0.514***</td>
<td>0.533***</td>
<td>0.543***</td>
</tr>
<tr>
<td>RBP4</td>
<td>0.088</td>
<td>0.112</td>
<td>0.022</td>
</tr>
</tbody>
</table>

*P < 0.005, †P < 0.01, ‡P < 0.05. †Significant after controlling for HOMA-IR.
lack of prospectively defined clinical end points such as the development of diabetes. Although the sample sizes were adequate for the analyses undertaken, a much larger population would be required to assess cardiovascular events. Our finding may not be applicable to men since they were not the target of the First Nations Bone Health Study. We used an indirect measure of insulin resistance, HOMA-IR, since it is well suited to epidemiological studies and shows a satisfactory correlation with other quantitative measures such as the euglycemic–hyperinsulinemic glucose clamp (17). Correlations were adjusted for trunk fat and total fat, but no adjustment was made for other potentially important covariates such as physical activity. Although we excluded women with known or biochemical evidence of type 2 diabetes, this is unlikely to have excluded all women who were destined to develop diabetes in the future, particularly since women with impaired fasting glucose were included in the study.

In summary, our observations in First Nation women, a population that is at high risk for vascular disease, suggest that these women are more insulin resistant due in part to their tendency to accumulate adipose tissue in the truncal region. However, despite the increase in insulin resistance, we observed no significant increase in inflammatory cytokines, with the exception of IL-6. Although limited by cross-sectional nature, these data suggest that inflammation is unlikely to be the primary cause of the insulin resistance, as has been suggested by some investigators (44,45).

Acknowledgments—This research was supported by funds from the Health Science Centre Foundation, Canadian Institutes for Health Research, and the Manitoba Health Research Council. L.J.M is a recipient of the Henry G. Friesen Chair in Endocrine and Metabolic Research.

We thank the rest of the First Nations Bone Health Study Research Group: Dr. C.R. Greenberg, Dr. L. Lix, Dr. C.J. Metge, Dr. J.D. O’Neil, Dr. A. Tenenhouse, Dr. H.A. Weiler, Dr. M. Doupe, Dr. J. Krahn, Dr. L. Roos, Dr. E.A. Salamon, A. Walker Young, and P. Wood Steinman. The authors are indebted to Health Information Management of Manitoba Health and to the First Nations and Inuit Health Branch and Indian and Northern Affairs Canada for permission to use the Status Verification System, as well as to the Health Information Research Committee of the Assembly for Manitoba Chiefs for actively supporting this work.

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42. Silha and Associates
Retinopathy Predicts Cardiovascular Mortality in Type 2 Diabetic Men and Women

Auni Juutilainen, MD1
Seppo Lehto, MD1
Tapani Rönnemaa, MD2
Kalevi Pyörälä, MD1
Markku Laakso, MD1

OBJECTIVE — To investigate the association of retinopathy with the risk of all-cause, cardiovascular disease (CVD), and coronary heart disease (CHD) mortality in type 2 diabetic subjects in a population-based 18-year follow-up study with particular emphasis on sex differences.

RESEARCH DESIGN AND METHODS — Our study cohort comprised 425 Finnish type 2 diabetic men and 399 type 2 diabetic women who were free of CVD at baseline. The findings were classified based on standardized clinical ophthalmoscopy to categories of no retinopathy, background retinopathy, and proliferative retinopathy. The study end points were all-cause, CVD, and CHD mortality.

RESULTS — Adjusted Cox model hazard ratios (95% CIs) of all-cause, CVD, and CHD mortality in men were 1.34 (0.98–1.83), 1.30 (0.86–1.96), and 1.18 (0.74–1.89), respectively, for proliferative retinopathy and 2.92 (1.41–6.06), 3.17 (1.38–7.30), and 4.98 (2.06–12.06), respectively, for proliferative retinopathy and in women 1.61 (1.17–2.22), 1.71 (1.17–2.51), and 1.79 (1.13–2.85), respectively, for background retinopathy and 2.92 (1.41–6.06), 3.17 (1.38–7.30), and 4.98 (2.06–12.06), respectively, for proliferative retinopathy.

CONCLUSIONS — Proliferative retinopathy in both sexes and background retinopathy in women predicted all-cause, CVD, and CHD death. These associations were independent of current smoking, hypertension, total cholesterol, HDL cholesterol, glycemic control of diabetes, duration of diabetes, and proteinuria. This suggests the presence of common background pathways for diabetic microvascular and macrovascular disease other than those included in the conventional risk assessment of CVD. The sex difference observed in the association of background retinopathy with macrovascular disease warrants closer examination.

Hypertension is the major determinant of the risk of microvascular complications of diabetes (1), whereas the evidence that hyperglycemia is a major risk factor for macrovascular complications of this disease is more limited (2,3). Population-based studies have shown that microvascular complications predict cardiovascular disease (CVD) mortality not only in type 1 (3,4) and type 2 (5–10) diabetic subjects but even in non diabetic subjects (10) and in general population samples, controlling for the effect of glucose status (11–14). These observations suggest similar underlying pathogenic processes in microvascular complications and in atherosclerotic CVD in diabetes.

It has been suggested that microvascular processes might be especially important in the development of coronary heart disease (CHD) in women (11,13). However, epidemiological data are largely missing with respect to possible sex differences in the association of diabetic retinopathy with CVD. We have performed an 18-year follow-up study of 824 Finnish subjects with type 2 diabetes (425 men and 399 women) who were free of CVD at baseline to evaluate the predictive value of retinopathy for all-cause, CVD, and CHD mortality by sex.

RESEARCH DESIGN AND METHODS — A detailed description of study participants has been published previously (15). Altogether, 1,059 subjects (581 men and 478 women) with type 2 diabetes, aged 45–64 years, were identified through a national drug reimbursement register. Subjects with type 1 diabetes were excluded based on the age of onset of diabetes, history of ketoadi- osis, and, if needed, on glucagon-stimulated C-peptide measurement. Subjects with prior CVD (prior myocardial infarction, prior stroke, or prior lower-extremity amputation for vascular causes) were excluded. The diagnosis of previous myocardial infarction was based on the modified World Health Organization criteria for definite or possible myocardial infarction (16) and that of stroke on World Health Organization criteria for stroke (17). The final study population included 425 men and 399 women (n = 824) for whom the data of ophthalmoscopic examination at baseline were available.

Baseline study

The baseline examination, conducted in 1982–1984, has been described in detail previously (15). Subjects were classified as having hypertension if they were receiving drug treatment for hypertension or if systolic blood pressure was ≥160 mmHg or diastolic blood pressure was ≥95 mmHg in the sitting position after a 5-min rest.

Biochemical methods

All laboratory specimens were taken after a 12-h fast at 0800 h. The analyses were performed in duplicate except for glycated hemoglobin (A1). Serum total cholesterol and triglycerides were determined enzymatically (Boehringer).
HDL cholesterol was determined enzymatically after precipitation of LDL and VLDL with dextran sulfate-MgCl₂. Plasma glucose was determined with the glucose oxidase method (Boehringer). A1C was determined by affinity chromatography (Isolab). Plasma insulin concentration was determined by a commercial radioimmunoassay method (antisera M8170 and 8309; Novo, Copenhagen, Denmark). Serum creatinine was determined by kinetic Jaffe method using the Hitachi 705 analyzer (Tokyo, Japan). Total urinary protein concentration was measured from the morning spot urine specimen with the Coomassie brilliant blue method (Bio-Rad Laboratories, Hercules, CA) (18). Creatinine clearance was estimated by the Cockroft-Gault formula (19).

**Ophthalmoscopic examination and classification of retinopathic changes**

Ophthalmoscopic examination of fundi was performed after pharmacological dilatation of pupils at the baseline visit by two experienced diabetologists (M.L. and T.R.). For the purpose of this study, retinal findings were classified into three categories according to the status of the worse eye: no retinopathic changes, background retinopathy (microaneurysms, microinfarcts, hard exudates, or hemorrhages), and proliferative retinopathy (neovascularization or previous laser coagulation therapy). Because of poor visibility of fundi caused by cataract, 19 subjects were excluded from further analyses. Consistency of retinopathy findings between the two observers was ascertained by the examination of the fundi of 40 patients by both diabetologists. The κ-coefficient between the retinopathy categories determined by the two observers was 0.84.

**Follow-up study**

The follow-up period lasted until 1 January 2001. Copies of death certificates of deceased participants were obtained from the Cause-of-Death Register (Statistics Finland). In the final classification of causes of death, hospital and autopsy records were also used if available.

**Definition of end points**

The end points used in this study were all-cause mortality, CVD mortality (ICD-9 codes 390–459), and CHD mortality (ICD-9 codes 410–414).

---

**Table 1—Baseline characteristics according to the grade of retinopathy**

<table>
<thead>
<tr>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>No background retinopathy</td>
<td>No background retinopathy</td>
</tr>
<tr>
<td>Proliferative retinopathy</td>
<td>Proliferative retinopathy</td>
</tr>
<tr>
<td>Value for linear trend</td>
<td>Value for linear trend</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>28.7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.9</td>
</tr>
<tr>
<td>Area (% east)</td>
<td>40.4</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>6.3</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>87</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>151</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.8</td>
</tr>
<tr>
<td>Fasting insulin (mU/l)</td>
<td>21.1</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>10.9</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.3</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.3</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.3</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.3</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.3</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.3</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Note: Data are means ± SD, unless otherwise indicated. *The significance of the group difference is tested with logarithmic transformation. †Patients with insulin treatment excluded. ‡From the Cockroft-Gault formula.
Retinopathy predicts cardiovascular mortality

Table 2—Event rate per 1,000 person-years for all-cause, CVD, and CHD deaths in 425 men and 399 women with type 2 diabetes without prior CVD at baseline during 18 years of follow-up according to the grade of retinopathy

<table>
<thead>
<tr>
<th></th>
<th>No retinopathy</th>
<th>Background retinopathy</th>
<th>Proliferative retinopathy</th>
<th>P value for linear trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>634</td>
<td>163</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>All-cause mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>51.3</td>
<td>57.1</td>
<td>93.7</td>
<td>0.066</td>
</tr>
<tr>
<td>Women</td>
<td>49.7</td>
<td>71.1</td>
<td>126.3</td>
<td>0.005</td>
</tr>
<tr>
<td>All</td>
<td>50.5</td>
<td>63.9</td>
<td>103.3</td>
<td>0.001</td>
</tr>
<tr>
<td>CVD mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>32.1</td>
<td>32.2</td>
<td>64.4</td>
<td>0.341</td>
</tr>
<tr>
<td>Women</td>
<td>32.6</td>
<td>51.1</td>
<td>98.2</td>
<td>0.007</td>
</tr>
<tr>
<td>All</td>
<td>32.3</td>
<td>41.3</td>
<td>74.4</td>
<td>0.012</td>
</tr>
<tr>
<td>CHD mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>24.5</td>
<td>23.9</td>
<td>41.0</td>
<td>0.841</td>
</tr>
<tr>
<td>Women</td>
<td>20.8</td>
<td>35.5</td>
<td>98.2</td>
<td>0.001</td>
</tr>
<tr>
<td>All</td>
<td>22.7</td>
<td>29.5</td>
<td>57.8</td>
<td>0.019</td>
</tr>
</tbody>
</table>

Approval of the ethics committees
The ethics committees of the Kuopio University Hospital and the Turku University Central Hospital approved the study. All study subjects had given informed consent.

Statistical methods
Data analyses were conducted with the SPSS 11.5.1 program (SPSS, Chicago, IL). The results for continuous variables were given as means ± SD and for categorical variables as percentages. The differences of continuous variables between the three categories of retinopathy were analyzed by test of linearity included in ANOVA. χ² test for trend (linear-by-linear association) was used to test the linear trend for cases compared with noncases by the three categories of retinopathy. Event rates per 1,000 person-years were calculated. In all statistical analyses, logarithmic transformations were used for triglycerides, fasting insulin, and urinary protein to correct their skewed distribution. Cox models for overall versus no retinopathy, proliferative versus no retinopathy, and background versus no retinopathy were produced with two levels of adjustment. The limit for P value of statistical significance was considered 0.05, except for analyses of interaction (P < 0.10).

RESULTS — Baseline characteristics according to the grade of retinopathy are given in Table 1. Men with retinopathy smoked less, received insulin treatment more frequently, were leaner, had higher HDL cholesterol, had lower triglycerides, had higher urinary protein, had lower estimated creatinine clearance, had lower plasma insulin, had higher A1C, and had longer duration of diabetes than men without retinopathy. Women with retinopathy received insulin treatment more frequently, had higher systolic blood pressure, had higher level of urinary protein, had lower plasma insulin, and had longer duration of diabetes than women without retinopathy.

During 18 years of follow-up, 287 (67.5%) men and 271 (67.9%) women died, and of those who died, 177 (61.7%) men and 183 (67.5%) women died of CVD and 133 (46.3%) men and 122 (45.0%) women died of CHD. The event rates of all end points according to the grade of retinopathy are given in Table 2. In men there were 32.1, 32.2, and 64.4 deaths of CVD per 1,000 person-years in the presence of no retinopathy, background retinopathy, and proliferative retinopathy, respectively. Respective CVD death rates in women were 32.6, 51.1, and 98.2, respectively.

Figure 1 shows Kaplan-Meier curves for CVD mortality by the grade of retinopathy in men and women during the 18 years of follow-up. Background retinopathy had an impact on CVD mortality in women but not in men. The impact of proliferative retinopathy was similar in both sexes, but in women with proliferative retinopathy the risk of CVD death was already dramatically increased during the first half of the follow-up.

Table 3 shows Cox model hazard ratios with their 95% CIs and sex × retinopathy interaction of all-cause, CVD, and CHD mortality for overall, background, and proliferative retinopathy at two levels of adjustment. In model I, the adjustment is performed for age, sex (in the pooled analyses of men and women), and area of residence and in model II additionally for A1, current smoking, hypertension, total cholesterol, HDL cholesterol, duration of diabetes, and urinary protein (log). Background retinopathy predicted all-cause, CVD, and CHD mortality in women but not in men. Proliferative retinopathy predicted all-cause and CVD mortality in both sexes in both models and CHD mortality in both models in women but only in model II in men (Table 3).

Statistically significant sex × retinopathy interaction was observed for CVD death with respect to overall retinopathy and for all-cause, CVD, and CHD death with respect to background retinopathy. Proliferative retinopathy predicted mortality similarly in both sexes. Further adjustment for A1 did not influence the hazard ratios of CVD mortality for overall and background retinopathy in men or in women and decreased the hazard ratio of CVD mortality for proliferative retinopathy (by 13.4%) in women but not in men (data not shown). Also adding other risk factors into the adjustment (model II) increased the hazard ratios of overall retinopathy for all-cause, CVD, and CHD death by 21.1, 29.6, and 24.5%, respectively, in men but decreased these by 2.9, 5.8, and 10.5%, respectively, in women, compared with model I.

CONCLUSIONS — Our study showed that proliferative retinopathy predicted all-cause, CVD, and CHD death in both sexes of type 2 diabetic subjects who were free of CVD at baseline. Furthermore, overall and background retinopathy predicted all these categories of mortality in women, suggesting a sex difference in the effect of nonproliferative retinopathy on mortality. The association between retinopathy and mortality was independent not only of conventional CVD risk factors but also of glycemic control, duration of diabetes, and proteinuria. Thus, our results agree with the concept that similar underlying processes are responsible for micro- and macrovascular complications in diabetes.

In previous studies (20–22), hyperglycemia, duration of diabetes, elevated blood pressure, dyslipidemia, and obesity have been associated with the development and progression of diabetic retinopathy. Therefore, it could be expected that
these factors would be common underlying factors for retinopathy and atherosclerotic CVD. However, in our study, adjusting for these factors did not markedly influence the hazard ratios of mortality for retinopathy.

Several studies have been published on retinopathy as a predictor of CVD risk in type 2 diabetes (Table 4), but limited data exist with respect to the sex difference. A sex difference was observed in our study in the association of background retinopathy with all-cause, CVD, and CHD death, with a significant association in women but not in men. This accords with the findings of two large population-based cohort studies, one from the U.S. (13) and one from Australia (11). They have shown that retinal arteriolar narrowing is more strongly associated with risk of CHD in women than in men (23).

Diabetic retinopathy and atherosclerosis include pathophysiological similarities. Both processes include components of endothelial dysfunction, inflammation, neovascularization, apoptosis, and the hypercoagulable state (24). The neovascularization of the vessel wall has been found to be a consistent feature of the development of atherosclerotic plaque (25), and vasa vasorum neovascularization precedes endothelial dysfunction (26). Endothelial dysfunction could be a feature linking retinopathy and large-vessel disease. However, in the Hoorn Study applying the method of flow-mediated vasodilatation, endothelial dysfunction–related mechanisms were not clearly associated with retinopathy (27). In the development of retinopathy, vascular endothelial growth factor acts as a primary regulator, and retinal hypoxia and hyperglycemia interact as promoting factors, with possible roles of IGF, transforming growth factor, tumor necrosis factor-α, and epidermal growth factor (28), as well as cyclooxygenase-2 and nitric oxide (29). Inflammation may be important in the pathogenesis of both macrovascular (30–34) and microvascular disease (35–37).

Elegant studies of Brownlee et al. (38) have shown that a single unifying process of diabetes complications is hyperglycemia-induced overproduction of superoxide by the mitochondrial electron transport chain. Mitochondrial overproduction of superoxide activates four damaging pathways: polyol pathway, hexosamine pathway, protein kinase C pathway, and advanced glycation end products formation. There is no doubt that these pathways lead to microvascular complications. However, in addition to hyperglycemia, other risk factors are operative in the development of macrovascular complications, among them “conventional risk factors” and insulin resistance. Insulin resistance is a characteristic finding in type 2 diabetes, but long-lasting hyperglycemia also induces insulin resistance in type 1 diabetes. High circulating free fatty acid levels induce mitochondrial overproduction of reactive oxygen species and activate protein kinase C pathway, and advanced glycation end products formation. There is no doubt that these pathways lead to microvascular complications. However, in addition to hyperglycemia, other risk factors are operative in the development of macrovascular complications, among them “conventional risk factors” and insulin resistance. Insulin resistance is a characteristic finding in type 2 diabetes, but long-lasting hyperglycemia also induces insulin resistance in type 1 diabetes. High circulating free fatty acid levels induce mitochondrial overproduction of reactive oxygen species and activate protein kinase C pathway, which leads to the formation of advanced glycation end products.

The major limitation of our study is...
Table 3—Cox model for all-cause, CVD, and CHD mortality in 425 type 2 diabetic men and 399 type 2 diabetic women without prior CVD at baseline with overall, background, and proliferative retinopathy compared with subjects with no retinopathy

<table>
<thead>
<tr>
<th></th>
<th>Overall versus no retinopathy</th>
<th>Background versus no retinopathy</th>
<th>P value for interaction sex × retinopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio (95% CI)</td>
<td>P value</td>
<td>Hazard ratio (95% CI)</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.23 (0.94–1.61)</td>
<td>0.127</td>
<td>1.09 (0.81–1.47)</td>
</tr>
<tr>
<td>Women</td>
<td>1.71 (1.31–2.25)</td>
<td>&lt;0.001</td>
<td>1.63 (1.23–2.17)</td>
</tr>
<tr>
<td>All</td>
<td>1.44 (1.19–1.74)</td>
<td>&lt;0.001</td>
<td>1.32 (1.08–1.62)</td>
</tr>
<tr>
<td>Model II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.49 (1.11–1.99)</td>
<td>0.007</td>
<td>1.34 (0.98–1.83)</td>
</tr>
<tr>
<td>Women</td>
<td>1.66 (1.22–2.26)</td>
<td>0.001</td>
<td>1.61 (1.17–2.22)</td>
</tr>
<tr>
<td>All</td>
<td>1.58 (1.28–1.95)</td>
<td>&lt;0.001</td>
<td>1.48 (1.19–1.84)</td>
</tr>
<tr>
<td>CVD mortality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.15 (0.81–1.62)</td>
<td>0.443</td>
<td>0.97 (0.65–1.43)</td>
</tr>
<tr>
<td>Women</td>
<td>1.91 (1.38–2.63)</td>
<td>&lt;0.001</td>
<td>1.80 (1.28–2.52)</td>
</tr>
<tr>
<td>All</td>
<td>1.49 (1.18–1.88)</td>
<td>0.001</td>
<td>1.34 (1.04–1.73)</td>
</tr>
<tr>
<td>Model II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.49 (1.03–2.17)</td>
<td>0.036</td>
<td>1.30 (0.86–1.96)</td>
</tr>
<tr>
<td>Women</td>
<td>1.80 (1.24–2.59)</td>
<td>0.002</td>
<td>1.71 (1.17–2.51)</td>
</tr>
<tr>
<td>All</td>
<td>1.65 (1.28–2.14)</td>
<td>&lt;0.001</td>
<td>1.52 (1.15–1.99)</td>
</tr>
<tr>
<td>CHD mortality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.06 (0.71–1.60)</td>
<td>0.771</td>
<td>0.94 (0.60–1.48)</td>
</tr>
<tr>
<td>Women</td>
<td>2.19 (1.49–3.22)</td>
<td>&lt;0.001</td>
<td>1.96 (1.30–2.96)</td>
</tr>
<tr>
<td>All</td>
<td>1.51 (1.15–2.00)</td>
<td>0.003</td>
<td>1.34 (0.99–1.81)</td>
</tr>
<tr>
<td>Model II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.32 (0.85–2.04)</td>
<td>0.220</td>
<td>1.18 (0.74–1.89)</td>
</tr>
<tr>
<td>Women</td>
<td>1.96 (1.27–3.04)</td>
<td>0.003</td>
<td>1.79 (1.13–2.85)</td>
</tr>
<tr>
<td>All</td>
<td>1.63 (1.20–2.20)</td>
<td>0.002</td>
<td>1.47 (1.06–2.03)</td>
</tr>
</tbody>
</table>

Two levels of adjustment are used (model I: age, sex [in the analysis for all], and area of residence; model II: model I + A1, current smoking, hypertension, total cholesterol, HDL cholesterol, duration of diabetes, and urinary protein [log]). Bold data indicate significant interaction at P < 0.10.
that the evaluation of retinopathy was based on fundoscopy. Although the \( \kappa \)-coefficient between the retinopathy categories determined by the two observers was high (0.84), it is possible that subtle changes may have been missed. On the other hand, the findings of our study were consistent with other studies evaluating retinopathy with more sophisticated techniques (Table 4). Moreover, if subtle retinopathy changes would have remained unnoticed, this would have weakened rather than strengthened our findings.

In conclusion, in type 2 diabetes proliferative retinopathy in men and background, proliferative, and overall retinopathy in women predicted all-cause, CVD, and CHD death. These associations were independent of conventional CVD risk factors, glycemic control, duration of diabetes, and proteinuria. Thus, it is likely that retinopathy indicates the presence of such common factors in the pathophysiology of diabetic microvascular and macrovascular disease that are not included in the conventional risk factor assessment of CVD. The sex difference observed in the association of background retinopathy with macrovascular disease warrants closer examination.

### Table 4—Studies on retinopathy predicting CVD in type 2 diabetes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study subjects</th>
<th>Follow-up, study end points</th>
<th>Relative risk (95% CI)</th>
<th>Adjusting factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miettinen et al. (8)</td>
<td>1,040 Finnish type 2 diabetic subjects</td>
<td>7-year follow-up of CHD events</td>
<td>Background 1.38 (0.95–2.00); proliferative 2.12 (1.02–4.39)</td>
<td>Age, area, sex, total cholesterol, HDL cholesterol, triglycerides, smoking, hypertension, urinary protein, A1C</td>
</tr>
<tr>
<td>Klein et al. (39)</td>
<td>The Wisconsin Epidemiologic Study of Diabetic Retinopathy: 1,370 subjects with age of onset of diabetes &gt;30 years</td>
<td>16-year follow-up of all-cause, CHD, and stroke mortality</td>
<td>All-cause mortality: mild nonproliferative 1.34 (1.29–1.71) and proliferative 1.89 (1.43–2.50); CHD mortality: mild nonproliferative 1.21 (0.95–1.53) and proliferative 1.43 (0.94–2.17); stroke mortality: mild nonproliferative 1.30 (0.92–1.85) and proliferative 1.88 (1.03–3.43)</td>
<td>Age, sex, duration of diabetes, A1C, systolic blood pressure, prior CVD, smoking (pack-years), diuretic use</td>
</tr>
<tr>
<td>Fuller et al. (5)</td>
<td>The World Health Organization Multinational Study of Vascular Disease in Diabetes: 1,390 type 2 diabetic subjects</td>
<td>12-year follow-up of CVD mortality</td>
<td>1.2 (0.8–1.8) in men and 2.7 (1.8–4.1) in women</td>
<td>Age, duration of diabetes, systolic blood pressure, cholesterol, smoking, proteinuria, electrocardiographic abnormalities, glucose</td>
</tr>
<tr>
<td>van Hecke et al. (4)</td>
<td>The Hoorn Study: 631 nondiabetic and diabetic subjects</td>
<td>10.7-year follow-up (median) of all-cause and CVD mortality</td>
<td>All-cause mortality in diabetic subjects 2.05 (1.23–3.44); CVD mortality in diabetic subjects 2.20 (1.03–4.70)</td>
<td>Age and sex</td>
</tr>
<tr>
<td>Cusick et al. (40)</td>
<td>The Early Treatment Diabetic Retinopathy Study (ETDRS): 2,267 type 2 diabetic subjects</td>
<td>5-year follow-up of all-cause mortality</td>
<td>Moderate nonproliferative 1.27 (0.94–1.72); severe nonproliferative 1.48 (1.03–2.15); mild proliferative 1.28 (0.80–2.06); moderate/high proliferative 2.02 (1.28–3.19)</td>
<td>Age, sex, BMI, A1C, total cholesterol, triglycerides, fibrinogen, cigarette smoking, daily insulin use, the use of antihypertensive medications, other baseline diabetes complications</td>
</tr>
<tr>
<td>Targher et al. (9)</td>
<td>The Valpolicella Heart Study: 248 type 2 diabetic subjects who developed CVD during follow-up and 496 type 2 diabetic control subjects</td>
<td>5-year follow-up of CVD events</td>
<td>Nonproliferative 1.8 (1.2–2.3); proliferative 4.1 (2.0–8.9)</td>
<td>Age, sex, BMI, smoking history, plasma lipids, A1C, diabetes duration, diabetes treatment</td>
</tr>
</tbody>
</table>
Retinopathy predicts cardiovascular mortality

References


16. McLeod DS, Lefer DJ, Merges C, Luty


How Reliable Is Estimation of Glomerular Filtration Rate at Diagnosis of Type 2 Diabetes?

OBJECTIVE — The Cockcroft-Gault (CG) and Modification of Diet in Renal Disease (MDRD) equations previously have been recommended to estimate glomerular filtration rate (GFR). We compared both estimates with true GFR, measured by the isotopic $^{51}$Cr-EDTA method, in newly diagnosed, treatment-naive subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS — A total of 292 mainly normoalbuminuric (241 of 292) subjects were recruited. Subjects were classified as having mild renal impairment (group 1, GFR <90 ml/min per 1.73 m$^2$) or normal renal function (group 2, GFR ≥90 ml/min per 1.73 m$^2$). Estimated GFR (eGFR) was calculated by the CG and MDRD equations. Blood samples drawn at 44, 120, 180, and 240 min after administration of 1 MBq of $^{51}$Cr-EDTA were used to measure isotopic GFR (iGFR).

RESULTS — For subjects in group 1, mean (±SD) iGFR was 83.8 ± 4.3 ml/min per 1.73 m$^2$. eGFR was 78.0 ± 16.5 or 73.7 ± 12.0 ml/min per 1.73 m$^2$ using CG and MDRD equations, respectively. Ninety-five percent CIs for method bias were –1.1 to –0.6 using CG and –14.4 to –7.0 using MDRD. Ninety-five percent limits of agreement (mean bias ± 2 SD) were –37.2 to 25.6 and –33.1 to 11.7, respectively. In group 2, iGFR was 119.4 ± 20.3 ml/min per 1.73 m$^2$. eGFR was 104.4 ± 26.3 or 92.3 ± 18.7 ml/min per 1.73 m$^2$ using CG and MDRD equations, respectively. Ninety-five percent CIs for method bias were –17.4 to –12.5 using CG and –29.1 to –25.1 using MDRD. Ninety-five percent limits of agreement were –54.4 to 24.4 and –59.5 to 5.3, respectively.

CONCLUSIONS — In newly diagnosed type 2 diabetic patients, particularly those with a GFR ≥90 ml/min per 1.73 m$^2$, both CG and MDRD equations significantly underestimate iGFR. This highlights a limitation in the use of eGFR in the majority of diabetic subjects outside the setting of chronic kidney disease.
nine, age, sex, and ethnic origin, is being widely advocated (22,24).

eGFR values derived by the MDRD and the traditional Cockcroft-Gault (CG) equations have been validated in CKD (25–30); however, there is concern that they underestimate GFR in the vast majority of individuals with normal or near-normal renal function (31,32). Recent work by Parving and colleagues (33) in type 2 diabetic subjects with incipient and established nephropathy found the performance of eGFR to be unacceptable for monitoring kidney function in type 2 diabetic patients. Our study was designed to explore the relationship between the CG- and MDRD-derived eGFR and the reference $^{51}$Cr-EDTA isotopically measured GFR in newly diagnosed, treatment-naïve subjects with type 2 diabetes.

**RESEARCH DESIGN AND METHODS** — Subjects for this study were recruited from a long-term, local ethics committee–approved follow-up study of type 2 diabetic subjects performed at the University Hospital of Wales, Heath Park, Cardiff, U.K., and Llandough Hospital, Cardiff, U.K. The study population consisted of 292 newly diagnosed, treatment-naïve type 2 diabetic subjects recruited between 1996 and 2005 who had a reference isotopic 51Cr-EDTA GFR (iGFR) measurement in our hospital was validated at the Welsh External Quality Assurance Scheme.

**Clinical methods**

At 0830 h, following an overnight fast, a urine specimen was collected, anthropometric measurements taken, and blood pressure measured in the recumbent position after 10 min rest. Subjects were intravenously cannulated and blood samples drawn for blood glucose, A1C, and serum creatinine. Subsequently, a single intravenous injection containing 1 MBq $^{51}$Cr-EDTA was administered at 0 min, with further blood sampling at 44, 120, 180, and 240 min. Blood was collected into heparinized tubes and centrifuged at 4°C.

**Laboratory methods**

The reference iGFR was obtained by a single-injection plasma clearance method corrected for body surface area (BSA). The simplified $^{51}$Cr-EDTA clearance method used has been validated against plasma clearance determined by multiple sampling (36). The four-sample method used allowed estimation using a two-compartment model. A close correlation between total plasma clearance of $^{51}$Cr-EDTA and insulin clearance determined by the classical technique has been shown previously (37).

Serum creatinine levels were determined using the OCD (J&J) dry-slide system. The Vitros 750 × RC and 950 analyzer. The laboratory reported that the coefficient of variation of the assay was 4.2% at a creatinine concentration of 103 μmol/l and 1.92% at a creatinine concentration of 516 μmol/l. Creatinine measurement in our hospital was validated at intervals by measurement of samples from the Welsh External Quality Assurance Scheme.

**Estimation of GFR**

To estimate GFR, the CG formula for creatinine clearance corrected for BSA and the four-variable abbreviated MDRD formula, as recommended for use in the U.K. CKD guidelines (24), were used. The formulas are as follows: 1) CG formula (27): eGFR (ml/min per 1.73 m²) = [140 – age (years)] × weight (kg) / (height (cm)1.23 × [serum creatinine (μmol/l)], where k is 1.23 for men and 1.04 for women and c adjusts for BSA (33). c = 1.73/BSA, with BSA calculated using the following DuBois (38) formula: BSA (m²) = [weight (kg)]0.425 × [height (cm)]0.725 × 0.007874; and 2) the abbreviated four-variable MDRD formula (23): eGFR (ml/min per 1.73 m²) = 186 × [serum creatinine (μmol/l)/88.4] –1.154 × [age (years)] –0.203 × (0.742 if female) × (1.210 if African American).

**Statistical analysis**

Data were assessed graphically for serial correlation. Subjects were grouped by iGFR, with group 1 having iGFR 60–89 ml/min per 1.73 m² and group 2 having iGFR ≥90 ml/min per 1.73 m². eGFR results derived by the CG and MDRD formulas were compared with iGFR by means of two-tailed, paired t tests (confirmed by nonparametric equivalents for nonnormal distributions). χ² test for proportions, linear regression, and the κ statistic for rater agreement. Regression goodness of fit and other statistical method assumptions were checked graphically and by use of relevant statistics as appropriate. All calculations were performed using SPSS (version 12.0.1; SPSS) and S-PLUS (version 7.0; Insightful) software packages. Results are presented as means ± SD (95% CI), unless otherwise indicated. P < 0.05 was taken to indicate statistical significance. Sample size calculations indicated that the study had at least 80% power to detect a mean difference in GFR of 5 ml/min per 1.73 m².

**RESULTS** — The demographic characteristics of the 292 study participants are summarized in Table 1. Study subjects were largely normoalbuminuric (241 of 292 [83%]). Table 1 shows that subjects in group 1 had a higher mean age than those in group 2. There was no significant difference in weight or BMI between the groups. However, consistent with a lower iGFR, mean serum creatinine concentration was greater in group 1 than group 2.

The performance of the CG and MDRD formula–derived eGFR to estimate iGFR is presented in Table 2. Performance was assessed by use of bias (mean difference between eGFR and iGFR), precision (SD of the bias), accuracy (proportion of eGFR results within 10 and 30% of

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Table 1—Demographic data

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th>Group 1 (eGFR &lt;90)</th>
<th>Group 2 (eGFR ≥90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>292</td>
<td>37</td>
<td>255</td>
</tr>
<tr>
<td>Men/women</td>
<td>219/73</td>
<td>25/12</td>
<td>194/61</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.3 ± 9.4</td>
<td>62.8 ± 6.4</td>
<td>54.2 ± 9.3*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>92.0 ± 17</td>
<td>88.0 ± 16.7</td>
<td>92.5 ± 17.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.5 ± 5.6</td>
<td>30.9 ± 6.0</td>
<td>31.6 ± 5.5</td>
</tr>
<tr>
<td>A1C (%)</td>
<td>7.79 ± 2.00</td>
<td>7.16 ± 1.83</td>
<td>7.88 ± 2.01*</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>9.70 ± 3.09</td>
<td>8.44 ± 2.49</td>
<td>9.87 ± 3.13*</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>79.9 ± 14.8</td>
<td>90.7 ± 16.1</td>
<td>78.4 ± 14.0*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>136 ± 20</td>
<td>144 ± 22</td>
<td>135 ± 19*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81 ± 10</td>
<td>80 ± 10</td>
<td>82 ± 10</td>
</tr>
</tbody>
</table>

Data are means ± SD. *P < 0.05 for difference between means of group 1 and group 2.
Table 2—Predictive performance of the CG and the MDRD formulae

<table>
<thead>
<tr>
<th>Formula</th>
<th>CG</th>
<th>MDRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR [median (interquartile range)]</td>
<td>101.1 (26.8)</td>
<td>89.9 (19.0)</td>
</tr>
<tr>
<td>Bias</td>
<td>-13.8 (-16.1 to -11.6)</td>
<td>-25.0 (-26.9 to -23.1)</td>
</tr>
<tr>
<td>Precision</td>
<td>19.5</td>
<td>16.6</td>
</tr>
<tr>
<td>95% limits of agreement</td>
<td>(-52.8 to 25.2)</td>
<td>(-58.2 to 8.2)</td>
</tr>
<tr>
<td>Accuracy 10%</td>
<td>29% (24–35)</td>
<td>15% (11–19)</td>
</tr>
<tr>
<td>Accuracy 30%</td>
<td>86% (81–89)</td>
<td>79% (74–83)</td>
</tr>
<tr>
<td>R²</td>
<td>0.490</td>
<td>0.476</td>
</tr>
<tr>
<td>Gradient</td>
<td>0.99 (0.52–0.65)</td>
<td>0.81 (0.71–0.91)</td>
</tr>
<tr>
<td>Intercept</td>
<td>55.7 (48.5–63.0)</td>
<td>41.9 (32.9–51.0)</td>
</tr>
</tbody>
</table>

Data are means ± SD (95% CI), unless otherwise indicated. All R values were positive and, with one exception*, significantly different from zero.

CONCLUSIONS

Formula-derived eGFR results have become widely used in clinical practice. The CG and MDRD equations have been validated in patients with CKD and are currently used to stratify CKD in Europe and North America (24,40,41). However, these equations do not measure GFR levels as well as eGFR, and are currently used to estimate GFR in clinical practice. The CG and MDRD equations have a tendency to significantly underestimate higher levels of GFR (26,31,33,42). Additionally, Parving and colleagues (33) demonstrated that in type 2 diabetic subjects with macroalbuminuria eGFR had a poor sensitivity to detect GFR values <60 ml/min per 1.73 m². In our study of newly diagnosed, treatment-naive type 2 diabetic subjects, statistically significant correlations between formula-derived eGFR and iGFR were observed. However, these correlations were weaker for the lower CG formula. These results suggest that the CG formula may not be as good as the MDRD formula in predicting GFR in type 2 diabetic subjects.
min per 1.73 m². These findings echo and extend those of Parving and colleagues (33) who studied type 2 diabetic subjects with incipient or overt nephropathy.

Both formulas lacked precision, with wide prediction intervals for iGFR based on eGFR as illustrated in Fig. 1. Overall, the performance was particularly poor for subjects with iGFR in the range of 60–89 ml/min per 1.73 m². While the difficulty in interpreting eGFR values has been demonstrated in other patient groups (33,44,45), this is the first large study to show this in newly diagnosed, treatment-naive and mainly normoalbuminuric type 2 diabetic subjects.

The eGFR formulae were designed for application in patients with GFR <60 ml/min per 1.73 m² (27,28). They also have been shown to be reliable in type 2 diabetic patients with severe renal impairment (46). However, in the U.K. the National Service Framework for Renal Services now recommends the use of eGFR for renal assessment in all diabetic patients (22). This may be problematic, as this is a different population from which the equations were derived. In our study, there was little agreement above chance between the eGFR-derived stage of CKD and that derived using iGFR, as reflected by the low \( \kappa \) scores.

Diabetic patients, although at higher risk of CKD than the normal population, generally have normal renal function. According to our results, use of these equations in isolation as a screening tool will lead to an overestimation of the number of diabetic patients with renal impairment. From our study, 14% of patients with GFR between 60 and 89 ml/min per 1.73 m² would be classed as stage 3 U.K. CKD with either formula. In isolation this would be misleading considering the low sensitivity of these tests to detect impaired renal function. Parving and colleagues (33) demonstrated only 72% sensitivity for the MDRD formula and 66% for the CG formula to detect GFR values <60 ml/min per 1.73 m².

Assessment of serum creatinine clearly plays a significant role in the eGFR formulae. There are reports (47–49) of the impact of variation in calibration of the creatinine assay having an adverse impact on the performance of eGFR to estimate GFR, particularly at low levels of serum creatinine (50). Creatinine assays can be calibrated by gas chromatography-isotope dilution mass spectrometry to give a gold standard creatinine value. In the U.S., the National Kidney Education Program has initiated a standardization program to minimize this variation of creatinine measurement (50), and the program is expected to be complete by 2008. Despite being more precise and accurate, these methods give serum creatinine values, which are lower compared with the widely used modified Jaffe method (50). Use of the standardized values would then give higher GFR estimates. This has led to the MDRD equation being reexpressed in 2005 for use with a standardized serum creatinine assay (51). The authors of the updated equation recommend that the original four-variable MDRD equation should continue to be used until the standardization of creatinine assays is complete (49). During the course of our study, creatinine measurements were standardized by measurement of common samples in the Welsh External Quality Assurance Scheme but were not calibrated to the MDRD laboratory. This is a potential limitation of our study; however, until standardization is widespread our results reflect current clinical practice.

Due to the recognized deficiencies of serum creatinine to detect mild renal impairment, even when used with prediction equations (26,31,33,42,43), there is interest in cystatin C, a nonglycosylated basic protein, as a potential endogenous filtration marker of GFR. There is supportive evidence that the reciprocal of cystatin C correlates more closely with isotopic GFR than the CG or MDRD equa-

![Figure 1](image)

**Figure 1**—Relation of CG- and MDRD-calculated eGFR with \( ^{51} \)Cr-EDTA–measured iGFR. The solid line represents the regression line, and the dotted lines represent 95% prediction intervals for iGFR based on eGFR.

**Table 3**—U.K. CKD staging of subjects in groups 1 and 2

<table>
<thead>
<tr>
<th>U.K. CKD</th>
<th>CG eGFR( \kappa = 0.24 )</th>
<th>MDRD eGFR( \kappa = 0.15 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>stage 3 (GFR &lt;60)</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>stage 2 (GFR 60–89)</td>
<td>100</td>
<td>156</td>
</tr>
<tr>
<td>stage 1 (GFR ≥90)</td>
<td>186</td>
<td>129</td>
</tr>
</tbody>
</table>

| Group 1 (GFR 60–89; n = 37) | 5/37 (14) | 5/37 (14) |
| Group 2 (GFR ≥90; n = 255) | 1/255 (1) | 2/255 (1) |

Data are n (%).
Glomerular filtration rate and type 2 diabetes

tions in subjects with mild renal impairment (52). However, concerns remain regarding inpatient variation and the effect of certain drugs and hormonal levels on cystatin C concentration (53). While it remains an interesting potential tool for clinical use, substantially more work is needed in different patient subgroups before cystatin C can be considered as an alternative to serum creatinine.

In summary, for patients with diabetes and preserved renal function we recommend that eGFR results not be considered in isolation but together with other indicators of CKD such as microalbuminuria, proteinuria, hematuria, or changes in creatinine concentration. It may be appropriate only to quantify eGFR results <60 ml/min per 1.73 m², since U.K. CKD stage 3 CKD has increased clinical significance, necessitating additional intervention. Unfortunately, even this is problematic due to the over diagnosis of CKD stage 3 by eGFR demonstrated in this study.

eGFR is being widely, but not universally, used in patients with and without CKD. The U.K. CKD guidelines advocate the four-variable MDRD equation because it is most accurate in CKD and most easily applicable in clinical practice. However, the current study highlights that while eGFR maybe useful in the assessment of CKD it does have significant limitations outside of this setting.

References

28. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D: A more accurate method of estimate glomerular filtration rate from serum creatinine: a new prediction equation: Modification of Diet in Re-
Effect of Periodontitis on Overt Nephropathy and End-Stage Renal Disease in Type 2 Diabetes

Wendy A. Shultis, PhD1
E. Jennifer Weil, MD1
Helen C. Looker, MBBS1
Jeffrey M. Curtis, MD, MPH1
Marc Shlossman, DDS, MS2,3
Robert J. Genco, DDS, PhD2
William C. Knowler, MD, DRPH1
Robert G. Nelson, MD, PhD1

OBJECTIVE — The purpose of this study was to investigate the effect of periodontitis on development of overt nephropathy, defined as macroalbuminuria, and end-stage renal disease (ESRD) in type 2 diabetes.

RESEARCH DESIGN AND METHODS — Individuals residing in the Gila River Indian Community aged ≥25 years with type 2 diabetes, one or more periodontal examination, estimated glomerular filtration rate ≥60 ml/min per 1.73 m², and no macroalbuminuria (urinary albumin-to-creatinine ratio ≥300 mg/g) were identified. Periodontitis was classified as none/mild, moderate, severe, or edentulous using number of teeth and alveolar bone score. Subjects were followed to development of macroalbuminuria or ESRD, defined as onset of renal replacement therapy or death attributed to diabetic nephropathy.

RESULTS — Of the 529 individuals, 107 (20%) had none/mild periodontitis, 200 (38%) had moderate periodontitis, 117 (22%) had severe periodontitis, and 105 (20%) were edentulous at baseline. During follow-up of up to 22 years, 193 individuals developed macroalbuminuria and 68 developed ESRD. Age- and sex-adjusted incidence of macroalbuminuria and ESRD increased with severity of periodontitis. After adjustment for age, sex, diabetes duration, BMI, and smoking in a proportional hazards model, the incidences of macroalbuminuria were 2.0, 2.1, and 2.6 times as high in individuals with moderate or severe periodontitis or those who were edentulous, respectively, compared with those with none/mild periodontitis (P = 0.01). Incidences of ESRD in individuals with moderate or severe periodontitis or in those who were edentulous were 2.3, 3.5, and 4.9 times as high, respectively, compared with those with none/mild periodontitis (P = 0.02).

CONCLUSIONS — Periodontitis predicts development of overt nephropathy and ESRD in individuals with type 2 diabetes. Whether treatment of periodontitis will reduce the risk of diabetic kidney disease remains to be determined.


Periodontitis is a common infection of the periodontal tissues (1) and a major cause of tooth loss in adults (2,3). The most prevalent form is chronic periodontitis (1). Individuals with diabetes are at increased risk of developing periodontitis, and, once established, periodontitis is more severe in people with diabetes (3–6). Poor glycemic control hastens the progression of periodontitis and, in turn, periodontitis appears to further impair glycemic control (6–8).

Periodontitis predicts cardiovascular disease risk (9) and deaths from diabetic kidney disease and cardiovascular disease in individuals with type 2 diabetes (10). However, the relationship between periodontitis and diabetic kidney disease has not been fully explored. Among nonobese individuals with type 2 diabetes in Japan, IgG titers for Porphyromonas gingivalis (a periodontal pathogen) were correlated with the urinary albumin-to-creatinine ratio (ACR), although analyses were not adjusted for confounding factors (11). In the Atherosclerosis Risk in Communities (ARIC) Study, a largely nondiabetic population, periodontitis was associated with a low estimated glomerular filtration rate (GFR) (<60 ml/min per 1.73 m²), and this finding remained after adjustment for the presence of diabetes and other potential confounding variables (12).

In the present study, we examined the effect of periodontitis on the development of overt nephropathy, defined by macroalbuminuria (ACR ≥300 mg/g), and end-stage renal disease (ESRD) in type 2 diabetes in an American-Indian population.

RESEARCH DESIGN AND METHODS — Data were collected as part of a longitudinal study of diabetes and its complications in the Gila River Indian Community of Arizona (4). The diabetes study was initiated in 1965, and members of the community are invited to attend a research clinic for examination and screening every 2 years. Ethical approval was obtained from the institutional review boards of the National Institute of Diabetes and Digestive and Kidney Diseases and from the Gila River Indian Council.

This analysis includes data from all individuals with diabetes whose heritage was at least half Pima or Tohono O’odham and who had one or more periodontal examinations after 25 years of age at which baseline ACR was <300 mg/g and baseline estimated GFR was ≥60 ml/min per 1.73 m². Diabetes was diagnosed according to World Health Organization criteria (13) by a 2-h postload plasma glucose concentration ≥200 mg/dl or from clinical records. Urinary albumin was measured by nephelometric immunnoassay (14). Urinary and serum creatinine were
measured by a modification of the Jaffe reaction (15). Serum creatinine values were calibrated to the Modification of Diet in Renal Disease (MDRD) Study laboratory, and these values were used to estimate GFR with the four-variable MDRD equation, categorizing individuals as of nonblack ethnicity (16). The correlation between MDRD-estimated GFR and iothalamate GFR in this American-Indian population from preliminary validation work is 0.80 (n = 201) (17). The cutoff of 60 ml/min per 1.73 m² GFR was chosen on the basis of the guidelines of the National Kidney Foundation to exclude individuals with preexisting impaired GFR (16). ESRD was defined as the requirement for renal replacement therapy due to diabetes or death from diabetic nephropathy. Data on ESRD were collected independently of the biennial research examinations and were complete to 31 December 2002.

Periodontal examinations were conducted between 1983 and 1990. Panoramic radiographs were evaluated and scored by a single examiner (M.S.). Alveolar bone loss was determined by scoring percent bone loss from the cementoenamel junction to the apex at the deepest point on the mesial or distal surfaces of each tooth present, excluding third molars. Periodontitis was classified in order of severity by the number of missing teeth and percent alveolar bone loss as 1) none/mild periodontitis, defined as ≥24 teeth, of which <6 had 25–49% bone loss and none had ≥50% bone loss; 2) moderate periodontitis, defined as ≥15 teeth, of which <7 had 50–74% bone loss and <4 had ≥75% bone loss; 3) severe periodontitis, defined as 1–14 teeth or greater bone loss than in previous categories; and 4) edentulous. In previous reports, 72% of the tooth loss in this population is attributable to periodontitis (3), and, as such, missing teeth were considered to be lost due to periodontitis, and edentulous individuals were considered to have the most severe manifestation of periodontitis. Although our definition of periodontitis does not permit us to differentiate between aggressive and chronic periodontitis, the majority of cases are likely to represent chronic periodontitis (1).

HbA1c was measured by agar gel electrophoresis until 1989, after which A1C was measured by high-performance liquid chromatography. A1C values were estimated from HbA1 values using the equation A1C = (0.99 × HbA1c) – 1.535 (18). BMI was calculated as weight in kilograms divided by the square of height in meters, and obesity was defined by a BMI ≥30 kg/m². Smoking status was self-reported as nonsmoking (<100 cigarettes in a lifetime), prior smoking (have not smoked for past year), currently smoking ≤1 pack/day, and currently smoking >1 pack/day. Only five individuals reported currently smoking >1 pack/day, and smoking was considered as current smoking “yes” or “no” for analysis. Blood pressure was measured at the first and fourth Korotkoff sounds with the subject in the supine position. Mean arterial pressure was calculated as two-thirds diastolic blood pressure + one-third systolic blood pressure. Hypertension was defined by diastolic blood pressure ≥80 mmHg, systolic blood pressure ≥130 mmHg, or current usage of antihypertensive medicine (19).

**Statistical analysis**

Characteristics of the individuals across categories of periodontitis were explored using the nonparametric Kruskal-Wallis test for continuous variables and the Mantel extension test for categorical variables. Incidence rates were computed as the number of new occurrences of macroalbuminuria or ESRD per 1,000 person-years at risk by age and sex and by periodontitis status, standardized to the 1980 community population. If subjects changed periodontitis strata during follow-up, their person-time was apportioned to the appropriate stratum. Follow-up accumulated only while individuals resided within the community and extended from the date of the first diabetes research examination after the age of 25 years that included a periodontal examination to development of macroalbuminuria or ESRD. For individuals who did not develop either outcome, follow-up time was censored: in the case of the macroalbuminuria analysis at the date of the last research examination and for the ESRD analysis at development of ESRD not due to diabetes, date of death from causes other than diabetic nephropathy if death occurred before 31 December 2002 or at 31 December 2002. Linear association was computed by the Mantel extension test, modified for person-time denominators.

The effect of periodontitis on incidence of macroalbuminuria and ESRD was also examined using time-dependent Cox proportional hazards models to control for the effects of potentially confounding variables. Final models were adjusted for age at baseline, sex, duration of diabetes at baseline, BMI, and current smoking. A1C was considered both a potential confounder and intermediary in the relationship of periodontitis to macroalbuminuria and ESRD, and final models were repeated with additional adjustment for A1C. Assumptions of proportionality were checked using log follow-up time interaction terms for each baseline variable. Residual versus linear predictor plots were checked for outliers. Product terms of predictor variables, including sex interactions, did not improve the models and were not included. To maintain proportionality assumptions, final models for macroalbuminuria were stratified by baseline age and BMI, and final models for ESRD were stratified by baseline BMI and current smoking. Such stratification controls for confounding by the variables but does not allow estimation of their effects. Time-dependent models were constructed to allow the values for periodontitis, A1C, and current smoking (macroalbuminuria only) to change with time. The overall effect of periodontitis on the incidence of macroalbuminuria and ESRD was assessed by likelihood ratio tests comparing time-dependent models with and without terms for periodontitis. Statistical analyses were performed using SAS software version 8 (SAS Institute, Cary, NC).

**RESULTS** — Among the 529 individuals (168 men and 361 women) included in this study, 107 (20%) had none/mild periodontis, 200 (38%) had moderate periodontitis, and 117 (22%) had severe periodontitis, and 105 individuals (20%) were edentulous at baseline (Table 1). Age, diabetes duration, A1C (all P < 0.0001), and hypertension (P = 0.01) at baseline were positively associated with severity of periodontitis, whereas BMI and obesity were negatively associated with severity of periodontitis (both P < 0.0001). During a median follow-up of 9.4 years (range 0.03–21.6 years), 193 individuals developed macroalbuminuria, and during a median follow-up of 14.9 years (0.03–21.8 years), 68 individuals developed ESRD. The unadjusted incidence of macroalbuminuria and ESRD were 37.5 cases/1,000 person-years and 9.0 cases/1,000 person-years, respectively. Incidence rates of macroalbuminuria and ESRD by age and sex are shown in Table 2.

Age- and sex-adjusted incidence rates of macroalbuminuria and ESRD were
Periodontitis and kidney disease

Table 1—Characteristics of the individuals in this analysis by periodontitis status at baseline

<table>
<thead>
<tr>
<th>Continuous variables</th>
<th>None or mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Edentulous</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>107</td>
<td>200</td>
<td>117</td>
<td>105</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes duration</td>
<td>33 (25–72)</td>
<td>44 (25–72)</td>
<td>49 (26–77)</td>
<td>55 (25–79)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>A1C (%)</td>
<td>1.9 (0.0–19.7)</td>
<td>4.3 (0.0–26.4)</td>
<td>7.9 (0.0–24.6)</td>
<td>12.3 (0.0–32.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>6.1 (3.8–13.0)</td>
<td>7.4 (2.7–13.4)</td>
<td>9.4 (2.8–15.9)</td>
<td>9.1 (4.2–14.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>38.6 (24.6–71.1)</td>
<td>33.3 (21.0–55.0)</td>
<td>30.2 (20.5–54.4)</td>
<td>29.8 (21.9–65.4)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Categorical variables

<table>
<thead>
<tr>
<th></th>
<th>None or mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Edentulous</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>24 (22.4)</td>
<td>80 (40.0)</td>
<td>39 (33.3)</td>
<td>25 (23.8)</td>
<td>0.66</td>
</tr>
<tr>
<td>Obese</td>
<td>93 (86.9)</td>
<td>141 (70.5)</td>
<td>61 (52.1)</td>
<td>51 (48.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>47 (43.9)</td>
<td>98 (49.8)</td>
<td>64 (55.7)</td>
<td>63 (60.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>Currently smoking</td>
<td>21 (19.6)</td>
<td>53 (26.5)</td>
<td>26 (22.2)</td>
<td>14 (13.5)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Data are median (range) or n (%). Total n = 529. Missing data: n = 3 for baseline mean arterial pressure (MAP), n = 13 for baseline A1C, n = 5 for baseline hypertension, and n = 1 for baseline smoking.

positively associated with severity of periodontitis (P = 0.0001 and P = 0.003, respectively; Fig. 1). Moderate and severe periodontitis and edentulousness predicted the development of macroalbuminuria in a dose-dependent manner after adjustment for age, sex, duration of diabetes, and BMI at baseline and updated periodontal and smoking status at each examination (Table 3). The hazard rate ratios (HRRs) for moderate or severe periodontitis and edentulousness were 2.0 (95% CI 1.2–3.5), 2.1 (1.2–3.8), and 2.6 (1.4–4.6), respectively (overall P = 0.01). This relationship was attenuated after adjustment for A1C, updated at each examination; HRR for moderate periodontitis was 1.3 (0.4–4.9), for severe periodontitis was 1.8 (0.5–6.7), and for edentulousness was 2.5 (0.7–9.4) (overall P = 0.21).

CONCLUSIONS — Periodontitis predicts the development of overt nephropathy and ESRD in a dose-dependent manner in individuals with little or no preexisting kidney disease after adjustment for age, sex, duration of diabetes, BMI, and current smoking. These findings confirm the emerging evidence of an independent association between periodontitis and the development of diabetic kidney disease. To our knowledge, only two studies have investigated the effect of periodontitis on early kidney disease (11,12). These studies were cross-sectional; one was in a largely nondiabetic population (12), and the other did not explore the effect of potential confounders (11). The present study, however, was prospective, conducted exclusively in individuals with diabetes, and included a proportionately large number of individuals with kidney disease. Data on change in periodontal status were collected (on up to four occasions for each individual), and models allowed periodontitis and A1C to change at each examination. Current smoking data were also updated at each examination in the macroalbuminuria analysis. Furthermore, all of the study participants had an estimated GFR ≥60 ml/min per 1.73 m² and did not have macroalbuminuria at baseline. Macroalbuminuria and not microalbuminuria was chosen as an outcome in our analysis because microalbuminuria is much more likely to regress than macroalbuminuria, and restricting our dataset to individuals without microalbuminuria at baseline reduced our sample size by approximately one-third.

Implicit in a time-dependent analysis

Table 2—Incidence of overt nephropathy, characterized by macroalbuminuria (MA), and ESRD per 1,000 person-years (PYRs) by age and sex in an American-Indian population with type 2 diabetes

<table>
<thead>
<tr>
<th>Age-group (years)</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>PYRs</td>
</tr>
<tr>
<td>MA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25–44</td>
<td>14</td>
<td>542</td>
</tr>
<tr>
<td>45–64</td>
<td>37</td>
<td>792</td>
</tr>
<tr>
<td>≥65</td>
<td>6</td>
<td>128</td>
</tr>
<tr>
<td>ESRD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25–44</td>
<td>3</td>
<td>658</td>
</tr>
<tr>
<td>45–64</td>
<td>15</td>
<td>1,265</td>
</tr>
<tr>
<td>≥65</td>
<td>3</td>
<td>320</td>
</tr>
</tbody>
</table>

n = 529.
are the assumptions that the study exposure (in this case, periodontitis) does not affect any covariates used as regressors and that there is no confounding within levels of the other covariates. These assumptions may not be valid when the exposure and the covariates vary over time, because a covariate may be affected by the exposure and also be a confounder. In the present analysis, the level of hyperglycemia may be a confounder because it is a known risk factor for kidney disease (20) and is associated with periodontitis (3,4,6). On the other hand, the level of glycemia also rises as a consequence of periodontitis (6,7). A1C may therefore be both a confounder and an intermediate variable on the causal pathway between periodontitis and kidney disease, and a proportional hazards analysis that includes A1C as a time-dependent covariate will underestimate the effect of periodontitis on the development of kidney disease. Nevertheless, exclusion of this variable may cause confounding. Accordingly, we examined the effect of periodontitis on kidney disease by using two models, but we favor the model that did not control for A1C (Table 3) because this variable is almost certainly an intermediate variable in the pathway of interest.

BMI was inversely associated with severity of periodontitis at baseline in this analysis (Table 1), in contrast to the positive association between BMI and periodontitis reported in the ARIC Study (12). However, the ARIC Study was conducted in a predominantly nondiabetic population. After diabetes diagnosis, BMI declines in Pima Indians, and this decline continues with increasing diabetes duration (21). Given that poor glycemic control hastens the progression of periodontitis (6,8) and glycemic control tends to worsen with increasing diabetes duration (22), an inverse association between BMI and severity of periodontitis in individuals with diabetes is expected. Indeed, after adjustment for age, diabetes duration, and A1C, the association between BMI and periodontitis at baseline was no longer significant (data not shown).

Smoking is associated with both periodontitis (23,24) and kidney disease (25,26) and may confound the relationship between these diseases. Nevertheless, fewer than 1% of adult Pima Indians smoke one pack or more of cigarettes per day. In this population, current smoking is not a prominent risk factor for fatal coronary heart disease (27) and did not explain the relationship between periodontitis and kidney disease in the present study. However, given our measures of smoking, the possibility of residual confounding due to smoking in this analysis cannot be excluded. High blood pressure is strongly associated with diabetic kidney disease (28); however, hypertension was not considered a potential confounding factor in our analysis, because when we adjusted our final time-dependent models for hypertension, the association of periodontitis with macroalbuminuria and ESRD remained unchanged (data not shown). Furthermore, to our knowledge there is no published evidence linking blood pressure with risk of development of periodontitis. The positive association between the presence of hypertension and severity of periodontitis at baseline in the present analysis was attributable, in part, to the higher average age of individuals who were edentulous or had severe periodontitis (data not shown). Finally, the effect of periodontitis on macroalbuminuria and ESRD may be underestimated to the extent that complete tooth loss was due to factors other than periodontitis.

A proposed mechanism for the effect of periodontitis on the development of kidney disease is systemic inflammation. Both periodontitis and kidney disease are associated with inflammatory markers such as C-reactive protein (29,30), and chronic low-level inflammation associated with periodontitis may lead to endothelial dysfunction, which plays a role in the pathogenesis of kidney disease (31,32). Periodontitis is treatable, and treatment by tooth extraction (33) or other mechanical procedures and locally administered antibiotics (34) lowers levels of C-reactive protein and other inflammatory markers. In edentulous individuals, the potentially deleterious effects of systemic inflammation are underestimated, in part, to the higher average age of individuals who were edentulous or had severe periodontitis (data not shown). Finally, the effect of periodontitis on macroalbuminuria and ESRD may be underestimated to the extent that complete tooth loss was due to factors other than periodontitis.

### Table 3—Time-dependent Cox proportional hazards models of the effect of periodontitis (PD) on the incidence of overt nephropathy, characterized by macroalbuminuria (MA), and ESRD in an American-Indian population with type 2 diabetes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Effect of PD on MA*</th>
<th>Effect of PD on ESRD†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate PD (vs. none/mild PD)</td>
<td>2.0 (1.2–3.5)</td>
<td>2.3 (0.6–8.1)</td>
</tr>
<tr>
<td>Severe PD (vs. none/mild PD)</td>
<td>2.1 (1.2–3.8)</td>
<td>3.5 (0.96–12.4)</td>
</tr>
<tr>
<td>Edentulous (vs. none/mild PD)</td>
<td>2.6 (1.4–4.6)</td>
<td>4.9 (1.4–17.4)</td>
</tr>
<tr>
<td>Age at baseline (10 years)</td>
<td>—</td>
<td>0.8 (0.6–1.0)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>0.8 (0.6–1.2)</td>
<td>0.8 (0.4–1.4)</td>
</tr>
<tr>
<td>Diabetes duration at baseline (10 years)</td>
<td>2.1 (1.6–2.7)</td>
<td>2.4 (1.5–3.6)</td>
</tr>
<tr>
<td>Smoking (no/yes)</td>
<td>1.2 (0.8–1.7)</td>
<td>—</td>
</tr>
</tbody>
</table>

n = 515. *Model stratified by baseline age and BMI because these variables violated the proportionality assumption. †Model stratified by baseline BMI and smoking because these variables violated the proportionality assumption. $P$ value for overall effect of PD on outcome obtained by likelihood ratio test comparing models with and without terms for PD.
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stemic inflammation on kidney function could occur during the period of active periodontal infection and accumulate during the lifetime of the individual. This hypothesis is supported by the observation that the strongest predictive effect of periodontal status on kidney disease was found in edentulous individuals (Table 3). Cardiovascular disease prevalence and risk is higher or equivalent in edentulous individuals compared with that in individuals with severe periodontitis in some (35,36) but not all studies (37).

In summary, periodontitis predicts the development of both overt nephropathy and ESRD in an American-Indian population with type 2 diabetes. Future studies investigating measures of inflammatory markers may help elucidate the potential mechanisms for the association between periodontitis and diabetic kidney disease. Whether successful management of periodontitis will reduce the risk of diabetic kidney disease, however, remains to be determined.

Acknowledgments — This research was supported by the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases and the National Institute of Dental and Craniofacial Research (grant no. DE-06514). R.G. received additional support from the Sunstar Company.

The authors are indebted to the members of the Gila River Indian Community for participating in this investigation and to the doctors, nurses, and support staff involved in collecting and processing the data.

References

6. Taylor GW: Bidirectional interrelation-
Prevalence of Candida glabrata and Its Response to Boric Acid Vaginal Suppositories in Comparison With Oral Fluconazole in Patients With Diabetes and Vulvovaginal Candidiasis

Debati Ray, MBBS
Ravinder Goswami, MD, DM
Uma Banerjee, MD
Vatsla Dadhwal, MD

OBJECTIVE — A large proportion of vulvovaginal candidiasis (VVC) in diabetes is due to non–albicans Candida species such as C. glabrata and C. tropicalis. Observational studies indicate that diabetic patients with C. glabrata VVC respond poorly toazole drugs. We evaluated the response to oral fluconazole and boric acid vaginal suppositories in diabetic patients with VVC.

RESEARCH DESIGN AND METHODS — A total of 112 consecutive diabetic patients with VVC were block randomized to receive either single-dose oral 150-mg fluconazole or boric acid vaginal suppositories (600 mg/day for 14 days). The primary efficacy outcome was the mycological cure in patients with C. glabrata VVC in the two treatment arms. The secondary outcomes were the mycological cure in C. albicans VVC, overall mycological cure irrespective of the type of Candida species, frequencies of yeast on direct microscopy, and clinical symptoms and signs of VVC on the 15th day of treatment. Intention-to-treat (ITT; n = 111) and per-protocol (PP; n = 99) analyses were performed.

RESULTS — C. glabrata was isolated in 68 (61.3%) and C. albicans in 32 (28.8%) of 111 subjects. Patients with C. glabrata VVC showed higher mycological cure with boric acid compared with fluconazole in the ITT (21 of 33, 63.6% vs. 10 of 35, 28.6%; P = 0.01) and PP analyses (21 of 29, 72.4% vs. 10 of 30, 33.3%; P = 0.01). The secondary efficacy outcomes were not significantly different in the two treatment arms in the ITT and PP analyses.

CONCLUSIONS — Diabetic women with C. glabrata VVC show higher mycological cure with boric acid vaginal suppositories given for 14 days in comparison with single-dose oral 150-mg fluconazole.

Diabetes Care 30:312–317, 2007

Patients with diabetes are at increased risk of developing vulvovaginal candidiasis (VVC) (1–8). Unlike nondiabetic women, these patients have a higher proportion of colonization/infection due to non–albicans Candida species such as C. glabrata and C. tropicalis (4, 6–8). In contrast, C. albicans VVC is more frequent in nondiabetic women (9). Moreover, diabetic patients with C. glabrata VVC respond poorly to single oral doses of 150 mg fluconazole (8). C. glabrata demonstrates intrinsically reduced susceptibility to azole drugs (10–13).

Redondo-Lopez et al. (10) and other investigators have reported the successful use of vaginal boric acid suppositories in the management of patients with C. glabrata (10,14,15) as well as C. albicans VVC (16–18). In view of the higher frequency of C. glabrata VVC among diabetic women and their poor response to fluconazole, there is a need for a therapeutic regimen that is effective against both C. glabrata and C. albicans. To the best of our knowledge, there is no previous study reporting the effect of boric acid vaginal suppositories in diabetic women with VVC. In the current open-label, randomized trial, we report the response to oral fluconazole and boric acid vaginal suppositories in diabetic patients with VVC.

RESEARCH DESIGN AND METHODS — This was a randomized, open-label comparison of 2 weeks’ duration between single-dose oral 150-mg fluconazole and 14 days of treatment with boric acid vaginal suppositories (600 mg/day) in 112 consecutive patients with diabetes (type 2 diabetes, n = 77 and type 1 diabetes, n = 35) and VVC conducted during 2004–2006. Diabetes was diagnosed as per the American Diabetes Association Expert Committee criteria (19). Patients already on insulin or oral hypoglycemic agents also were included. Any patient with onset of diabetes before age 30 years who had received continuous insulin treatment since diagnosis was considered to have type 1 diabetes. Subjects excluded were those who were pregnant, sexually inactive girls, those aged >65 years, those with renal insufficiency (serum creatinine >1.8 mg/dl), and those on steroid therapy. Patients who did not give consent for pelvic examination, those treated for vaginal discharge during the past 3 months, and...
symptomatic patients in whom *Candida* growth was not detected on fungal culture were also excluded. A1C was measured in all the subjects to assess their glycemic control (normal range: 5.4–7.0%) at visit 1. The institutional ethics committee of the All India Institute of Medical Sciences approved the study protocol, and all the subjects were examined after their informed consent.

**Diagnosis of VVC**

A large number of diabetic patients were assessed for symptoms and signs of VVC including vaginal discharge, vulval pruritus, burning sensation, vulval edema, and vaginal congestion at visit 1 (20). Two sterile cotton-tipped commercial swabs were used to collect discharge from high vagina and transported to the mycology laboratory without delay. One of the swabs was used to determine the presence of yeast by direct microscopy, while the other was used for fungal culture. The VVC was diagnosed in the presence of clinical signs and symptoms and growth of *Candida* species on culture of the high vaginal swab (HVS).

Detection and identification of the yeast up to the species level was done in the mycology laboratory of the All India Institute of Medical Sciences as per the standard protocols (21,22). The criteria for laboratory diagnosis were 1) direct demonstration of the yeast by Gram’s staining and 2) culture on Sabouraud’s dextrose agar supplemented with 2 mg/ml gentamicin, with and without cycloheximide (0.5%). Identification of *Candida* species was performed by examining colony morphology, germ tube test, hyphal morphology, and chlamydospore formation on cornmeal agar, triphenyl tetrazolium reduction test, fermentation and assimilation of different sugars, and cycloheximide susceptibility.

**Randomization and concealment**

Following identification of *Candida* growth on HVSs, subjects were assigned to receive single-dose oral 150-mg fluconazole or boric acid vaginal suppositories (600 mg/day) for 14 days in a 1:1 ratio using a randomization list with random permuted blocks, length of 4, at the clinic site (visit 2). The randomization list was generated using a Microsoft Excel spreadsheet as described in detail (available at http://www.childrens-mercy.org/stats/plan/random.asp). Block randomization was used to ensure the balance in the number of patients in the two treatment arms.

A statistician generated the entire randomization sequence list in advance, and allocations were sequentially numbered from the beginning to end. A female clinical investigator (D.R.) carried out genital examinations for VVC, including an HVS for fungal culture for all consecutive diabetic patients attending the endocrine outpatient clinic and gave a serial number to all the patients examined. Following a report of yeast growth, two clinicians (D.R. and R.G.) assigned patients to a treatment arm, as per the serial order of the clinical examination and randomization list sequence number, in an irreversible manner. One of these two clinicians (R.G.) was not involved with clinical examination. Patients were allocated to treatment arm following reports of yeast growth but before the results of *Candida* species were available. The serial order of the randomization list was not disclosed to the patients until the treatment was assigned.

The intake of fluconazole was supervised to ensure compliance. Patients receiving boric acid were instructed to insert one boric acid–filled gelatin capsule (600 mg) intravaginally every night for 14 uninterrupted days either by a plastic applicator or dispensed by hand as per their preference. Two extra capsules were given, and unused capsules were counted on the follow-up visit to assess compliance. Patients in both treatment arms were reassessed on the 15th day of therapy (visit 3). To minimize interobserver variation, the same investigator carried out the repeat clinical assessment at visit 3 when HVSs were taken again for demonstration of yeast by direct microscopy and fungal culture. All the HVSs were of the same color and size and sent to the mycological laboratory in identical containers labeled with a numerical identity. The plan of block randomization, patients’ treatment assignments, the pre- and post-nature of the specimen and clinical findings were not disclosed to the mycologist and the laboratory staff who performed fungal culture and species identification till the completion of the 2-year study.

**Efficacy outcomes**

The primary objective was to test the hypothesis that diabetic patients with *C. glabrata* VVC when treated with boric acid vaginal suppositories (600 mg/day) for 14 days show a higher mycological cure rate in comparison with a single oral dose of 150 mg fluconazole. Mycological cure was defined as the absence of *Candida* growth on the HVS culture on the 15th day of therapy. The primary efficacy outcome was the mycological cure in patients with *C. glabrata* VVC in the two treatment arms. The secondary efficacy outcomes were mycological cure in *C. albicans* VVC, overall mycological cure irrespective of the type of *Candida* species isolated, presence of yeast on direct microscopy, and frequency of clinical symptoms and signs of VVC on the 15th day in the two treatment arms.

**Adverse effects**

Treatment-emergent adverse events were defined as those occurring after receiving the first dose of treatment.

**Sample size and power calculations**

Sample size estimation for the assessment of primary efficacy outcome was based on our previously published mycological cure rate of 18.7% in diabetic patients with *C. glabrata* VVC after a single oral dose of 150 mg fluconazole therapy (8). The sample size was calculated using a statistical program (EPI-Info 2002; Centers for Disease Control and Prevention, Atlanta, GA), assuming the persistence of the above trend and three times higher response rate (56.1%) with boric acid therapy. Calculations revealed that 30 subjects with *C. glabrata* infection in each of the two treatment arms would be required to give a study power of 80% at 95% CI. With a 54.1% prevalence rate of *C. glabrata* infection, as observed in our previous study in diabetic subjects with VVC (8), a sample size of 112 diabetic patients with VVC was estimated to give us 60 subjects with *C. glabrata* VVC.

**Statistical analysis**

Both intention-to-treat (ITT) and per-protocol (PP) analyses were performed as efficacy analyses. Patients included in the ITT analysis included all those who were assigned to the two treatment arms and in whom at least baseline HVS culture showed *Candida* growth. To be included in the PP analysis, patients also had to comply with the assigned treatment regimen and follow-up as per the study protocol on day 15 (visit 3). For determination of the primary and secondary efficacy outcomes in the ITT analysis, missing data were imputed by explicit allocation of poor outcome in both treatment arms. The differences in the mean age, BMI, and A1C between patients in...
the two treatment arms were analyzed using Student’s t test. Logistic regression analysis, with mycological cure as the dependent variable and treatment group and A1C as the independent variable, was used to determine the significance of difference in the primary and secondary efficacy outcomes in the two treatment groups. The differences in the parametric and nonparametric indexes between subjects who grew *C. albicans* and *C. glabrata* on HVS culture were compared using a Student’s t test and χ² test, respectively. All P values calculated were two tailed, and P < 0.05 was considered significant. SPSS 7.5.1 statistical package was used for analysis.

RESULTS — Of 112 subjects randomized to the two treatment arms, 111 were included in the ITT analysis and 99 were analyzed in the PP analysis. One patient was excluded from the ITT analysis because she fell into exclusion criteria (sexually inactive, in whom a HVS was not taken). Twelve patients were dropped for PP analysis because of the following reasons: two stopped boric acid therapy due to vaginal burning sensation appearing on the 7th day of therapy, two did not come for follow-up because of diabetic foot and pulmonary consolidation (fluconazole arm), and eight patients were lost to follow-up for unknown reasons (four in each of the two treatment arms).

The most common species isolated in the cohort of 111 patients included in the ITT analysis was *C. glabrata* (n = 68, 61.3%), followed by *C. albicans* (n = 32, 28.8%) and *C. tropicalis* (n = 4, 3.6%). The baseline demographic and clinical characteristics, including the proportion of patients with type 1 and type 2 diabetes, frequencies of *C. glabrata* and *C. albicans* species isolated on HVS culture, number of postmenopausal women, and those using commercially available protective sanitary napkins, were comparable in both the treatment arms in the ITT and PP analyses groups (Table 1). Only two women were on oral contraceptive pills (one in each of the two treatment arms). The mean A1C value at baseline was lower in the boric acid treatment arm than in the fluconazoole arm in the ITT as well as in the PP analyses group. The primary and secondary efficacy outcomes were, therefore, adjusted for A1C at baseline (Table 2).

The mycological cure rate in patients with *C. glabrata* VVC was significantly higher in the boric acid treatment arm compared with the fluconazoole arm in both the ITT and PP analyses (P values = 0.01 for both after adjustment for baseline A1C). The odds in favor of mycological cure with boric acid vaginal suppositories in diabetic patients with *C. glabrata* VVC was 4.0 (95% CI 1.4–11.6). The goodness of fit of the logistic regression model was assessed by Nagelkerke R². Although the Nagelkerke R² value for primary outcome was only 15.3%, the model should not be interpreted as inadequate (23). No significant difference in mycological cure was observed in the two treatment arms in subjects with *C. albicans* VVC. The overall mycological cure on the 15th day, when analyzed irrespective of the type of *Candida* species isolated at baseline HVS culture, tended to be higher in those receiving boric acid in the ITT (P = 0.07) and PP (P = 0.06) analyses. The improvement in vaginal pruritus, discharge, and other clinical signs and symptoms was comparable in the two treatment arms in the ITT or PP analyses.

The duration of different symptoms and signs related to VVC, yeast positivity on direct microscopy, and mean A1C values were comparable in diabetic subjects who grew *C. albicans* and *C. glabrata* in the ITT and PP analyses groups. The mean A1C tended to be higher in subjects who continued to grow *Candida* on repeat HVS culture compared with those who did not grow *Candida* in the ITT (9.3 ± 2.0% vs. 8.6 ± 2.1%; P = 0.11) and PP groups (9.5 ± 2.2% vs. 8.6 ± 2.1%; P = 0.06).

**Adverse effects**

Two of the patients in the boric acid treatment arm who stopped the treatment due to vaginal burning sensation were considered to demonstrate the adverse effect, as this improved after stopping the drug.

**CONCLUSIONS** — The 59.9% prevalence of *C. glabrata* infection observed in the current study confirms the findings of our earlier studies that non-albicans VVC is frequent in diabetic women (4,8). de Leon et al. (6) observed the 54% vaginal carriage rate of *C. glabrata* in type 2 diabetes. The comparable frequency of clinical symptoms and signs between diabetic

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**Table 1—Baseline clinical characteristics, symptoms, and signs related to VVC, yeast positivity on direct microscopy, and mean A1C values in the ITT and PP analyses**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ITT analysis</th>
<th>P value</th>
<th>PP analysis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluconazole</td>
<td>Boric acid</td>
<td>Fluconazole</td>
<td>Boric acid</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.2 ± 10.7</td>
<td>41.2 ± 11.3</td>
<td>0.63</td>
<td>40.2 ± 9.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 ± 5.4</td>
<td>24.9 ± 4.5</td>
<td>0.64</td>
<td>24.6 ± 5.6</td>
</tr>
<tr>
<td>A1C (%)</td>
<td>9.36 ± 2.5</td>
<td>8.5 ± 1.6</td>
<td>0.04</td>
<td>9.35 ± 2.8</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>21 (38.1)</td>
<td>17 (30.3)</td>
<td>0.50</td>
<td>19 (38.8)</td>
</tr>
<tr>
<td>Use of commercial sanitary napkins during menstruation</td>
<td>14 (41.2)</td>
<td>22 (56.4)</td>
<td>0.28</td>
<td>13 (43.3)</td>
</tr>
<tr>
<td>VVC symptoms for &gt;1 month</td>
<td>50 (90.9)</td>
<td>47 (83.9)</td>
<td>0.41</td>
<td>44 (89.8)</td>
</tr>
<tr>
<td>Vulval pruritus</td>
<td>45 (81.8)</td>
<td>41 (73.2)</td>
<td>0.39</td>
<td>40 (81.6)</td>
</tr>
<tr>
<td>Vulval edema</td>
<td>5 (9.0)</td>
<td>13 (23.2)</td>
<td>0.08</td>
<td>5 (10.2)</td>
</tr>
<tr>
<td>Vaginal congestion</td>
<td>39 (70.9)</td>
<td>43 (76.7)</td>
<td>0.62</td>
<td>33 (67.3)</td>
</tr>
<tr>
<td>Direct microscopy positivity</td>
<td>35 (63.6)</td>
<td>30 (53.5)</td>
<td>0.37</td>
<td>29 (59.2)</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>35 (63.6)</td>
<td>33 (58.9)</td>
<td>0.75</td>
<td>30 (61.2)</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>14 (25.4)</td>
<td>18 (32.1)</td>
<td>0.95</td>
<td>13 (26.5)</td>
</tr>
<tr>
<td>Other <em>Candida</em> species</td>
<td>6 (10.9)</td>
<td>5 (8.9)</td>
<td>0.97</td>
<td>6 (12.2)</td>
</tr>
</tbody>
</table>

Data are means ± SD for age, BMI, and A1C and n (%) for other indices in the two treatment arms in ITT and PP groups. P values test differences in the means or proportions observed in the two treatment arms.
women with C. glabrata or C. albicans infection is similar to that reported by Geiger et al. (24) in nondiabetic women. Increased prevalence of C. glabrata infection in diabetic women has clinical relevance because poor therapeutic response and innate resistance to azoles has been reported for C. glabrata VVC in non-diabetic women (10–13). Similar information is lacking in diabetic subjects, as they are often excluded in antifungal efficacy studies (8,10,25–27). Poor mycological cure in diabetic women with C. glabrata VVC to single-dose oral 150-mg fluconazole, observed in the current study, is in accordance with our earlier case-control study (8). Mechanisms implicated for resistance of C. glabrata to fluconazole include increased fungal ergosterol synthesis and up to eightfold higher expression of azole efflux pump protein coded by CgCDR1 transporter genes (26,28). Diabetic patients with VVC also respond poorly to itraconazole and ketoconazole (29,30).

In the current study, the higher mycological cure (72.4%) to boric acid therapy in diabetics with C. glabrata VVC is similar to that reported in nondiabetic individuals (10,14–18). In 1974, Swate and Weed (16) first reported boric acid to be a “safe, effective and inexpensive form of therapy” for VVC. Sobel and Chaim (14) reported clinical improvement in 81% and mycological cure in 77% of non-diabetic patients with symptomatic C. glabrata/ Torulopsis vaginitis treated with vaginal boric acid. There are also reports of better therapeutic response to boric acid compared with topical nystatin and miconazole in nondiabetic women with C. albicans VVC (17,18). Otero et al. (31) have reported a greater in vitro susceptibility of C. albicans to boric acid compared with C. glabrata.

Boric acid or boracic [B(OH)3] is a weak acid, and its mode of antifungal action is not clear. Shohama and Tasker (15) proposed that its acidic properties lead to disruption of the fungal cell wall. The pH of boric acid–Sabouraud’s broth (5.0–5.09) required for in vitro inhibition of C. albicans growth has been reported to be similar to the vaginal pH in untreated C. albicans women (5). A study by Weisberg and Weinberg (32) also has acidic properties (32). The dose of the boric acid used to treat VVC ranged from 600 mg daily for 14 days (18) to twice daily (10) to twice daily for 14 days (18). We used 600 mg boric acid or boracic acid (5 g) in all our experiments in the ITT and PP groups. Patients with mixed infection and non-albicans candidiasis other than C. glabrata were excluded in the study because of the limited numbers of patients available in each group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ITT Analysis</th>
<th>PP Analysis</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycological cure in C. glabrata VVC</td>
<td>10/35 (28.6%)</td>
<td>21/33 (63.6%)</td>
<td>4.4</td>
<td>1.6–12.1</td>
</tr>
<tr>
<td>Yeast on direct microscopy</td>
<td>12 (85.7%)</td>
<td>11 (61.1%)</td>
<td>0.3</td>
<td>0.04–1.5</td>
</tr>
<tr>
<td>Overall mycological cure</td>
<td>25 (45.4%)</td>
<td>37 (66.1%)</td>
<td>2.1</td>
<td>0.9–4.5</td>
</tr>
<tr>
<td>Yeast on direct microscopy</td>
<td>15 (30.6%)</td>
<td>7 (14.0%)</td>
<td>0.48</td>
<td>0.13–1.1</td>
</tr>
<tr>
<td>Vaginal congestion</td>
<td>34 (61.8%)</td>
<td>25 (44.6%)</td>
<td>0.54</td>
<td>0.2–1.1</td>
</tr>
</tbody>
</table>

**Table 2—Primary and secondary efficacy outcomes in the two treatment arms in the ITT and PP groups.**
acid daily for 14 days because >90% mycological cure has been reported with this schedule in nondiabetic patients with symptomatic \textit{C. albicans} (17) or \textit{C. glabrata} VVC (14).

Boric acid therapy (600 mg/day for 14 days) has been found to be safe (14,17), except for local burning sensation and vestibular erythema in occasional patients (14,17,18). Two of our patients also reported this side effect. Improvement in clinical features of VVC was comparable in two treatment arms. However, we cannot exclude an element of bias in the analysis of this secondary outcome, as the same investigator carried out the clinical examination following treatment.

In our previous studies, poor glycemic control was linked with \textit{Candida} growth (4) but was unrelated to the type of species isolated or response to the fluconazole therapy (8). In the current study, there was a trend of higher mean A1C levels in those showing persistence of \textit{Candida} growth following either of the two therapies. The cause of increased \textit{C. glabrata} isolation in diabetic women is not clear but may involve frequent use of antifungal drugs leading to its reduced susceptibility to azoles (33) and consequent polarization/homing in diabetic women. Feng et al. (34) reported lesser susceptibility of \textit{C. glabrata} in comparison with \textit{C. albicans} to \textit{β}-defensins, natural cationic antimicrobial/antifungal peptides expressed in human epithelia. In diabetic milieu, \textit{β}-defensins expression is reduced (35). Reduced expression of defensins in association with resistance of \textit{C. glabrata} to fungicidal activity of drugs like fluconazole may also explain the high prevalence of \textit{C. glabrata} VVC in diabetic women.

One of the relevant findings in this study is the importance of species identification of \textit{Candida} isolates for proper management of VVC in diabetic women. It is emphasized that conventional inexpensive testing procedures, though time consuming, are good enough to identify \textit{Candida} up to species level and would help in the management of diabetic women with VVC. As an alternative, boric acid therapy could be considered as the frontline therapy for treating VVC in diabetic women because it is effective against both \textit{C. albicans} and \textit{C. glabrata} compared with fluconazole, which is effective against \textit{C. albicans} only.

Acknowledgments — The authors are thankful to the financial support by ICMR, New Delhi, for this study.

References


Insulin Resistance as Estimated by Homeostasis Model Assessment Predicts Incident Symptomatic Cardiovascular Disease in Caucasian Subjects From the General Population

The Bruneck Study

Enzo Bonora, MD, PhD
Stefan Kiechl, MD
Johann Willeit, MD
Friedrich Oberhollenzer, MD

Georg Egger, MD
James B. Meigs, MD, MPH
Riccardo C. Bonadonna, MD
Michele Muggio, MD

OBJECTIVE — The purpose of this study was to evaluate whether insulin resistance is associated to cardiovascular disease (CVD) and to understand whether this association can be explained by traditional and novel CVD risk factors associated with this metabolic disorder.

RESEARCH DESIGN AND METHODS — We examined a sample representative of the population of Bruneck, Italy (n = 919; aged 40–79 years). Insulin-resistant subjects were those with a score in the top quartile of the homeostasis model assessment (HOMA) for insulin resistance (HOMA-IR). Risk factors correlated with insulin resistance included BMI, A1C, HDL cholesterol, triglycerides, blood pressure, high-sensitivity C-reactive protein (hsCRP), fibrinogen, oxidized LDL, vascular cell adhesion molecule-1 (VCAM-1), and adiponectin. Subjects without CVD at baseline were followed up for 15 years for incident CVD, a composite end point including fatal and nonfatal myocardial infarction and stroke, transient ischemic attack, and any revascularization procedure.

RESULTS — During follow-up, 118 subjects experienced a first symptomatic CVD event. Levels of HOMA-IR were higher at baseline among subjects who developed CVD (2.8) compared with those remaining free of CVD (2.5) (P < 0.05). Levels of HOMA-IR also were significantly correlated (P < 0.05) with most CVD risk factors we evaluated. In Cox proportional hazard models, insulin-resistant subjects had an age-, sex-, and smoking-adjusted 2.1-fold increased risk (95% CI 1.3–3.1) of incident symptomatic CVD relative to non-insulin-resistant subjects. After sequential adjustment for physical activity and classic risk factors (A1C, LDL cholesterol, and hypertension) as well as BMI, HDL cholesterol, triglycerides, and novel risk factors, including fibrinogen, oxidized LDL, hsCRP, VCAM-1, and adiponectin, the association between HOMA-IR and incident CVD remained significant and virtually unchanged (hazard ratio 2.2 [95% CI 1.4–3.6], P < 0.001).

CONCLUSIONS — HOMA-estimated insulin resistance is associated with subsequent symptomatic CVD in the general population independently of all classic and several nontraditional risk factors. These data suggest that insulin resistance may be an important target to reduce CVD risk.

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founding role of novel CVD risk factors has been done. In other words, the association between insulin resistance and CVD, if observed, might be attenuated or abolished after adjustment for novel, insulin resistance–related risk factors.

The effect of ameliorating insulin resistance on CVD outcomes has been tested in only one intervention trial (32). However, although pioglitazone reduced the risk for CVD events in a post hoc subgroup analysis, the trial was conducted among diabetic patients and the suggested benefit of insulin sensitization may have been related to the observed reduction of risk factors (hyperglycemia, dyslipidemia, and hypertension) known to improve with pioglitazone therapy. Thus, the role of insulin resistance in the pathogenesis of CVD remains an open question.

With these issues in mind, in the present study we evaluated whether insulin resistance was associated with new cases of symptomatic CVD independently of traditional and nontraditional risk factors known to cluster with this metabolic disorder in a large sample from the general population of Bruneck, Italy.

RESEARCH DESIGN AND METHODS — The Bruneck Study is a long-term prospective population-based survey of atherosclerosis and its risk factors. It is being conducted in Bruneck, a small town of ~13,500 people, located in Northeastern Italy, close to the Austrian border. As reported previously (33), a baseline evaluation was performed between July and November 1990. Among the 1,000 randomly selected men and women of the 4,793 Caucasians subjects aged 40–79 years, 936 volunteered after the purposes and modalities of the study had been carefully presented. As 17 subjects had incomplete data collection, the sample we used for most statistical analyses included 919 subjects. Insulin measurements were performed in 888 subjects because 2 subjects were receiving insulin treatment, and 29 subjects had no serum available for the measurement of insulin. After exclusion of 49 subjects with preexisting CVD, 839 subjects (416 men and 423 women) remained for the current analysis. The main clinical features of the study population and the subset with insulin measurements available have been reported in previous publications (1,4,17,33–35).

Reevaluations were performed every 5 years, i.e., in 1995, 2000, and 2005. In this period 210 of the 839 subjects died. Follow-up was 100% complete for clinical end points.

The protocol was approved by the ethics committee of the University of Verona. All subjects gave an informed consent.

Clinical data

The following data were collected with a standardized questionnaire: cigarette smoking, alcohol consumption, physical activity, socioeconomic status, previous diseases, and drug prescriptions. BMI, waist circumference, and blood pressure were assessed with standard techniques. Overweight was defined by a BMI from 25 to 29.9 kg/m² and obesity by a BMI ≥30 kg/m². Hypertension was diagnosed when systolic blood pressure was ≥140 mmHg, diastolic blood pressure was ≥90 mmHg, or antihypertensive treatment was ongoing. At baseline, all subjects underwent a standard oral glucose tolerance test. Details on the methodology have been reported previously (1,4,17,33–35).

Laboratory data

In the morning after an overnight fast, venous blood was sampled for the measurement of A1C, as well as the plasma concentrations of glucose and the serum concentrations of total and HDL cholesterol, triglycerides, insulin, adiponectin, high-sensitivity C-reactive protein (hsCRP), fibrinogen, oxidized LDL, and vascular adhesion molecule-1 (VCAM-1). LDL cholesterol was calculated by the formula of Friedewald. Details on analytical procedures have been reported previously (1,4,17,33–35). Impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and diabetes were diagnosed according to current criteria.

Assessment of insulin resistance

The degree of insulin resistance at baseline was estimated by the homeostasis model assessment (HOMA) of insulin resistance (HOMA-IR) (36). In a recent article, we reported on the good reliability of the HOMA for estimating insulin resistance (37). Subjects in the top quartile of HOMA-IR distribution values were considered to be insulin resistant.

Assessment of CVD

The present report focuses on symptomatic CVD, an aggregate end point that included cardiovascular death, nonfatal myocardial infarction and stroke, transient ischemic attack (TIA), and coronary, carotid, or lower limb revascularization. Myocardial infarction was deemed confirmed when World Health Organization criteria for definite disease status were met (38). Ischemic stroke and TIA were classified according to the criteria of the National Survey of Stroke (39). Vascular mortality included deaths due to myocardial infarction and stroke and sudden cardiac deaths. All of these events or procedures were ascertained by examining the medical records of the local general practitioners and confirmed by reviewing the files of Bruneck Hospital. Cardiovascular deaths were identified by reviewing death certificates. Actual event dates were used, and only the first event was considered in this analysis. Major advantages of the Bruneck Study cohort are that virtually all subjects living in the area of Bruneck are referred to the local hospital and that the network existing between the local hospital and the general practitioners allows retrieval of virtually all medical information on people living in the area.

Statistical analysis

Statistical analyses were performed with SPSS-X and BMDP software. Skewed variables were loge-transformed to improve the approximation to a Gaussian distribution. Nonparametric tests yielded very similar results (data not presented). Reported P values are two-sided.

Overall, missing values for the variables adiponectin, hsCRP, oxidized LDL, and VCAM-1 were rare (<5%) and occurred randomly. They were replaced by estimates derived from the “regression procedure” of the SPSS missing value approach.

The correlations of demographic and behavioral variables, as well as laboratory parameters with loge-transformed insulin resistance (HOMA-IR) were expressed by standard correlation coefficients. Partial correlation coefficients were corrected for sex, age, smoking, and BMI.

Cox proportional hazard models were used to assess whether baseline insulin resistance was an independent predictor of incident CVD. For this purpose, HOMA-IR was modeled as a categorical variable, and subjects were stratified into those belonging to the top quartile (insulin-resistant subjects) versus those belonging to the other three quartiles (non–insulin-resistant subjects). Five nested models were run: the first one included sex, age, smoking, and HOMA-IR; the second model included model 1 variables, physical activity, and the three
most classic risk factors (hyperglycemia, here represented by A1C, LDL cholesterol, and hypertension); the third model included model 2 variables and traditional risk factors strongly related to insulin resistance (BMI, HDL cholesterol, and triglycerides); the fourth model included model 3 variables as well as novel risk factors related to insulin resistance, including fibrinogen (prothrombotic state), oxidized LDL (oxidative stress), hsCRP (inflammation), and VCAM-1 (endothelial dysfunction); and the fifth model included model 4 variables as well as adiponectin. In the principal analyses, smoking, hypertension, and HOMA-IR were modeled as categorical variables and the others as continuous variables. Proportional hazard assumptions were satisfied in all models.

RESULTS — At baseline, the prevalences of diabetes, IFG, and IGT were 6.8, 8.6, and 9.2%, respectively; those of overweight and obesity were 27.5 and 8.6, respectively; and that of hypertension was 61.9%. Table 1 displays baseline clinical features of subjects with and without CVD during follow-up. Those who developed CVD had a higher risk profile at baseline. After adjustments for sex, age, smoking, and BMI, log$_{10}$-transformed HOMA-IR was significantly correlated to A1C, LDL cholesterol and HDL cholesterol, triglycerides, hsCRP, fibrinogen, oxidized LDL, VCAM-1, and adiponectin (Table 2).

During the 15 years of follow-up, 118 subjects experienced one or more symptomatic CVD events. In particular, we observed 58 cases of nonfatal and fatal myocardial infarction and 58 cases of fatal and nonfatal stroke and TIA. Forty-four subjects underwent coronary, carotid, or lower limb revascularization. Cox models revealed that insulin-resistant subjects had an increased risk of incident symptomatic CVD compared with non–insulin-resistant subjects (Table 3). This result was found in the model including only sex, age, and smoking; in the model also including physical activity and classic risk factors (A1C, LDL cholesterol, and hypertension); and in the model also including BMI, HDL cholesterol, and triglycerides. Moreover, when the models also included nontraditional risk factors (fibrinogen, oxidized LDL, hsCRP, VCAM-1, and adiponectin), the association between HOMA-IR and CVD remained significant and virtually unchanged (Table 3). With model 5, which also included nontraditional risk factors, the hazard ratios (HRs) for CVD in the different HOMA-IR quartiles were 1.0 (quartile 1, reference), 0.9 ([95% CI 0.5–1.5], quartile 2), 0.9 ([0.5–1.6], quartile 3), and 2.1 ([1.1–3.9], quartile 4) (P for trend = 0.005). Therefore, there was no dose-response relation but a clear binary relation. Accordingly, when HOMA-IR was used as a continuous variable, no significant association was found with HOMA-IR (in model 5 per 1 unit change in HOMA-IR the HR was 1.3 [0.9–1.7], NS).

Results did not change when we used systolic blood pressure instead of hypertension (model 5, HR 2.4 [95% CI 1.5–3.8], P < 0.001) or number of cigarettes/day instead of smoking (model 5, 2.5 [1.5–4.0], P < 0.001) or when waist circumference replaced BMI (model 5, 2.4 [1.5–3.8], P < 0.001) or the presence/absence of IFG/IGT/diabetes replaced A1C (model 5, 2.5 [1.5–4.0], P < 0.001).

### Table 1—Clinical features of subjects in the cohort at baseline

<table>
<thead>
<tr>
<th>Feature</th>
<th>CVD negative at follow-up</th>
<th>CVD positive at follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>721</td>
<td>118</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>47.6</td>
<td>61.9*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.2 ± 11.2</td>
<td>65.8 ± 9.8*</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>24.1</td>
<td>30.5*</td>
</tr>
<tr>
<td>Physical activity (score)</td>
<td>4.4 ± 1.5</td>
<td>4.3 ± 1.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.9 ± 3.7</td>
<td>25.1 ± 4.3</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>87 ± 10</td>
<td>89 ± 10</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.50 ± 0.92</td>
<td>5.89 ± 1.67*</td>
</tr>
<tr>
<td>A1C (%)</td>
<td>5.5 ± 0.6</td>
<td>5.8 ± 0.9*</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>75 (49–111)</td>
<td>78 (40–127)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.5 (1.7–3.9)</td>
<td>2.8 (1.4–4.8)*</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.52 ± 0.97</td>
<td>3.72 ± 0.91*</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.47 ± 0.36</td>
<td>1.43 ± 0.37</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.22 (0.90–1.78)</td>
<td>1.33 (1.08–1.82)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>144 ± 21</td>
<td>155 ± 24*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>88 ± 10</td>
<td>92 ± 10*</td>
</tr>
<tr>
<td>hsCRP (mg/l)</td>
<td>1.4 (0.8–2.8)</td>
<td>2.0 (1.1–4.2)*</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>2.56 (2.21–2.92)</td>
<td>2.71 (2.35–3.03)*</td>
</tr>
<tr>
<td>Oxidized LDL (units/l)</td>
<td>30.4 (22.4–36.9)</td>
<td>33.0 (27.9–41.9)*</td>
</tr>
<tr>
<td>VCAM-1 (ng/ml)</td>
<td>60.5 (488–808)</td>
<td>669 (553–817)*</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>10.9 (7.6–15.6)</td>
<td>11.1 (7.4–17.5)</td>
</tr>
</tbody>
</table>

Data are means ± SD or median (interquartile range). n = 839. *P < 0.05.

### Table 2—Simple and multiple-adjusted (partial) correlations of log$_{10}$-transformed HOMA-IR with selected variables

<table>
<thead>
<tr>
<th>Feature</th>
<th>Simple P value</th>
<th>Multiple adjusted P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.44 (0.001)</td>
<td>—</td>
</tr>
<tr>
<td>Waist</td>
<td>0.31 (&lt;0.001)</td>
<td>0.07 (0.048)</td>
</tr>
<tr>
<td>A1C</td>
<td>0.26 (0.001)</td>
<td>0.24 (0.001)</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.09 (0.012)</td>
<td>0.07 (0.037)</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>−0.29 (&lt;0.001)</td>
<td>−0.21 (&lt;0.001)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.41 (&lt;0.001)</td>
<td>0.38 (&lt;0.001)</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.22 (&lt;0.001)</td>
<td>0.07 (0.063)</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.23 (&lt;0.001)</td>
<td>0.04 (0.256)</td>
</tr>
<tr>
<td>hsCRP</td>
<td>0.19 (&lt;0.001)</td>
<td>0.09 (0.012)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.21 (&lt;0.001)</td>
<td>0.12 (&lt;0.001)</td>
</tr>
<tr>
<td>Oxidized LDL</td>
<td>0.09 (0.012)</td>
<td>0.08 (0.032)</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>0.09 (0.009)</td>
<td>0.09 (0.011)</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>−0.18 (&lt;0.001)</td>
<td>−0.16 (&lt;0.001)</td>
</tr>
</tbody>
</table>

Data are correlation coefficient (P value) for simple correlations and partial correlation coefficient (P value) for multiple-adjusted correlations. Multiple adjustment included sex, age, smoking, and BMI. n = 839.
When subjects were stratified according to sex, results were confirmed separately in men and women (Table 3). Results were also confirmed separately in those with normal fasting glucose and normal glucose after an oral glucose tolerance test and in those with abnormal glucose regulation (IFG, IGT, or diabetes), as well as in those without and with type 2 diabetes (Table 3). Moreover, the introduction of an interaction term between insulin resistance (HOMA-IR quartile 4) and abnormal glucose regulation (IFG/IGT/diabetes) in a model including these variables yielded similar results, and the interaction term was not significant (model 5, P = 0.63).

Results were similar when subjects developing TIA or undergoing revascularization where excluded and when the analysis was restricted to those with fatal and nonfatal myocardial infarction or stroke (model 5, HR 2.2 [95% CI 1.3–3.9], P = 0.006). In subjects with insulin resistance the 15-year risks of CVD were 17.5 and 35%, according to the absence or presence of diabetes, respectively (P = 0.02 for difference).

When we used insulin rather than HOMA-IR results were quite similar. The HR for CVD in subjects belonging to the top versus the other three quartiles of fasting plasma insulin was 2.0 [95% CI 1.2–3.2], P < 0.005) in model 5. When insulin was modeled as a continuous variable, no association was found with CVD (model 5, per 1 unit change of insulin level, 1.3 [0.99–1.02], P = 0.5).

**CONCLUSIONS** — The main finding of this study is that insulin resistance, as estimated by a simple method based on the measurement of plasma glucose and serum insulin in a single fasting blood sample, was associated with incident symptomatic CVD in a cohort extracted from a population with a low prevalence of diabetes and obesity. The association of insulin resistance with CVD was independent of classic risk factors (including hypertension and obesity, as well as in those without and with type 2 diabetes (model 3). Moreover, the introduction of an interaction term between insulin resistance (HOMA-IR quartile 4) and abnormal glucose regulation (IFG/IGT/diabetes) in a model including these variables yielded similar results, and the interaction term was not significant (model 5, P = 0.63).

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**CONCLUSIONS** — The main finding of this study is that insulin resistance, as estimated by a simple method based on the measurement of plasma glucose and serum insulin in a single fasting blood sample, was associated with incident symptomatic CVD in a cohort extracted from a population with a low prevalence of diabetes and obesity. The association of insulin resistance with CVD was independent of classic risk factors (including hyperglycemia, hypertension, high LDL cholesterol, smoking, and physical activity) and of other components of the metabolic syndrome (obesity, hypertriglyceridemia), and low HDL cholesterol. Most importantly and originally, the association remained significant and virtually unchanged after accounting for novel risk factors related to insulin resistance, including adiponectin and biomarkers indicating a prothrombotic state (high fibrinogen), increased oxidative stress (high circulating oxidized LDL), endothelial dysfunction (high VCAM-1), and chronic mild inflammation (increased hsCRP).

In previous articles, it was reported that several nontraditional risk factors are related to insulin resistance (17–20). In the present article, we confirm and extend these observations. Nontraditional risk factors might represent further links (or intermediate phenotypes) between insulin resistance and CVD. Accordingly, in vitro and in vivo data suggest that insulin reduces platelet aggregation (10) and fibrinogen synthesis (12), possesses anti-inflammatory and antioxidant properties (13,14), and favorably influences the endothelial function and the physiology of the vascular wall (11,15,16). If we assume that insulin resistance is not confined to glucose metabolism but encompasses many, if not all, biological effects of the hormone, these effects of insulin would be blunted in insulin-resistant states. Insulin resistance, therefore, might be viewed as a common denominator and perhaps a cofactor of several metabolic and nonmetabolic disorders representing cardiovascular risk factors. This mechanistic interpretation is strongly supported by studies reporting that an improvement in insulin resistance yields a correction of diverse metabolic abnormalities. This has been observed with lifestyle changes (40,41), as well as with chronic treatment with drugs such as metformin (42) and, to a greater extent, glitazones (43). Interestingly, metformin was the only pharmacological agent achieving a significant prevention of CVD in the UK Prospective Diabetes Study (UKPDS) (44), and glitazones have been shown to reduce carotid intima-media thickness (45) and to prevent coronary restenosis after angioplasty (46) and perhaps reduce risk for CVD in type 2 diabetes (32).

In this article, we report the novel observation that, although it is statistically and perhaps causally related to diverse novel metabolic and nonmetabolic abnormalities, insulin resistance remains independently associated with incident CVD even after accounting for them. The probable interpretation of this finding is that insulin resistance contributes to the development of CVD by pathophysiological mechanisms that are, at least in part, distinct from those that we tried to gauge in the present study. For example, we did not measure plasminogen activator inhibitor-1. Fibrinolytic abnormalities might be a major link between insulin resistance and CVD (47). Other intermediates in the link might be free fatty acids, which are higher in subjects with insulin resistance (48), impair endothelial function (49), and predict CVD (50). A further intermediate abnormality might be proinsulin, which is generally increased in insulin-resistant states (51) and is related to CVD (52).

Further, we recognize that a single assessment of a given biochemical or clinical parameter might be insufficient to fully describe its association with insulin resistance and CVD and that more accu
Insulin resistance and CVD

rate assessments of risk factor (e.g., long-term blood pressure monitoring instead of three spot measurements) or the choice of other parameters reflecting endothelial dysfunction, oxidative stress, or chronic inflammation may better identify their possible role as intermediates between insulin resistance and CVD. Therefore, the hypothesis that insulin resistance might also lead to CVD through its deleterious impact on glucose and lipid metabolism, blood pressure, coagulation abnormalities, inflammation, oxidant stress, and endothelial dysfunction cannot be definitely ruled out.

The present results on the association between insulin resistance and CVD agree with data generated by using our same methodology for estimating insulin resistance (i.e., HOMA) in large samples from the general populations of the U.S. (23,26,31), Finland (22), and Sweden (25). They also are consistent with reports in which insulin sensitivity was more directly assessed with the glucose clamp (29) and the insulin suppression test (21). Of note, our findings extend and strengthen these observations and, for the first time, point out that the association between insulin resistance and CVD is also confirmed when one allows for a greater number of potential confounders, including adiponectin and biomarkers of thrombophilia, inflammation, oxidant stress, and endothelial dysfunction.

The increased cardiovascular risk in subjects with insulin resistance was observed separately in those with and without diabetes. In the latter, however, the HRs increased across the various models and the 95% CIs were broad. This finding is reasonably attributable to the small number of events and sample size relative to the number of variables in the model. The increased risk in diabetic subjects with higher HOMA-IR scores is consistent with results we have found in a study focusing on a large number of patients (24) and in diabetic subjects recruited in the Veterans Affairs HDL Intervention Trial (VA-HIT) (26). Also they are consistent with data generated by insulin tolerance testing in a large sample of Japanese type 2 diabetic patients (28).

In two studies carried out in nondiabetic American Indians (27) and in diabetic Caucasians (30), HOMA-IR was not a predictor of CVD. These discrepancies with all other studies, including ours, might be attributed to differences in the study population, in the statistical methods (in these studies HOMA-IR was modulated as a continuous variable), and to the imperfect estimate of insulin resistance by HOMA. In other words, in some situations HOMA might underestimate the true association between insulin resistance and CVD. In our experience, however, the variability of clamp-measured insulin resistance explained by HOMA-IR was 65% (37). On the other hand, from the clinical perspective, HOMA-IR has the potential to be useful, whereas glucose clamp or other more direct methods are not suitable.

In the discussion of future applications in clinical routine, a suggestion from our study is that insulin resistance might reasonably be included among the metabolic parameters that the physician should evaluate to quantify the overall cardiovascular risk. However, before giving this measure a strong recommendation, further studies are required to confirm our findings and to prove that adding HOMA-IR (or other surrogate measures of insulin resistance) to clinical testing indeed improves prediction accuracy. Moreover, before translation of our results to clinical practice, a standardization of insulin assay and, therefore, HOMA-IR is warranted.

In a mechanistic interpretation of our results, insulin resistance might be reasonably considered among targets for a specific intervention, and the population might be strongly recommended to adopt a lifestyle capable of ameliorating insulin sensitivity. For instance, physical exercise, which was proved to successfully improve insulin sensitivity (53), should be encouraged. In this regard, it might be hypothesized that the lower CVD risk observed in subjects who are less sedentary or who exercise regularly (54) could be attributed also to their higher insulin sensitivity.

In summary, in a general population sample, insulin resistance conferred a greater risk for CVD independently of diverse potential confounders, including traditional and novel risk factors. The identification of intermediate abnormalities linking insulin resistance to CVD deserves further studies. Improvement of insulin sensitivity might be an additional goal in prevention of cardiovascular risk.

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The skillful technical assistance of Federica Moschetta and Monica Zardini is gratefully acknowledged.

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Insulin resistance and CVD


OBJECTIVE — We compared and contrasted cardiovascular disease (CVD) risk factors, subclinical manifestations of CVD, incident coronary heart disease (CHD), and all-cause mortality by categories of impaired glucose regulation in nondiabetic individuals.

RESEARCH DESIGN AND METHODS — The study included 6,888 participants aged 52–75 years who had no history of diabetes or CVD. All-cause mortality and incident CHD were ascertained over a median of 6.3 years of follow-up.

RESULTS — Agreement between fasting and postchallenge glucose impairment was poor: 3,048 subjects (44%) had neither impaired fasting glucose (IFG) nor impaired glucose tolerance (IGT), 1,690 (25%) had isolated IFG, 1,000 (14%) had isolated IGT, and 1,149 (17%) had both IFG and IGT. After adjustment for age, sex, race, and center, subjects with isolated IFG were more likely to smoke, consume alcohol, and had higher mean BMI, waist circumference, LDL cholesterol, and fasting insulin and lower HDL cholesterol than those with isolated IGT, while subjects with isolated IGT had higher mean triglycerides, systolic blood pressure, and white cell counts. Measures of subclinical CVD and rates of all-cause mortality and incident CHD were similar in isolated IFG and isolated IGT.

CONCLUSIONS — Neither isolated IFG nor isolated IGT was associated with a more adverse CVD risk profile.

Type 2 diabetes imposes an increased burden of atherosclerotic cardiovascular disease (CVD), particularly of the coronary arteries, peripheral arteries, and cerebrovascular system (1). However, evidence of CVD risk can also be traced to glucose regulation abnormalities antecedent to diabetes status (2,3). The American Diabetes Association (ADA) and the World Health Organization both recognize “impaired” glucose categories, metabolic stages of glucose homeostasis intermediary between normal and diabetes (4,5). Impaired glucose tolerance (IGT) is defined by both organizations as a 2-h postchallenge glucose level ≥7.8 mmol/l (140 mg/dl) but <11.1 mmol/l (200 mg/dl). Although both organizations originally defined impaired fasting glucose (IFG) as a fasting glucose level between 6.1 mmol/l (110 mg/dl) and 6.9 mmol/l (125 mg/dl) (4,5), the ADA recommended in 2003 that the lower cut point for IFG be reduced to 5.6 mmol/l (100 mg/dl) (6). Studies (7,8) in diverse populations worldwide have reported substantial disagreement between fasting and postchallenge glucose impairment categories, although few studies (9–13) have investigated the impact of the lower cut point of 5.6 mmol/l for IFG.

Possible differences in CVD morbidity and mortality between IFG and IGT remain unclear, although the current evidence indicates that IGT entails greater risk of CVD (2,14). The DECODE (Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe) investigators pooled data from a large number of prospective studies conducted in Europe and found that 2-h glucose was a better predictor than fasting glucose for all-cause and CVD mortality (15,16). Individual prospective studies (17–22) have reported similar findings. None of these studies investigated the impact of the lower cut point of 5.6 mmol/l for IFG on the association between glucose impairment and CVD risk.

The purpose of this investigation was to compare and contrast CVD risk factors, subclinical manifestations of CVD, incident coronary heart disease (CHD), and all-cause mortality by categories of fasting and postchallenge glucose impairment in nondiabetic individuals. Special attention was given to direct comparisons of discordant categories (i.e., isolated IFG and isolated IGT), as such comparisons are more likely to reveal possible differences in the etiology and risk associated with fasting and postchallenge glucose impairment (23).

RESEARCH DESIGN AND METHODS — The Atherosclerosis Risk in Communities (ARIC) Study is a multicenter, prospective investigation of CVD risk factors, subclinical atherosclerosis, and clinical CVD end points. The study was initiated between 1987 and
1989 with 15,792 men and women, aged 45–64 years, drawn from four U.S. communities: Forsyth County, North Carolina; Jackson, Mississippi; the northwest suburbs of Minneapolis, Minnesota; and Washington County, Maryland (24). In addition to the baseline exam (visit 1), a total of three follow-up exams (visits 2–4) were conducted at ∼3-year intervals.

A standardized oral glucose tolerance test (OGTT) was administered during visit 4 between 1996 and 1998. Of 11,656 subjects attending visit 4 (74% of the original cohort), 1,183 were ineligible for the OGTT because they reported pharmacological treatment for diabetes, 237 because they had not fasted at least 10 h before the OGTT was scheduled to begin, and 192 because they had partial removal of the stomach or small intestine or were on kidney dialysis. Of the eligible subjects, 9,126 (86%) were willing to participate in the OGTT. We excluded 107 subjects because of technical difficulties with the OGTT or glucose assays and 65 subjects because they had not fasted at least 8 h before the initial (fasting) blood draw. Participants were also excluded from this analysis if they had prevalent diabetes, based on 2-h postchallenge glucose ≥11.1 mmol/l or fasting glucose ≥7.0 mmol/l (n = 1,153) or self-reported physician diagnosis (n = 34). Subjects with a history of CHD (n = 630) or stroke or transient ischemic attacks (n = 207) were excluded based on self-report and active surveillance of the cohort for hospitalized events between visits 1 and 4. Due to insufficient numbers, 18 participants of racial/ethnic groups other than black or white were excluded as well as 24 black subjects in either the Minneapolis and Washington County centers. After all exclusions, 6,888 participants were available for this study.

CVD risk factors and glucose

Unless otherwise indicated, CVD risk factors were measured at visit 4. Cigarette smoking and alcohol drinking status were categorized as current and not current (former and never). Current use of lipid-lowering and antihypertensive medications was determined by questionnaire. Educational attainment was categorized dichotomously as no college versus some college based on interviews at visit 1. BMI was derived from measured height and weight, and waist circumference was measured at the umbilical level. Blood samples were drawn from an antecubital vein with minimal trauma at fasting and 2-h postchallenge. Glucose levels were determined by a hexokinase assay procedure. The reliability coefficient was 0.99 based on blinded duplicate samples collected from 430 ARIC subjects at visit 4. Total cholesterol (25) and triglycerides (26) were measured by enzymatic methods. HDL cholesterol was measured after dextran-magnesium precipitation of non-HDL cholesterol lipoproteins (27), and LDL cholesterol was estimated (28). Insulin was measured by enzyme-linked immunosorbent assay (Boehringer Mannheim, Indianapolis, IN). Blood pressure was measured three times using a random-zero sphygmomanometer. The mean of the last two measurements was used for analysis. White cell counts were determined by local reference laboratories using automated particle counters. Only the Forsyth County and Washington County field centers elected to measure white cell counts. For each subject, we determined the number of metabolic syndrome abnormalities based on the National Cholesterol Education Program Adult Treatment Panel III definition (29). We excluded elevated fasting glucose from these counts because it was used to classify subjects into glucose impairment categories that formed the basis for comparison.

Subclinical CVD

Mean intima-media thickness (IMT) and presence of atherosclerotic plaque were both ascertained by B mode ultrasound at six 1-cm segments of the carotid artery: the left and right internal, bifurcation, and common. Trained ultrasound readers evaluated carotid IMT (in millimeters) for each site and secondarily indicated whether there was presence of a lesion (plaque) at any site, based on published criteria (30). Measurements of IMT at all six carotid segments were not attained for every participant and were imputed by maximum likelihood methods. The number (percentage) of subjects with imputed values for zero, one, two, three, four, and five segments was 1,129 (28%), 1,008 (25%), 771 (19%), 594 (15%), 396 (10%), and 191 (5%), respectively. Details of the measurement and imputation methods are described elsewhere (31,32). Resting ankle and brachial blood pressure readings divided by the average of two brachial systolic blood pressure readings (33). Peripheral arterial disease was defined as ankle-brachial index of ≤0.9 for men and ≤0.85 for women (34). The ultrasonic and ankle-brachial measurements were obtained on approximately half of the ARIC cohort at visit 4. Left ventricular hypertrophy was determined electrocardiographically by Cornell voltage criteria ≥28 mm for men and ≥22 mm for women (35,36).

All-cause mortality and incident CHD

Vital status was determined through annual follow-up contacts with cohort members and searches of local hospital records and the National Death Index. Incident CHD was determined by contacting participants annually to identify hospitalizations during the previous year and by surveying discharge lists from local hospitals and death certificates from state vital statistics offices for potential CVD events and validated by computer algorithm and physician review. Details on quality assurance for ascertainment and classification of CHD events have been published elsewhere (24,37). For this analysis, incident CHD was defined as definite or probable myocardial infarction, fatal CHD, or cardiac procedure. Follow-up time was computed as the time between visit 4 and the first event (i.e., death or incident CHD, depending on analysis), loss to follow-up, or 31 December 2003, whichever was earliest.

Statistical analysis

Data management and analysis were performed with SAS version 9.1 (SAS Institute, Cary, NC). Adjusted means of demographic and behavioral factors, physiologic factors, and subclinical disease measures among glucose impairment categories were determined by ANCOVA using the LS MEANS option in SAS PROC GLM. Multiple logistic regression was performed to obtain adjusted proportions for dichotomous risk factors. Cox proportional hazards regression was used to evaluate associations between glucose categories and all-cause mortality or incident CHD.

RESULTS — Among 6,888 participants, 47% (3,255) were white women, 36% (2,461) were white men, 11% (776) were black women, and 6% (396) were black men. Over half of the participants (56%) were classified as having IFG, IGT,
or both (Table 1). IFG ($n = 2,839$, 42%) was more common, overall, than IGT ($n = 2,150$, 31%), with isolated IFG much more common than isolated IGT (25 vs. 14%, respectively). There were substantial differences in the distribution of glucose categories by sex and race: for example, the ratio of isolated IFG to isolated IGT were higher in black women (2.3), white men (3.4), and black men (6.5) compared with white women (0.77). Kappa coefficients evaluating agreement between IFG and IGT categories were highest for black women (0.24), followed by white women (0.20), black men (0.15), and white men (0.14). Kappa coefficients were slightly lower when the higher 6.1 mmol/l cut point for fasting glucose was used to define IFG. The Pearson correlation coefficient between fasting and 2-h glucose was 0.27.

In general, subjects with both IFG and IGT had a more adverse CVD risk factor profile than those with neither condition (Table 2). However, neither isolated IFG nor isolated IGT was associated with a consistently worse pattern of CVD risk factors. Subjects with isolated IFG were younger, on average, than subjects with isolated IGT. After adjusting for age, sex, race, and center, subjects with isolated IFG were more likely to smoke, consume alcohol, and had higher mean BMI.

### Table 1—Distribution of fasting and postchallenge glucose impairment by sex and race

<table>
<thead>
<tr>
<th>Glucose category</th>
<th>Normal*</th>
<th>Isolated IFG†</th>
<th>Isolated IGT‡</th>
<th>IFG/IGT§</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>White women</td>
<td>1,623 (50)</td>
<td>502 (15)</td>
<td>649 (20)</td>
<td>481 (15)</td>
<td>3,255</td>
</tr>
<tr>
<td>Black women</td>
<td>334 (43)</td>
<td>199 (26)</td>
<td>86 (11)</td>
<td>157 (20)</td>
<td>776</td>
</tr>
<tr>
<td>White men</td>
<td>940 (38)</td>
<td>834 (34)</td>
<td>242 (10)</td>
<td>445 (18)</td>
<td>2,461</td>
</tr>
<tr>
<td>Black men</td>
<td>151 (38)</td>
<td>155 (39)</td>
<td>24 (6)</td>
<td>66 (17)</td>
<td>396</td>
</tr>
<tr>
<td>Total</td>
<td>3,048 (44)</td>
<td>1,690 (25)</td>
<td>1,001 (14)</td>
<td>1,149 (17)</td>
<td>6,888</td>
</tr>
</tbody>
</table>

Data are n (%). *Fasting glucose <5.6 mmol/l and postchallenge glucose <7.8 mmol/l. †Fasting glucose between 5.6 and 6.9 mmol/l and postchallenge glucose <7.8 mmol/l. ‡Fasting glucose <5.6 mmol/l and postchallenge glucose between 7.8 and 11.0 mmol/l. §Fasting glucose between 5.6 and 6.9 mmol/l and postchallenge glucose between 7.8 and 11.0 mmol/l.

### Table 2—Adjusted means ± SEs or percentages of CVD risk factors and subclinical disease by fasting and postchallenge glucose impairment

<table>
<thead>
<tr>
<th>Risk factor *</th>
<th>Glucose category</th>
<th>Normal†</th>
<th>Isolated IFG‡</th>
<th>Isolated IGT§</th>
<th>IFG/IGT§</th>
<th>P value¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3,048</td>
<td>1,690</td>
<td>1,001</td>
<td>1,149</td>
<td>6,888</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>61.8 ± 0.1</td>
<td>61.8 ± 0.1</td>
<td>63.5 ± 0.2</td>
<td>63.3 ± 0.2</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>15.1</td>
<td>15.0</td>
<td>11.6</td>
<td>11.8</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Alcohol intake (%)</td>
<td>55.2</td>
<td>56.6</td>
<td>50.3</td>
<td>53.1</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Education (%)</td>
<td>44.7</td>
<td>39.9</td>
<td>38.3</td>
<td>38.2</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.9 ± 0.1</td>
<td>29.2 ± 0.1</td>
<td>28.1 ± 0.2</td>
<td>30.4 ± 0.1</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>96.5 ± 0.2</td>
<td>102.8 ± 0.3</td>
<td>100.3 ± 0.4</td>
<td>105.9 ± 0.4</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.40 ± 0.01</td>
<td>1.31 ± 0.01</td>
<td>1.36 ± 0.01</td>
<td>1.23 ± 0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.16 ± 0.02</td>
<td>3.33 ± 0.02</td>
<td>3.14 ± 0.03</td>
<td>3.24 ± 0.03</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.20</td>
<td>1.39</td>
<td>1.51</td>
<td>1.61</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Lipid-lowering medication (%)</td>
<td>8.4</td>
<td>9.5</td>
<td>13.2</td>
<td>12.6</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.12 ± 0.01</td>
<td>5.86 ± 0.01</td>
<td>5.18 ± 0.01</td>
<td>6.00 ± 0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>2-h glucose (mmol/l)</td>
<td>5.73 ± 0.02</td>
<td>6.12 ± 0.03</td>
<td>8.91 ± 0.03</td>
<td>9.13 ± 0.03</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>57.0</td>
<td>72.7</td>
<td>61.5</td>
<td>86.5</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>123.5 ± 0.3</td>
<td>125.4 ± 0.4</td>
<td>127.7 ± 0.6</td>
<td>129.0 ± 0.5</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>70.7 ± 0.2</td>
<td>71.1 ± 0.2</td>
<td>71.3 ± 0.3</td>
<td>72.1 ± 0.3</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>Antihypertensive medication (%)</td>
<td>23.1</td>
<td>28.7</td>
<td>31.4</td>
<td>39.4</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Metabolic syndrome abnormalities**</td>
<td>1.50 ± 0.02</td>
<td>1.92 ± 0.03</td>
<td>1.95 ± 0.04</td>
<td>2.35 ± 0.03</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>White cell count ($10^9$ cells/l)††</td>
<td>6.0 ± 0.1</td>
<td>6.3 ± 0.1</td>
<td>6.6 ± 0.1</td>
<td>6.5 ± 0.1</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Carotid IMT (mm)††</td>
<td>0.760 ± 0.005</td>
<td>0.781 ± 0.007</td>
<td>0.779 ± 0.008</td>
<td>0.802 ± 0.008</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Carotid plaque at any site (%)††</td>
<td>35.4</td>
<td>39.2</td>
<td>37.0</td>
<td>35.9</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Peripheral artery disease (%)††</td>
<td>4.3</td>
<td>5.3</td>
<td>4.0</td>
<td>3.5</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Left ventricular hypertrophy (%)††</td>
<td>1.8</td>
<td>2.3</td>
<td>3.2</td>
<td>3.3</td>
<td>0.27</td>
<td></td>
</tr>
</tbody>
</table>

*All means or proportions except age adjusted for age, sex, race, and center. †Fasting glucose <5.6 mmol/l and postchallenge glucose <7.8 mmol/l. ‡Fasting glucose between 5.6 and 6.9 mmol/l and postchallenge glucose <7.8 mmol/l. §Fasting glucose between 5.6 and 6.9 mmol/l and postchallenge glucose between 7.8 and 11.0 mmol/l. ¶Test for difference in mean or percentage between isolated IFG and isolated IGT. #Geometric means. **Number of metabolic syndrome abnormalities (between 0 and 4), excluding elevated fasting glucose. ††Only measured on a subset of subjects at visit 4. Sample sizes are 3,587 for white cell count, 4,089 for carotid IMT, 3,716 for carotid plaque, 3,895 for peripheral artery disease, and 4,950 for left ventricular hypertrophy.
Glucose impairment and cardiometabolic risk

Table 3—All-cause mortality and CHD incidence by fasting and postchallenge glucose impairment

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Glucose category</th>
<th>Normal*</th>
<th>Isolated IFG†</th>
<th>Isolated IGT‡</th>
<th>IFG/IGT§</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause mortality</td>
<td>Rate (per 1,000 person-years)</td>
<td>7.0</td>
<td>7.6</td>
<td>8.9</td>
<td>8.5</td>
</tr>
<tr>
<td>Deaths</td>
<td>19,417</td>
<td>10,697</td>
<td>6,298</td>
<td>7,201</td>
<td></td>
</tr>
<tr>
<td>Incident CHD</td>
<td>Events</td>
<td>151</td>
<td>104</td>
<td>46</td>
<td>73</td>
</tr>
<tr>
<td>Rate (per 1,000 person-years)</td>
<td>8.0</td>
<td>10.0</td>
<td>7.4</td>
<td>10.4</td>
<td></td>
</tr>
<tr>
<td>Hazard ratio (95% CI), model 1</td>
<td>1.00 (reference)</td>
<td>0.92 (0.70–1.22)</td>
<td>1.19 (0.87–1.63)</td>
<td>0.98 (0.72–1.32)</td>
<td></td>
</tr>
<tr>
<td>Hazard ratio (95% CI), model 2</td>
<td>1.00 (reference)</td>
<td>0.93 (0.70–1.24)</td>
<td>1.16 (0.83–1.60)</td>
<td>1.03 (0.75–1.42)</td>
<td></td>
</tr>
</tbody>
</table>

*Fasting glucose <5.6 mmol/l and postchallenge glucose <7.8 mmol/l. †Fasting glucose between 5.6 and 6.9 mmol/l and postchallenge glucose <7.8 mmol/l. ‡Fasting glucose <5.6 mmol/l and postchallenge glucose between 7.8 and 11.0 mmol/l. §Fasting glucose between 5.6 and 6.9 mmol/l and postchallenge glucose between 7.8 and 11.0 mmol/l. ¶Adjusted for age, sex, race, and center. ‡‡Adjusted for model 1 variables and smoking status, hypertension, LDL cholesterol, HDL cholesterol, triglycerides, use of lipid-lowering medications, BMI, and waist circumference.

CONCLUSIONS—We observed poor agreement between IFG and IGT using current ADA definitions for glucose impairment in nondiabetic individuals. Approximately 39% of subjects in the ARIC cohort without diabetes or a history of CVD were discordant on glucose impairment categories (i.e., isolated IFG or isolated IGT). In cross-sectional analysis, subjects with combined glucose impairment (IFG/IGT) had the least favorable pattern of CVD risk factors. Isolated IFG and isolated IGT had differing patterns of risk factors, but neither category had a consistently worse CVD risk profile or excess of metabolic syndrome abnormalities. Measures of subclinical CVD and rates of all-cause mortality and incident CHD did not differ significantly between isolated IFG and isolated IGT.

In 2003 the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus recommended that the cut point for IFG be lowered from 6.1 to 5.6 mmol/l (6). The primary rationale for the change was to make the sensitivity and specificity of IFG similar to that of IGT in predicting future risk of type 2 diabetes (6,38). The change has been controversial (9,38–42). Population-based data from the U.S. and other countries indicate that the prevalence of IFG increased two- to fourfold upon application of the new criteria (9,12,39,43,44), with the largest increases in IFG in younger age-groups (39). A recent report from the National Health and Nutrition Examination Survey 1999–2002 estimated that 26 million U.S. adults have IFG using the lower cut...
point (45). In the present study, the overall prevalence of isolated IFG was higher than the prevalence of isolated IGT among subjects without a history of CVD, suggesting that the new definition of IFG does not necessarily produce equivalent numbers of subjects in the IFG and IGT categories. Furthermore, the lower cut point for IFG produced only slightly better agreement between IFG and IGT categories.

The prevalence of isolated IFG and isolated IGT differed by sex and, to a lesser extent, by race. More specifically, isolated IFG was more common than isolated IGT among black men and women and white men but not among white women. Studies with the higher (11,46) and lower (11,13) cut point for IFG have reported, at least in relative terms, that women are more likely to have isolated IGT and men are more likely to have isolated IFG. Whether there are important differences according to race or ethnicity is less clear. Based on World Health Organization definitions, IGT was more common than IFG in both non-Hispanic white and non-Hispanic black subjects in the U.S., but discrepancies between IGT prevalence and IFG prevalence were most evident in non-Hispanic white women (47).

Cross-sectional studies (11,13,48–51), most using the higher IFG cut point, have found inconsistent differences in CVD risk factors between isolated IFG and isolated IGT categories. Although we found statistically significant differences between isolated IFG and isolated IGT for most CVD risk factors, absolute differences were generally small and not consistently higher in one category or the other. An Expert Consensus Workshop of the International Diabetes Federation recently concluded that IFG is characterized by raised hepatic glucose output and deficits in early insulin secretion, while IGT is characterized by peripheral insulin resistance (2). Data from the present study suggest that subjects with IFG are more insulin resistant if fasting insulin is interpreted as a surrogate measure of insulin resistance. However, studies using direct measures of insulin resistance (i.e., euglycemic-hyperinsulinemic clamp or frequently sampled intravenous glucose tolerance test) have found that subjects with isolated IGT have similar (51), if not greater (48,50), deficiencies in insulin action compared with subjects with isolated IFG.

Neither isolated IFG nor isolated IGT was more strongly associated with measures of subclinical CVD in the ARIC cohort. Our data appear to contrast with that of the RIAD (Risk Factors in IGT for Atherosclerosis and Diabetes) Study, which found that carotid IMT was more strongly associated with IGT than IFG in middle-aged subjects (52). We are unsure why patterns of association between IFG, IGT, and carotid IMT differ in the two studies. Associations between fasting glucose and carotid IMT among nondiabetic subjects were weak in ARIC at visit 1 (53); our data corroborate those earlier findings.

Unlike the present study, a meta-regression analysis of 20 prospective studies found a significant graded relationship between CVD events and glucose level, both fasting and postchallenge (3). Some studies (44,54,55) have reported a J-shaped relation between fasting or 2-h postchallenge glucose and CVD or total mortality, with subjects having the lowest glucose levels having slightly increased risk relative to those in low-normal categories. However, the lack of association between fasting glucose and incident CHD in our study is consistent with earlier reports from the ARIC cohort. In one analysis, fasting glucose levels <6.4 mmol/l at the baseline exam (visit 1) were not associated with incident CHD over 4–7 years of follow-up (56). In another analysis, fasting glucose measured at visit 2 was not associated with incident CHD over 8–10 years of follow-up among non-diabetic subjects (57). By contrast, A1C levels >4.6% at visit 2 demonstrated a positive, graded association with incident CHD among individuals without diabetes (57).

It is unclear why the present study failed to find an association between postchallenge glycemia and all-cause mortality or incident CHD that has been reported elsewhere (13,15–22,58,59). The median follow-up of 6.3 years may have been too short for an association to emerge, as many earlier studies had longer follow-up for nonfatal and fatal outcomes. In the Whitehall Study of British men, decreased survival among glucose-intolerant subjects only became apparent between 5 and 10 years of follow-up (58). However, in an analysis of 14 European cohorts by the DECODE group, hazard ratios for CVD death associated with IGT and/or IFG were lower after 10 years of follow-up compared with 5 years of follow-up (60). It is possible that in studies with longer follow-up, a high proportion of individuals with IGT at baseline develop diabetes as an intermediate condition before onset of CHD or death. Associations between baseline IGT and these long-term outcomes may therefore be explained by greater risk of diabetes among those with IGT. However, development of diabetes during follow-up was not found to be an intermediate factor linking baseline IGT and incident CHD in a Finnish cohort study (61).

Approximately 14% of subjects without prevalent CVD and diabetes were excluded from our analysis for other reasons, mainly because they refused the OGTT. Our results may have underestimated the association between glucose impairment and all-cause mortality or incident CHD if subjects with glucose impairment who developed these outcomes were more likely to be excluded. Subjects who were excluded were more likely to be African American and smoke and had lower BMI, higher HDL cholesterol, and higher systolic blood pressure than those not excluded. After adjusting for age, sex, race, and center, subjects who were excluded had higher rates of all-cause mortality (hazard ratio 1.53 [95% CI 1.21–1.94]) but similar rates of incident CHD (1.16 [0.88–1.52]) compared with subjects not excluded. However, mean fasting glucose was similar in excluded subjects compared with those not excluded, and the magnitude of association between fasting glucose and all-cause mortality or incident CHD was similar in the two groups, suggesting that the results are representative for all subjects in the cohort without CVD and diabetes.

Possible differences in the etiology and long-term risk associated with IFG and IGT are important to delineate in light of the population impact of CVD morbidity and mortality and inconsistent use of the OGTT in clinical settings. However, neither IFG nor IGT were important predictors of incident CHD or all-cause mortality in the ARIC cohort. The relatively poor agreement between fasting and postchallenge glucose levels and differing patterns of association with CVD risk factors suggest that IFG and IGT do not represent metabolically equivalent categories.

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High Risk of Cardiovascular Mortality in Individuals With Impaired Fasting Glucose Is Explained by Conversion to Diabetes

The Hoorn Study

JOHNS M. RIJKELIJKHUIZEN, PHD1
GIEI. NJPELS, MD, PHD1,2
ROBERT J. HEINE, MD, PHD1,3

LEX M. BOUTER, PHD1
COEN D.A. STEHOUWER, MD, PHD1,4
JACQUELINE M. DEKKER, PHD1

OBJECTIVE — To optimize identification of future diabetic patients, the American Diabetes Association (ADA) introduced criteria for impaired fasting glucose (IFG) in 1997 (IFG 6.1 mmol/l [IFG6.1]) and lowered the threshold from 6.1 to 5.6 mmol/l (IFG5.6) in 2003. Our aim was to assess the consequences of lowering the IFG cutoff on the risk of cardiovascular disease (CVD) mortality and to evaluate whether this risk is explained by a conversion to type 2 diabetes within 6.4 years.

RESEARCH DESIGN AND METHODS — In a population-based cohort, the Hoorn Study, plasma glucose was determined in 1989 and 1996 (n = 1,428). Subjects were classified in 1989 according to 1997 and 2003 ADA criteria. Subjects with IFG in 1989 were further classified according to diabetes status in 1996. Hazard ratios for CVD mortality (n = 81) in the period 1996–2005 were adjusted for age and sex.

RESULTS — Subjects with IFG6.1, but not IFG5.6, had a significantly higher CVD mortality risk than normal fasting glucose (NFG) subjects. Subjects who converted from IFG to diabetes (IFG6.1: 42%; IFG5.6: 21%) had a more than twofold risk of CVD mortality (IFG6.1: 2.47 [1.17–5.19]; IFG5.6: 2.14 [1.12–4.10]) than subjects with NFG. IFG subjects who did not develop diabetes did not have significantly higher CVD mortality risks (IFG6.1: 1.50 [0.72–3.15]; IFG5.6: 1.15 [0.69–1.93]).

CONCLUSIONS — The lower cutoff for IFG (ADA 2003 criteria) results in a category of IFG that no longer represents a high-risk state of CVD. Furthermore, only subjects who convert from IFG to diabetes have a high risk of CVD mortality.


Impaired glucose regulation is a high-risk state for type 2 diabetes and cardiovascular disease (CVD) (1). Impaired glucose regulation can be either impaired fasting glucose (IFG) or impaired glucose tolerance (IGT), i.e., elevated glucose levels 2 h after the 75-g oral glucose tolerance test (OGTT). Both conditions contribute independently to the high risk of diabetes and CVD but show limited agreement (2,3). When the American Diabetes Association (ADA) introduced fasting diagnostic criteria in 1997 to avoid the OGTT (4), it has been shown that the elevated CVD risk was also present in subjects who remained IGT or returned to normal glucose tolerance (6). Such information is not yet available for subjects with IFG.

The aims of the present study were 1) to assess the impact of lowering the IFG cutoff on the prevalence of IFG and the incidence of diabetes among subjects classified as IFG in 1989, 2) to assess the impact of lowering the IFG cutoff on the associated risk of all-cause and CVD mortality, and 3) to study if the association between IFG and the risk of all-cause and CVD mortality is independent of the conversion to diabetes during follow-up.

RESEARCH DESIGN AND METHODS

Study population
The Hoorn Study is a population-based cohort study of glucose intolerance in a general Dutch population, which started in 1989. The study design has been described in detail elsewhere (7). The study cohort consisted of 2,484 Caucasian men and women in 1989. A follow-up examination was performed between January 1996 and December 1998 (mean follow-up duration was 6.4 years). Of the
initial cohort, 150 subjects had died and 108 subjects had moved out of Hoorn. A total of 140 other subjects were not invited because of logistic reasons. Of the remaining 2,086 subjects, 1,513 participated in the follow-up study in 1996. Details of this follow-up study have been described previously (3). All subjects have been followed with respect to mortality. The study was approved by the Ethics Committee of the VU University Medical Center. Informed consent was obtained from all participants.

**Glucose intolerance classification**
Both in 1989 and 1996, a 75-g OGTT was administered after an overnight fast. Fasting plasma glucose (FPG) and 2-h postload plasma glucose levels were determined with a glucose dehydrogenase method (Merck, Darmstadt, Germany) in 1989 and with the hexokinase method (Boehringer Mannheim, Mannheim, Germany) in 1996. Subjects (n = 41) who had already been using insulin, blood glucose-lowering agents, or a diet for diabetes were marked as “known diabetes mellitus” and were excluded from the analyses. Furthermore, 44 subjects were excluded because of missing information of plasma glucose values. Therefore, the analyses in the present study were performed on 1,428 subjects who completed both the measurements in 1989 and 1996. The prevalence of IFG according to both the 1997 ADA criteria (IFG6.1: IFG with FPG 6.1–7.0 mmol/l) and 2003 ADA criteria (IFG5.6: IFG with FPG 5.6–7.0 mmol/l) was determined for 1989 and 1996. Furthermore, all subjects were classified with 1997 and 2003 ADA criteria as normal fasting glucose (NFG), impaired fasting glucose (IFG), or newly discovered diabetes according to their glucose status in 1989. For each set of criteria, for subjects with IFG status in 1989, we distinguished between individuals who had and who had not converted to diabetes at the follow-up examination in 1996–1998. For a timeline of the design of the present study, see Fig. 1.

**Measurements**
Weight and height were measured with subjects wearing underwear only. BMI was calculated as the ratio of weight and squared height. Waist and hip circumferences were measured according to a standardized procedure (8). Waist-to-hip ratio was defined as waist circumference divided by hip circumference. Triglycerides, total cholesterol, and HDL cholesterol were determined from fasting blood samples by enzymatic techniques (Boehringer Mannheim). The Friedewald formula was used to calculate the level of LDL cholesterol (9).

**Follow-up of mortality**
There is a continuous registration of the mortality of participants of the Hoorn Study performed in cooperation with the municipal registry of the city of Hoorn. Information about causes of death was extracted from medical records of general practitioners and the local hospital. Causes of death were coded according to the ICD-9 (10). Cardiovascular mortality was defined as ICD-9 codes 390–459 (diseases of the circulatory system) or 798 (sudden death, cause unknown), because sudden death is generally caused by CVD (11). The vital status of subjects who had moved out of Hoorn was obtained from the municipal registries of the cities to which they had moved. Follow-up data of the period from the physical examination in 1996 until 1 January 2005 were used to calculate CVD mortality risks (Fig. 1).

**Statistical analysis**
Relative risks of all-cause and CVD mortality during the period after the physical examination in 1996 were estimated by Cox proportional hazards analyses. For both 1997 and 2003 ADA diagnostic criteria, hazard ratios and 95% CIs for all-cause and CVD mortality were obtained for subjects with IFG status or diabetes relative to those with NFG status. Because this is a descriptive study, all models were adjusted for age and sex only. Values are presented as means ± SD. Statistical analyses were performed using standard software (SPSS 12.0.2). P values ≤0.05 were considered significant.

**RESULTS**
The characteristics of the population classified according to both 1997 and 2003 ADA criteria in 1989 are shown in Table 1. Subjects with IFG5.6 had a healthier profile than subjects with IFG6.1 (e.g., lower blood pressure and waist circumference). The prevalence of IFG5.6 was 33.2% (95% CI 30.8–35.6) and 10.1% (8.6–11.6) for IFG6.1. The mean FPG level of the study population was 5.6 ± 1.3 mmol/l in 1989 and, after a mean follow-up of 6.4 years, had increased to 6.2 ± 1.3 mmol/l in 1996. As a result, the prevalence of IFG5.6 increased to 55.7% (95% CI 53.2–58.2) and 23.6% (23.4–27.8) for IFG6.1.

Of the subjects with IFG6.1 in 1989, 42% had progressed to diabetes (Table 2) and the incidence rate was 66.5/1,000 person-years (95% CI 49.9–83.0). Of the IFG6.1 subjects, only 21% developed diabetes (Table 2), with an incidence rate 32.7/1,000 person-years (26.3–39.1).

In the period from the physical examination in 1996 until 1 January 2005, 192 subjects died. For 184 of the deceased (95.8%), the cause of death could be retrieved; 81 subjects died from CVD. Age- and sex-adjusted hazard ratios for all-cause and CVD mortality are shown in Table 3. The hazard ratio of CVD mortality for subjects with IFG6.1 was 1.87 (1.07–3.25) relative to the reference category with normal glucose levels (NFG). The hazard ratio for CVD mortality for the IFG5.6 group was lower and not statistically significant: 1.37 (0.87–2.16). The associations with all-cause mortality showed similar trends for both criteria: the all-cause and CVD mortality risks for subjects classified as IFG were both significantly higher than for NFG subjects and similar to the risks for newly detected diabetic subjects.
Fasting glucose and cardiovascular mortality

Table 1—Characteristics of the population classified according to both 1997 and 2003 ADA criteria in 1989

<table>
<thead>
<tr>
<th></th>
<th>1997 ADA criteria</th>
<th>2003 ADA criteria</th>
<th>Both criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NFG</td>
<td>IFG</td>
<td>NFG</td>
</tr>
<tr>
<td>n</td>
<td>1,217</td>
<td>149</td>
<td>878</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.1 ± 6.8</td>
<td>62.5 ± 6.9</td>
<td>59.8 ± 6.9</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>44.5</td>
<td>54.4</td>
<td>41.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.1 ± 8.6</td>
<td>169.1 ± 8.6</td>
<td>168.8 ± 8.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.7 ± 10.7</td>
<td>78.9 ± 11.2</td>
<td>73.7 ± 10.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1 ± 3.1</td>
<td>27.6 ± 3.6</td>
<td>25.8 ± 3.0</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>6.6 ± 1.1</td>
<td>6.7 ± 1.1</td>
<td>6.5 ± 1.1</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.36 ± 0.37</td>
<td>1.30 ± 0.34</td>
<td>1.38 ± 0.37</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>4.6 ± 1.0</td>
<td>4.6 ± 1.1</td>
<td>4.5 ± 1.0</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.5 ± 0.8</td>
<td>1.7 ± 0.8</td>
<td>1.4 ± 0.7</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>101.5 ± 6.2</td>
<td>102.8 ± 7.1</td>
<td>101.2 ± 6.0</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>89.0 ± 10.0</td>
<td>95.2 ± 10.7</td>
<td>87.7 ± 9.8</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.88 ± 0.08</td>
<td>0.93 ± 0.07</td>
<td>0.87 ± 0.08</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81.4 ± 10.1</td>
<td>84.9 ± 9.7</td>
<td>80.6 ± 9.9</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>131.4 ± 19.2</td>
<td>144.5 ± 19.6</td>
<td>129.5 ± 18.2</td>
</tr>
</tbody>
</table>

Data are means ± SD unless otherwise indicated. Type 2 diabetes represents newly discovered type 2 diabetes.

When comparing subjects with IFG status in 1989 who did and who did not convert to diabetes in 1996, Cox regression revealed that the risks for all-cause and CVD mortality were not significantly higher than for NFG for subjects who had not converted to diabetes (Table 3). Subjects with IFG5.6 or IFG6.1 status who had converted to diabetes during the 6.4 years of follow-up had higher all-cause and CVD mortality risks than subjects who were classified as newly discovered diabetes in 1989 and had significantly (up to 2.5 times) higher all-cause and CVD mortality risks than subjects with normal glucose status at that time.

**CONCLUSIONS** — The present study showed that lowering the cutoff for IFG from 6.1 to 5.6 mmol/l increases the prevalence of IFG and reduces the incidence rate of diabetes. Furthermore, the hazard ratios of all-cause and cardiovascular mortality were lower for IFG5.6 than for IFG6.1. In addition, the association between IFG and the risk of all-cause and CVD mortality is only present in subjects who had converted to diabetes during follow-up.

**Limitations of the study**

A number of possible study limitations needs to be discussed. In this study, we used a subpopulation of the Hoorn Study. The baseline cohort of the Hoorn Study in 1989 was a random sample of the population of the municipality of Hoorn, aged 50–70 years. For both the examinations in 1989 and 1996, ~70% response was reached; thus, the rate of participation suggests good representativeness. In population studies, however, generally, participants are healthier than nonparticipants. Therefore, both the prevalence and the incidence of diabetes might be somewhat underestimated. A limitation of the present study is the small number of cases in the subgroups, thus limiting the power of our study. However, the large difference in the estimates of those who did and those who did not convert, along with the CIs, show a very clear picture. Furthermore, conclusions about conversion to diabetes and about mortality were based on those subjects who survived the 6.4 years between the first (1989) and second (1996) physical examination. Thus, numbers of conversion and mortality may have been slightly higher. In addition, this is one of very few population studies with repeated glucose testing (after 6.4 years) and subsequently 10 years of follow-up of mortality.

**Consequences of lowering the IFG cutoff for the prevalence of IFG and incidence rate of diabetes**

We found a considerably higher prevalence of IFG5.6 than IFG6.1. Similar results have also been described by Borch-Johnsen et al. (12) and Davidson et al. (13), with IFG ranging from 24.1 to 38.8%. One of the reasons for proposing the 2003 ADA criteria was to increase sensitivity for the prediction of future diabetes. However, this is at the cost of specificity (5). According to our analyses, the fraction of IFG5.6 subjects developing diabetes decreased from 40 to 20% compared with IFG6.1. Vaccaro and Riccardi (14) studied the risk of progression from IFG to diabetes in the Italian Telephone Company (with a lower mean age than in our study) and found an even lower percentage of 12.5% when subjects were classified by 2003 ADA criteria. Thus, with the new criteria, the prevalence of IFG is increased considerably, while this is accompanied by a lower risk of future diabetes.

Table 2—A 6.4-year change in glucose tolerance status of subjects classified as IFG in 1989

<table>
<thead>
<tr>
<th></th>
<th>1997 ADA criteria</th>
<th>2003 ADA criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conversion to NFG</td>
<td>28 (18.8)</td>
<td>33 (6.8)</td>
</tr>
<tr>
<td>Remained IFG</td>
<td>59 (39.6)</td>
<td>354 (72.5)</td>
</tr>
<tr>
<td>Conversion to type 2 diabetes</td>
<td>62 (41.6)</td>
<td>101 (20.7)</td>
</tr>
<tr>
<td>Total</td>
<td>149 (100)</td>
<td>488 (100)</td>
</tr>
</tbody>
</table>

Data are n (%).
Consequences of lowering the IFG cutoff for CVD risk
Several studies have shown that IGT is a risk factor for CVD mortality (6,15,16), but there is debate whether this is also true for IFG (17). Qiao et al. (6) reported that IGT is a risk predictor for CVD morbidity and mortality and for all-cause mortality independent of the development of overt diabetes. We and others have previously shown that high 2-h glucose after an OGTT is more closely associated with an adverse CVD risk profile and CVD risk (2,18). In the present study, we showed that optimizing the cutoff for IFG for identification of future diabetes does not correspond to the risk of all-cause and CVD mortality and that the CVD mortality risk of IFG subjects is only present in those subjects who convert to diabetes. Subjects with IFG5.6 at baseline who had diabetes at follow-up had significantly lower HDL cholesterol and higher triglycerides, waist circumference, waist-to-hip ratio, and systolic blood pressure than subjects who did not have diabetes at follow-up (data not shown), indicating large differences in CVD risk profile between subjects who do and do not convert to diabetes. In subjects with IFG6.1, differences were smaller and only statistically significant for triglycerides levels (data not shown), indicating a more homogeneous group of IFG subjects with less relatively healthy subjects included.

We previously reported lower CVD mortality risk in subjects with FPG between 5.6 and 6.0 mmol/l (18). Now, with an even longer follow-up duration, we find that CVD mortality risk in the IFG5.6 group is not significantly higher than NFG subjects. Balkau et al. (19) reported J-shaped relationships with fasting glucose concentrations and all-cause mortality in men in the Paris Prospective Study, with a lowest mortality around FPG levels of 5.5 mmol/l. The DECODE (Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Europe) study group also found that the 7-year risk of coronary heart disease was lower in women with a fasting glucose value between 5.6 and 6.0 mmol/l compared with women with NFG (20). Because of such findings, the lower cutoff of the 2003 ADA criteria for IFG has been questioned (21,22). This reported J-shape and the results of the present study are strong reasons to reconsider the recommendation to lower the IFG cutoff to 5.6 mmol/l.

Consequences of lowering the IFG cutoff for early prediction of CVD and diabetes
The high incidence of type 2 diabetes in recent years has led to focus on the possibilities of prevention of diabetes. Indeed, lifestyle intervention studies have convincingly shown that in subjects with IGT, risk of diabetes and development of abnormal levels of cardiovascular risk factors could be reduced (23–25). However, the IFG5.6 group includes a very large, relatively healthy group of subjects with a different metabolic profile than IGT subjects. It is questionable if prevention strategies would be similarly effective in IFG as in IGT subjects with high risk of developing diabetes. In the present study, one-third of the population would be classified as IFG5.6, while the risk of developing diabetes is lower and the CVD mortality risk is similar to that of NFG subjects. The fact that high CVD mortality risk is only present in those subjects who will develop diabetes within 6 years indicates that our focus should lie on treatment of CVD risk factors and early detection of diabetes.

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Physical Activity and Metabolic Risk in Individuals With a Family History of Type 2 Diabetes

ULF EKELUND, PHD
SIMON J. GRIFFIN, DM
NICHOLAS J. WAREHAM, MD, PHD
ON BEHALF OF THE PROACTIVE RESEARCH GROUP

OBJECTIVE — We sought to examine the independent associations between different dimensions of physical activity with intermediary and clustered metabolic risk factors in overweight individuals with an increased risk of type 2 diabetes to inform future preventive action.

RESEARCH DESIGN AND METHODS — We measured total body movement and other subcomponents of physical activity by accelerometry in 258 adults (aged 30–50 years) with a family history of type 2 diabetes. We estimated aerobic fitness from an incremental treadmill exercise test. We measured body composition by bioimpedance and waist circumference, blood pressure, fasting triglycerides, HDL cholesterol, glucose, and insulin with standard methods. We constructed a standardized continuously distributed variable for clustered risk.

RESULTS — Total body movement (counts · day$^{-1}$) was significantly and independently associated with three of six risk factors (fasting triglycerides, insulin, and HDL) and with clustered metabolic risk ($P = 0.004$) after adjustment for age, sex, and obesity. Time spent at moderate- and vigorous-intensity physical activity (MVPA) was independently associated with clustered metabolic risk ($P = 0.03$). Five- and 10-min bouts of MVPA, time spent sedentary, time spent at light-intensity activity, and aerobic fitness were not significantly related with clustered risk after adjustment for confounding factors.

CONCLUSIONS — Total body movement is associated with intermediary phenotypic risk factors for cardiovascular disease and metabolic disease and with clustered metabolic risk independent of aerobic fitness and obesity. Increasing the total amount of physical activity in sedentary and overweight individuals may have beneficial effects on metabolic risk factors.

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Abbreviations: CVD, cardiovascular disease; FFM, fat-free mass; MVPA, moderate- and vigorous-intensity physical activity; PAEE, physical activity energy expenditure.

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Activity and metabolic risk in diabetes

previously (17). Briefly, potential participants were identified via diabetes register and medical records of family history in 20 general practices in East Anglia. To exclude very active individuals, participants completed a screening activity questionnaire describing occupational and leisure activity, based on published questionnaires (18,19).

Of the 465 eligible individuals, 399 were recruited for baseline measurements. Complete data on aerobic fitness, anthropometrics, and biochemistry at baseline were available for 365 individuals. Complete data on aerobic fitness, and leisure activity, based on published questionnaire describing occupational pants completed a screening activity to exclude very active individuals, participants and medical records of family history (Datascope, Cambridge, U.K.).

Cross-reactivity with intact proinsulin is <0.5% at 2,736 pmol/l, with 32–33 split proinsulin is 1% at 2,800 pmol/l, and with C-peptide is <0.1% at 5,280 pmol/l. Typical interassay coefficients of variance are 3.1% at 29.0 pmol/l, 2.1% at 79.4 pmol/l, 1.9% at 277 pmol/l, and 2.0% at 705 pmol/l, respectively (n = 174 in each case).

Clustered metabolic risk
We constructed a standardized continuously distributed variable (zMS) for clustered metabolic risk, which we have described in detail previously (15,16). This variable was derived by standardizing and then summing the following continuously distributed indexes of obesity (waist circumference), hypertension ([systolic blood pressure + diastolic blood pressure]/2), hyperglycemia (fasting plasma glucose), insulin resistance (fasting insulin), inverted fasting HDL cholesterol, and hypertriglyceridemia to create a Z score. The purpose of using a continuously distributed variable was to maximize statistical power (23).

Assessment of aerobic fitness and physical activity
Aerobic fitness (V02max) was predicted as oxygen uptake at maximal heart rate (220 minus age) by extrapolation of the regression line established during the individual calibration for the relationship between oxygen consumption and heart rate during a submaximal graded treadmill exercise test. Oxygen uptake and CO2 production were continuously measured by indirect calorimetry throughout the test (Vista XT metabolic system; Vacumed, Ventura, CA). Participants breathed through a face mask (Hans Rudolph, Kansas City, MO), and expired air was measured with a turbine flowmeter, carbon dioxide concentration with an infrared sensor, and oxygen concentration with a fast differential paramagnetic sensor. Gas analyzers were calibrated with gases of known composition, and the turbine flow meter was calibrated with a 3-l syringe before each measurement.

Data on free-living physical activity was assessed with an MTI ActiGraph (formerly known as the CSA activity monitor) model WAM 7164 (Manufacturing Technology, Fort Walton Beach, FL) accelerometer over 4 consecutive days. The participants wore the accelerometer in an elastic waistband at the lower back during daytime, except while bathing and during other aquatic activities. Participants who did not manage to record at least 500 min/day of activity for at least 3 days were excluded from further analyses (n = 7). The outcome variables were total body movement (counts • day−1), which is an indicator of the total volume of physical activity, and time (minutes • day−1) spent at different activity intensity categories averaged per day over the measurement period. These were calculated to determine which subcomponents of activity, if any, are associated with individual and clustered metabolic risk and to establish whether these associations were related in a dose-response manner. Intensity thresholds for moderate (1,952–5,724 counts • min−1) and vigorous intensity activity (>5,725 counts • min−1) were defined according to Freedson et al. (24). Because >60% of participants did not accumulate any time in vigorous intensity physical activity, we constructed a single variable by combining accumulated time in MVPA. Sedentary behavior was defined as <100 counts • min−1 and light intensity activity as 101–1,951 counts • min−1.

The cutoff for sedentary behavior is an arbitrary threshold, which we have used previously (25). We also calculated the average number of 5- and 10-min bouts of sustained physical activity at the MVPA level. In these analyses we allowed 1 min to drop below the threshold for MVPA in each 5-min bout. We calculated the percentage of participants accumulating 30 min or more per day at MVPA according to the recommendations from the Centers for Disease Control and Prevention and the American College of Sports Medicine (26). Data reduction, cleaning, and analyses of accelerometer data were performed using a specially written program (MAHУffe; see www.mrc-epid.cam.ac.uk).

Statistics
Descriptive summary statistics were calculated using means ± SD. Fasting insulin and triglycerides were logarithmically transformed owing to their skewed distribution. Geometric mean and reference intervals (1.96 × SD) are presented in the Results.

We modeled the associations be-
between all physical activity subcomponents (total counts, time spent sedentary, at light intensity activity, and at MVPA, and bouts of MVPA) and the phenotypes of the metabolic syndrome and clustered metabolic risk in separate models. These analyses were adjusted for age and sex. When obesity was not the outcome of interest, we assessed whether the subcomponents of physical activity were associated with each intermediary phenotype per se after adjustment for waist circumference, age, and sex. We then examined whether the different physical activity subcomponents were associated with clustered metabolic risk (zMS) in separate models. Finally, stepwise multiple linear regression analysis was used to examine which of the accelerometry-derived time estimates of physical activity variables contributed to the explained variation in clustered metabolic risk after adjustment for sex, age, and measurement time. We controlled for multicollinearity by calculating the correlation coefficients between the different time-derived variables and by calculating the tolerance and variance inflation factors. All data were analyzed in their continuous form although data are stratified by quartiles of total body movement (counts · day⁻¹) for illustrative purposes. All analyses were conducted using SPSS for Windows (version 11; SPSS, Chicago, IL). P < 0.05 denotes statistical significance.

**RESULTS** — Table 1 shows the descriptive characteristics of study participants. Thirty-two percent of participants were classified as normal weight, 40% were overweight, and an additional 27% were obese. Age, weight, height, BMI, waist circumference, fat mass, FFM, systolic blood pressure, fasting glucose, and the summary score of the metabolic syndrome and clustered metabolic risk in separate models. These analyses were adjusted for age and sex. We controlled for multicollinearity by calculating the correlation coefficients between the different time-derived variables and by calculating the tolerance and variance inflation factors. All data were analyzed in their continuous form although data are stratified by quartiles of total body movement (counts · day⁻¹) for illustrative purposes. All analyses were conducted using SPSS for Windows (version 11; SPSS, Chicago, IL). P < 0.05 denotes statistical significance.

**Table 1—Descriptive characteristics of participants**

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
<th>P value by ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>103</td>
<td>155</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.9 ± 6.4</td>
<td>40.7 ± 6.4</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>90.4 ± 16.4</td>
<td>73.7 ± 14.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.78 ± 0.07</td>
<td>1.64 ± 0.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.4 ± 4.6</td>
<td>27.4 ± 5.1</td>
<td>NS</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>22.5 ± 9.4</td>
<td>26.6 ± 10.0</td>
<td>0.01</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>67.9 ± 8.7</td>
<td>47.1 ± 6.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>101.4 ± 12.0</td>
<td>88.1 ± 11.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>127.3 ± 11.2</td>
<td>120.8 ± 13.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81.9 ± 8.8</td>
<td>76.2 ± 9.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting insulin (mmol · L⁻¹)</td>
<td>55.7 (50.2–61.8)</td>
<td>46.5 (43.4–49.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>Fasting glucose (mmol · L⁻¹)</td>
<td>5.10 ± 0.86</td>
<td>4.78 ± 0.52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides (mmol · L⁻¹)</td>
<td>1.45 (1.31–1.61)</td>
<td>1.05 (0.98–1.11)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL (mmol · L⁻¹)</td>
<td>1.22 ± 0.32</td>
<td>1.57 ± 0.38</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VO_{2max} (ml FFM · min⁻¹)</td>
<td>59.6 ± 11.1</td>
<td>57.8 ± 10.4</td>
<td>NS</td>
</tr>
<tr>
<td>Time sedentary (min · day⁻¹)</td>
<td>442 ± 97</td>
<td>419 ± 77</td>
<td>0.03</td>
</tr>
<tr>
<td>Time light (min · day⁻¹)</td>
<td>309 ± 80</td>
<td>320 ± 68</td>
<td>NS</td>
</tr>
<tr>
<td>Time moderate and vigorous (min · day⁻¹)</td>
<td>30 ± 18</td>
<td>24 ± 16</td>
<td>0.003</td>
</tr>
<tr>
<td>No. of 5-min bouts of MVPA</td>
<td>0.9 ± 0.9</td>
<td>0.8 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>No. of 10-min bouts of MVPA</td>
<td>0.3 ± 0.5</td>
<td>0.2 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Total counts (× 1,000 · day⁻¹)</td>
<td>283 ± 104</td>
<td>264 ± 98</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SD or geometric mean (95% CI). n = 258.
**Table 2** Independent associations of patterns of physical activity from accelerometry and intermediary phenotypic risk factors and clustered metabolic risk in middle-aged adults with a family history of type 2 diabetes.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Sedentary</th>
<th>Light</th>
<th>MVPA</th>
<th>Total counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist (cm)</td>
<td>0.04</td>
<td>0.21</td>
<td>0.09</td>
<td>0.003 to 0.013</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>0.07</td>
<td>0.75</td>
<td>0.53</td>
<td>0.14 to 0.07</td>
</tr>
<tr>
<td>Insulin (mol l⁻¹)</td>
<td>0.08</td>
<td>0.60</td>
<td>0.37</td>
<td>0.05 to 0.16</td>
</tr>
<tr>
<td>Glucose (mmol l⁻¹)</td>
<td>0.002</td>
<td>0.27</td>
<td>0.14</td>
<td>0.003 to 0.012</td>
</tr>
<tr>
<td>Inverted HDL (mmol l⁻¹)</td>
<td>0.01</td>
<td>0.28</td>
<td>0.28</td>
<td>0.06 to 0.17</td>
</tr>
<tr>
<td>Triglycerides (mmol l⁻¹)</td>
<td>0.06</td>
<td>0.59</td>
<td>0.59</td>
<td>0.17 to 0.08</td>
</tr>
</tbody>
</table>

Data on insulin and triglycerides are log transformed. Data are adjusted for sex and age. Data on total counts from accelerometry are additionally adjusted for monitoring time. All subcomponents except waist circumference are additionally adjusted for waist circumference.

**CONCLUSIONS**

We show here that the association between total body movement and an increase in everyday physical activity is strongly associated with insulin resistance, dyslipidemia, and clustered metabolic risk independent of sex, age, and clustered metabolic risk. Promoting overall body movement and an increase in everyday physical activity may therefore have potential to be considered when interpreting the results of the present study. Although we cannot assert causality and its direction, these findings from this study suggest that total body movement and aerobic fitness are important for reducing the risk of type 2 diabetes.

There are several limitations that need to be considered when interpreting the results of this study, including: (1) the potential for recall bias; (2) the cross-sectional study design; (3) the potential for underestimation of physical activity; (4) the potential for overestimation of metabolic risk; (5) the potential for underestimation of factors (sex, age, socioeconomic status, and early life programming), which may explain our findings.
find these activities unappealing and these targets hard to reach. Our results corroborate our previous findings suggesting that higher levels of PAEE measured by individual calibrated heart rate monitoring are favorably associated with features of the metabolic syndrome (15,16).

Study participants recorded, on average, fewer than one 5-min bout of MVPA per day, and bouts of MVPA were not associated with any of the intermediary phenotypic risk factors. It may be that the accumulated time at MVPA and particularly total body movement is more important in relation to these risk factors in sedentary individuals. However, we cannot exclude the possibility that bouts of activity are more strongly associated with intermediary phenotypic risk factors in a more heterogeneous population. Our data also suggest a linear dose-response association between the total amount of activity with clustered metabolic risk.

Thus, sedentary individuals may benefit from accumulating physical activity throughout the day or in shorter bouts (i.e., <5 min), and more activity accumulated leads to greater metabolic health benefits.

Previous epidemiological studies have clearly demonstrated that self-reported physical activity predicts cardiovascular morbidity and mortality end points (32–34) and that both walking and vigorous exercise are associated with risk reduction in the incidence of cardiovascular events (35–37). Further, both physical activity and obesity are independent predictors of all-cause mortality (38). However, these studies have not been able to characterize minute-by-minute patterns of physical activity because of reliance on self-reported measures of activity and lack of controls for aerobic fitness. From a public health perspective, this fact is important because if the association between physical activity and CVD and metabolic risk factors is mediated through aerobic fitness, it is likely that more vigorous exercise is needed to prevent CVD morbidity and mortality. Individual and population-based interventions would need to reflect this possibility.

In summary, total body movement is associated with intermediary phenotypic risk factors for CVD and metabolic disease and with clustered metabolic risk independent of aerobic fitness and obesity. Increasing the total amount of physical activity in sedentary and overweight individuals may have beneficial effects on these metabolic risk factors.

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The ProActive research team includes, besides the authors, Kate Williams, Julie Grant, Tom Fanahawse, A. Toby Prevost (principal investigator), Wendy Hardman (principal investigator), William Hollingworth, David Spiegelhalter (principal investigator), Stephen Sutton (principal investigator), and Ann Louise Kinninmonth (principal investigator).

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Should Central Obesity Be an Optional or Essential Component of the Metabolic Syndrome?

Ischemic heart disease risk in the Singapore Cardiovascular Cohort Study

JEANNETTE LEE, MBBS1
STEFAN MA, PhD2
DERRICK HENG, MBBS2
CHEE-ENG TAN, PhD3

SUOK-KAI CHEW, MSC2
KENNETH HUGHES, DM1
E-SHYONG TAI, MB, CHB7

OBJECTIVE — The International Diabetes Federation (IDF) proposes that central obesity is an “essential” component of the metabolic syndrome, while the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) proposes that central obesity is an “optional” component. This study examines the effect of the metabolic syndrome with and without central obesity in an Asian population with ischemic heart disease (IHD).

RESEARCH DESIGN AND METHODS — From the population-based cohort study (baseline 1992–1995), 4,334 healthy individuals were grouped by the presence or absence of the metabolic syndrome and central obesity and followed up for an average of 9.6 years by linkage with three national registries. Cox’s proportional hazards model was used to obtain adjusted hazard ratios (HRs) for risk of a first IHD event.

RESULTS — The prevalence of metabolic syndrome was 17.7% by IDF criteria and 26.2% by AHA/NHLBI criteria using Asian waist circumference cutoff points for central obesity. Asian Indians had higher rates than Chinese and Malays. There were 135 first IHD events. Compared with individuals without metabolic syndrome, those with both central obesity/metabolic syndrome and no central obesity/metabolic syndrome were at significantly increased risk of IHD, with adjusted HRs of 2.8 (95% CI 1.8–4.2) and 2.5 (1.5–4.0), respectively.

CONCLUSIONS — Having metabolic syndrome either with or without central obesity confers IHD risk. However, having central obesity as an “optional” rather than “essential” criterion identifies more individuals at risk of IHD in this Asian cohort.

R
cently, two new criteria diagnosing the metabolic syndrome have been proposed, with both allowing three of five components (central obesity, high fasting triglyceride, low HDL cholesterol, hypertension, glucose intolerance).

However, the International Diabetes Federation (IDF) proposes that central obesity is an “essential” component (1), while the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) proposes that central obesity is an “optional” component, like the other factors (2). Notably, in particular for Asians, there is agreement for the waist circumference cutoffs between the two criteria. Thus, in Asians, these proposals identify three groups of individuals: 1) no metabolic syndrome, 2) central obesity and metabolic syndrome, and 3) no central obesity and metabolic syndrome (this latter group meets the criteria for metabolic syndrome according to AHA/NHLBI but not IDF). In effect, the IDF criteria identified a subset of Asian individuals who have been identified as having the metabolic syndrome by the AHA/NHLBI criteria.

It has been suggested that the proportion of individuals without central obesity who have three or more components of the metabolic syndrome is small (3, 4). It is also felt that in the U.S., for the most part, the same individuals will be identified by either definition so that differences in the definitions are probably insignificant (2). However, this has not been assessed in various populations, particularly in populations comprising ethnic groups from Asia. Furthermore, the impact of central obesity as an essential component of the metabolic syndrome has not been extensively assessed in relation to the risk of ischemic heart disease (IHD).

Only one study (5) has shown that the rate of cardiovascular disease (CVD) mortality increased with increasing waist circumference in the presence of two or more other components but not with less than two other components. There have been no such studies in Asian populations. These studies are critical, given that the reason for defining the metabolic syndrome is to provide one practical definition that would be useful for the identification of individuals with increased risk of CVD (6–10) and diabetes (11, 12).

The aims of this study are to determine the different prevalence of the metabolic syndrome according to the IDF and AHA/NHLBI definitions and the impact of central obesity as an “essential” rather
than “optional” component of the metabolic syndrome on the risk of IHD in a healthy Asian population.

**RESEARCH DESIGN AND METHODS** — The Singapore Cardiovascular Cohort Study is a population-based prospective study combining two cross-sectional surveys. These are the 1992 National Health Survey (13) and the National University of Singapore Heart Study (1993–1995) (14). The methodologies of these surveys were described in detail and are only briefly described here.

Both surveys were a random sample of all Singapore residents, with disproportionate sampling by ethnic group to increase the number of Malays and Asian Indians relative to Chinese. Consent was obtained from all participants before conduct of study. This study has also been approved by the National University of Singapore institutional review board.

**Baseline measurements**

Ethnicity was self-reported and classified into Chinese, Malay, or Asian Indian. Two readings of blood pressure were taken after adequate resting using a standard mercury sphygmomanometer. If the two readings differed (diastolic by >15 mmHg or systolic >25 mmHg), a third reading was performed. The mean values of the closest two readings were calculated. Measurements were made of waist circumference (narrowest part of the body below the costal margin), weight, and height. Smoking was categorized as non- or current smoker, and alcohol intake as less than once a month or greater than or equal to once a month. Individuals were asked if they had ever been diagnosed as having preexisting IHD, cerebrovascular disease, diabetes, or hypertension and whether medication was prescribed.

All subjects were examined in the morning following a 10-h fast. Serum total cholesterol, triglyceride, and HDL cholesterol were measured using Kodak Ektachem Clinical Chemistry Slides (Kodak, Rochester, NY), and LDL cholesterol was calculated using the Friedewald formula. Plasma glucose was measured by the glucose oxidase method using blood collected in fluoride oxalate tubes. Individuals with type 2 diabetes were determined from medical history or if the fasting blood glucose was ≥7.0 mmol/l during the physical examination.

**Metabolic syndrome criteria**

The central obesity/metabolic syndrome status of individuals was obtained using the criteria set out by IDF (1) and AHA/NHLBI (2): 1) elevated triglycerides: >150 mg/dl (1.7 mmol/l), 2) reduced HDL cholesterol: <40 mg/dl (1.03 mmol/l) in male subjects and <50 mg/dl (1.29 mmol/l) in female subjects, 3) elevated blood pressure: systolic ≥130 mmHg or diastolic ≥85 mmHg or on treatment for hypertension, 4) elevated fasting plasma glucose: ≥100 mg/dl (5.6 mmol/l) or on treatment for type 2 diabetes, and 5) central obesity, using the waist circumference for South Asians/Asians: ≥90 cm in male subjects and ≥80 cm in female subjects.

Using these five metabolic score components, individuals were categorized into three central obesity/metabolic syndrome groups: 1) no metabolic syndrome, which includes individuals with less than three metabolic syndrome components; 2) central obesity and metabolic syndrome, which includes individuals with elevated waist circumference and two or more other components; and 3) no central obesity and metabolic syndrome, which includes individuals with low waist circumference but three or more other components.

**IHD events**

Data regarding IHD events were obtained by linking individual records (using unique identity card numbers) to three national registries of the Singapore Ministry of Health: 1) Registry of Births and Deaths, 2) Hospital Inpatient Discharge Database (this captures inpatient discharge information from all public and private hospitals, including day-surgery revascularization procedures such as coronary artery angioplasty or coronary stent placement), and 3) Singapore Myocardial Infarct Registry (this has comprehensive nationwide coverage of acute myocardial infarctions). An IHD event was defined as admission or death due to acute myocardial infarction or IHD (codes 410–414 of the ICD-9). For confidentiality, all personal identifiers were removed from the dataset before analysis.

**Data analysis**

Statistical analyses were performed using SPSS (version XIII SPSS for Windows, release 13.0.1, 2004; Chicago, IL). Categorical variables were expressed in percentages and continuous variables in means ± SD unless otherwise specified. Incidence rates for first IHD events were calculated for each of the central obesity/metabolic syndrome groups, and Cox proportional hazards regression was used to obtain adjusted hazard ratios (HRs) for first IHD events. The time-to-IHD event was the difference between the date of the first IHD event and the date of entry into the study. Subjects without IHD were censored at 31 December 2002 or the date of non-IHD death, whichever occurred first. HRs were adjusted for age, sex, ethnic group, study, LDL cholesterol, smoking, and alcohol intake. Interaction terms, created using the three central obesity/metabolic syndrome groups, with ethnic group (P = 0.387), sex (P = 0.911), and study (P = 0.081) analyzed separately, showed no significant interaction in the model; thus, the analysis was done with ethnic group, sex, and study combined. An interaction term consisting of follow-up time and the three central obesity/metabolic syndrome groups was used to test the proportional hazards assumption (15) for occurrence of IHD events and was found not to be significant (P = 0.351), indicating proportional hazard over time. The attributable percent among those with metabolic syndrome (AP%: the percent of risk IHD among those with metabolic syndrome that is due to metabolic syndrome) and attributable percent among the total population (AP%: that is the percent of risk of IHD among the whole population that is due to the metabolic syndrome) was also calculated for both AHA/NHLBI and IDF criteria with and without diabetes.

**RESULTS** — There were 4,334 participants after excluding 92 participants with preexisting IHD and 27 with missing data. These comprised 2,546 Chinese, 909 Malays, and 879 Asian Indians. There were 2,087 male subjects and 2,247 female subjects. The mean duration of follow-up was 9.6 ± 1.5 years and totaled 41,400 person-years. A total of 135 first IHD events were reported.

Table 1 shows that the prevalence of the three central obesity/metabolic syndrome groups were: no metabolic syndrome, 73.8%; central obesity/metabolic syndrome, 17.7%; and no central obesity/metabolic syndrome, 8.5%. Using the Asian criteria for waist circumference, the prevalence of metabolic syndrome according to the IDF was 17.7% and 26.2% according to the AHA/NHLBI. The prevalence of the three groups was also different among the ethnic groups, with Asian
TABLE 1—CHARACTERISTICS OF STUDY POPULATION BY CENTRAL OBESITY/METABOLIC SYNDROME GROUPS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No metabolic syndrome</th>
<th>Central obesity/metabolic syndrome</th>
<th>No central obesity/metabolic syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>3,200 (73.8)</td>
<td>766 (17.7)</td>
<td>368 (8.5)</td>
</tr>
<tr>
<td>Ethnic group*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>2,017 (79.2)</td>
<td>309 (12.1)</td>
<td>220 (8.6)</td>
</tr>
<tr>
<td>Malays</td>
<td>637 (70.1)</td>
<td>206 (22.7)</td>
<td>66 (7.3)</td>
</tr>
<tr>
<td>Asian Indians</td>
<td>546 (62.1)</td>
<td>251 (28.6)</td>
<td>82 (9.3)</td>
</tr>
<tr>
<td>Sex*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1,505 (72.1)</td>
<td>339 (16.2)</td>
<td>243 (11.6)</td>
</tr>
<tr>
<td>Female</td>
<td>1,695 (75.4)</td>
<td>427 (19.0)</td>
<td>125 (5.6)</td>
</tr>
<tr>
<td>Age (years)†</td>
<td>36.8 ± 11.9</td>
<td>48.8 ± 12.0</td>
<td>47.9 ± 12.2</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)†</td>
<td>5.2 ± 1.0</td>
<td>5.9 ± 1.1</td>
<td>5.8 ± 1.1</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)†</td>
<td>3.4 ± 0.9</td>
<td>3.9 ± 1.0</td>
<td>3.8 ± 1.4</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)†</td>
<td>1.3 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Triglycerides†</td>
<td>1.0</td>
<td>1.9</td>
<td>2.2</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)†</td>
<td>5.3 ± 1.0</td>
<td>6.5 ± 2.4</td>
<td>6.9 ± 2.8</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)†</td>
<td>114.6 ± 15.2</td>
<td>137.4 ± 21.9</td>
<td>136.3 ± 20.3</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)†</td>
<td>67.6 ± 10.9</td>
<td>81.9 ± 11.9</td>
<td>80.9 ± 11.5</td>
</tr>
<tr>
<td>Waist circumference (cm)†</td>
<td>73.1 ± 9.4</td>
<td>92.6 ± 8.4</td>
<td>79.8 ± 6.6</td>
</tr>
<tr>
<td>BMI (kg/m²)†</td>
<td>22.1 ± 3.4</td>
<td>29.0 ± 3.8</td>
<td>23.8 ± 2.5</td>
</tr>
<tr>
<td>Diabetes†</td>
<td>81.2 ± 2.5</td>
<td>195 ± 25.5</td>
<td>102 ± 27.7</td>
</tr>
<tr>
<td>Current smoker*</td>
<td>580 (18.1)</td>
<td>119 (15.6)</td>
<td>95 (25.8)</td>
</tr>
<tr>
<td>Alcohol intake*</td>
<td>316 (9.9)</td>
<td>69 (9.0)</td>
<td>55 (14.9)</td>
</tr>
<tr>
<td>Study*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHS 92</td>
<td>2,684 (83.9)</td>
<td>434 (56.7)</td>
<td>291 (79.1)</td>
</tr>
<tr>
<td>NUHHS</td>
<td>516 (16.1)</td>
<td>332 (43.3)</td>
<td>77 (20.9)</td>
</tr>
</tbody>
</table>

Data are n (%) or means ± SD. *Categorical variables; for ethnic group and sex, percentages are shown. †Continuous variables; for triglycerides, median values are shown. NHS 92, 1992 National Heart Study; NUHHS, National University of Singapore Heart Study.

Individuals having the highest prevalence of metabolic syndrome in both the presence (28.6%) and absence (9.3%) of central obesity (Table 1). Table 1 further describes the characteristics of the three central obesity/metabolic syndrome groups. Compared with those with three or more metabolic syndrome components without central obesity, those with three or more components with central obesity were older, more obese, and had higher blood pressure. In addition, they were more likely to be female and of Malay or Asian-Indian ethnicity. However, in contrast, individuals with three or more metabolic syndrome components without central obesity had higher plasma triglyceride and higher fasting glucose values than those with three or more components with central obesity. Also, in this group, there was a higher proportion of current smokers (25.8%) compared with the group with three or more components with central obesity (15.6%) or no metabolic syndrome (18.1%).

Table 2 shows the risk of IHD for the three central obesity/metabolic syndrome groups including and excluding individuals with type 2 diabetes. The highest incidence rates were for the central obesity/metabolic syndrome and no central obesity/metabolic syndrome groups, which included diabetic patients at 8.8 and 9.5 per 1,000 person-years, respectively. Compared with the no metabolic syndrome group, individuals with central obesity/metabolic syndrome and no central obesity/metabolic syndrome had significantly increased risks for IHD with adjusted HRs of 2.8 (95% CI 1.8–4.2) and 2.5 (1.5–4.0), respectively. A comparison of the central obesity/metabolic syndrome and no central obesity/metabolic syndrome groups showed no significant difference in risk of IHD between them, with HR 1.0 (95% CI 0.6–1.5) and an absolute rate difference of 0.7 (−4.7 to +3.2).

The exclusion of diabetic patients did not greatly reduce the risk of IHD for both central obesity/metabolic syndrome or no central obesity/metabolic syndrome groups (adjusted HR 2.5 [95% CI 1.6–4.2] and 1.9 [1.0–3.3], respectively), and there was not a significant difference between the two groups.

Individuals with the metabolic syndrome using either the IDF or AHA/NHLBI criteria were found to have an increased risk of IHD (Table 3). The exclusion of diabetic patients did not remarkably change the risk estimates for either the IDF or AHA/NHLBI criteria and adjusted HRs were similar (HR 2.3 [95% CI 1.5–3.6] using both criteria). The AP_E among those with metabolic syndrome according AHA/NHLBI criteria was higher (84%) than the IDF criteria (76%). Similarly, the AP_T was also found to be higher when the AHA/NHLBI criteria were used (57.6%) compared with the IDF criteria (36.4%). Although the AP_E did not change when diabetic patients were excluded from the analyses, the AP_T was lowered to 48.0 and 28.0% for the AHA/NHLBI and IDF criteria, respectively.

CONCLUSIONS—Our study showed that the prevalence of the metabolic syndrome is 17.7% based on IDF criteria but 26.2% based on AHA/NHLBI criteria. This meant that 8.5% of the population had three or more metabolic syndrome components in the absence of central obesity while using the Asian definition. Thus, making central obesity an “essential” rather than “optional” component in diagnosing metabolic syndrome fails to identify a fairly large proportion of individuals who otherwise would be classed as having the metabolic syndrome.

Our study also showed that individuals in central obesity/metabolic syndrome and no central obesity/metabolic syndrome groups are at similar risk of IHD. This suggests that including central obesity as an “essential” component for the diagnosis of metabolic syndrome, as proposed by IDF, does not add more to the identification of individuals at increased risk of IHD. These findings from our study suggest, at least in this Asian popu-
Central obesity/metabolic syndrome and IHD risk

Table 2—Association of central obesity/metabolic syndrome groups with risk of IHD

<table>
<thead>
<tr>
<th></th>
<th>n of events (%)</th>
<th>Person-years</th>
<th>Incidence rate (per 1,000 person-years)</th>
<th>HR (95% CI)*</th>
<th>HR (95% CI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Including diabetic patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No MetS</td>
<td>44 (1.4)</td>
<td>31,318</td>
<td>1.4 (0.9–1.8)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>CO/MetS</td>
<td>59 (7.7)</td>
<td>6,716</td>
<td>8.8 (6.6–11.0)</td>
<td>6.1 (4.1–9.0)</td>
<td>2.8 (1.8–4.2)</td>
</tr>
<tr>
<td>No CO/MetS</td>
<td>32 (8.7)</td>
<td>3,366</td>
<td>9.5 (6.5–13.4)</td>
<td>6.7 (4.2–10.6)</td>
<td>2.5 (1.5–4.0)</td>
</tr>
<tr>
<td>CO/ MetS vs. no CO/MetS</td>
<td></td>
<td></td>
<td></td>
<td>0.9 (0.6–1.4)</td>
<td>1.0 (0.6–1.3)</td>
</tr>
<tr>
<td>Excluding diabetic patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No MetS</td>
<td>40 (1.3)</td>
<td>30,541</td>
<td>1.3 (0.9–1.7)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>CO/MetS</td>
<td>38 (6.7)</td>
<td>5,013</td>
<td>7.6 (5.2–10.0)</td>
<td>5.6 (3.6–8.8)</td>
<td>2.5 (1.6–4.2)</td>
</tr>
<tr>
<td>No CO/MetS</td>
<td>18 (6.8)</td>
<td>2,453</td>
<td>7.3 (4.3–11.5)</td>
<td>5.5 (3.2–9.7)</td>
<td>1.9 (1.0–3.3)</td>
</tr>
<tr>
<td>CO/MetS vs. no CO/MetS</td>
<td></td>
<td></td>
<td></td>
<td>1.0 (0.6–1.8)</td>
<td>1.2 (0.8–1.5)</td>
</tr>
</tbody>
</table>

*Unadjusted HRs. †Adjusted HRs for age, study, ethnic group, sex, LDL cholesterol, smoking (nonsmoker vs. current smoker), and alcohol intake (none/occasional vs. ≥1/month). CO, central obesity; MetS, metabolic syndrome.

The IDF based their recommendation on the strength of the evidence linking waist circumference with CVD and the other components of the metabolic syndrome (16,17) and states that central obesity is an early step in the etiological cascade leading to the full metabolic syndrome. Our findings refute neither of these premises. Indeed, one study (18) in Japan has shown that visceral adiposity was a crucial determinant on the degree of insulin resistance associated with the presence of other metabolic syndrome components. In that study, the presence of three or more metabolic syndrome components was associated with a lesser degree of insulin resistance if visceral adiposity was not one of the three components (versus if it was). However, in relation to identifying individuals at increased risk of IHD, it does appear that central obesity adds to the risk of IHD in much the same way as the other four risk factors. This is in line with the findings of Katzmarzyk et al. (5), who showed that increasing waist circumference was associated with increased risk of CVD mortality when added to the other components of the metabolic syndrome. However, the presence of central obesity as one of the components of metabolic syndrome does not appear to alter the association between the presence of other multiple components and the risk of IHD. Thus, while making central obesity an essential requirement may make etiological sense and may be relevant to the identification of the insulin-resistant individual, the evidence that this approach is important for the identification of individuals at risk of IHD is limited at this time.

Several factors could also explain our findings. First, central obesity may not cause IHD directly but rather through the associated risk factors and thus may not have a strong influence on the risk of IHD.

Table 3—Risk of IHD for individuals with the metabolic syndrome according to IDF and AHA criteria

<table>
<thead>
<tr>
<th></th>
<th>n of events (%)</th>
<th>Person-years</th>
<th>Incidence rate (per 1,000 person-years)</th>
<th>HR (95% CI)*</th>
<th>HR (95% CI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Including diabetic patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDF criteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No MetS</td>
<td>76 (2.1)</td>
<td>34,685</td>
<td>2.1 (1.7–2.7)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>MetS</td>
<td>59 (7.7)</td>
<td>6,716</td>
<td>8.8 (6.6–11.0)</td>
<td>3.9 (2.8–5.5)</td>
<td>2.1 (1.4–3.1)</td>
</tr>
<tr>
<td>AHA criteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No MetS</td>
<td>44 (1.4)</td>
<td>31,319</td>
<td>1.4 (0.9–1.8)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>MetS</td>
<td>91 (8.0)</td>
<td>10,082</td>
<td>9.0 (7.2–10.9)</td>
<td>6.3 (4.4–9.0)</td>
<td>2.7 (1.8–4.0)</td>
</tr>
<tr>
<td>Excluding diabetic patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDF criteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No MetS</td>
<td>58 (1.7)</td>
<td>32,994</td>
<td>1.8 (1.3–2.2)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>MetS</td>
<td>38 (6.7)</td>
<td>5,013</td>
<td>7.6 (5.2–10.0)</td>
<td>4.2 (2.8–6.3)</td>
<td>2.3 (1.5–3.6)</td>
</tr>
<tr>
<td>AHA criteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No MetS</td>
<td>40 (1.3)</td>
<td>30,541</td>
<td>1.3 (0.9–1.7)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>MetS</td>
<td>56 (6.7)</td>
<td>7466</td>
<td>7.5 (5.3–9.5)</td>
<td>5.6 (3.7–8.4)</td>
<td>2.3 (1.5–3.6)</td>
</tr>
</tbody>
</table>

*Unadjusted HRs. †Adjusted HRs for age, study, ethnic group, sex, LDL cholesterol, smoking (nonsmoker vs. current smoker), and alcohol intake (none/occasional vs. ≥1/month). MetS, metabolic syndrome.
in this study until after the other CVD (metabolic syndrome) risk factors associated with central obesity have developed. Second, waist circumference is an imperfect surrogate of abdominal adiposity (19), and using it might lead to misclassification of individuals. Finally, central obesity is important, but the threshold for Asians may need to be further lowered.

The underlying purpose for diagnosis of the metabolic syndrome is to identify individuals who are at increased risk of developing diabetes and CVD and to apply preventive measures (1,2). We found in Asians that both individuals with and without central obesity and other metabolic syndrome components are at similar risk of IHD. The current AHA/NHLBI (2) proposal includes all of these individuals, while a sizable number who do not have central obesity but have the metabolic syndrome are omitted by the IDF (1) criteria and thus identifies a greater proportion of those at increased risk of IHD. However, we cannot comment at this time on the relevance of central obesity as an essential component of the metabolic syndrome in relation to the risk of diabetes due to lack of follow-up data. It may well be that the impact differs from that for IHD. Finally, of note is the high proportion of current smokers in the group of individuals without central obesity but with the metabolic syndrome compared with the other two groups. Although this has been adjusted for in the analysis to determine the risk of IHD for each group, it is still important to remember that the focus on the metabolic syndrome should not lead to negligence of the other CVD risk factors that need to be addressed at the individual level.

Possible limitations of our study should be noted. Measurement error of variables, especially waist circumference, could have occurred, though these are likely to be nondifferential leading to an underestimate of risks. Ascertainment of events was done only by data linkage, though three different population-based registers were used allowing for good coverage and case ascertainment. The study comprises two different cross-sectional surveys, though the participants of both were random samples selected using similar methodology.

In conclusion, this study has shown that the risk of IHD is increased in individuals with the metabolic syndrome with or without central obesity. However, the prevalence of metabolic syndrome is increased by 8.5% if central obesity is “optional” rather than “essential” and thus identifies more individuals at risk of IHD. Apart from metabolic syndrome, other CVD risk factors in individuals should also be considered and appropriately managed.

Acknowledgments — This study was supported by grants from the Biomedical Research Council (grant no.: 03/127/18/216) and National Medical Research Council (grant no.: 0.838/2004), Singapore.

References

Visceral Fatness and Insulin Sensitivity in Women With a Previous History of Gestational Diabetes Mellitus

Soo Lim, MD1
Sung Hee Choi, MD1
Young Joo Park, MD1
Kyong Soo Park, MD, PhD1

Hong Kyu Lee, MD, PhD1
Hak C. Jang, MD, PhD1
Nam H. Cho, PhD2
Boyd E. Metzger, MD3

OBJECTIVE — The purpose of this study was to investigate the insulin sensitivity and visceral fatness in women with previous gestational diabetes mellitus (GDM), who are prone to develop type 2 diabetes.

RESEARCH DESIGN AND METHODS — A 75-g oral glucose tolerance test (OGTT) performed 1 year postpartum identified 21 GAD+ women with previous GDM and impaired glucose tolerance (GDM-IGT). Forty age- and BMI-matched women with normal glucose tolerance (GDM-NGT) were selected by 1:3 matching to the GDM-IGT group. Another 18 women with normal glucose metabolism during a previous pregnancy and no family history of diabetes were recruited as the normal control group. Age and BMI matching was performed using a range with normal glucose metabolism during a previous pregnancy and no family history of diabetes were recruited as the normal control group. Age and BMI matching was performed using a range of ±1.0 years and ±1.0 kg/m², respectively. Total body fat was measured by tetrapolar bioelectrical impedance, and visceral fat was determined using a single cut of a computed tomography scan. Insulin sensitivity was determined by the minimal model technique using the frequently sampled intravenous glucose tolerance test.

RESULTS — One year postpartum, visceral fat was greater in the GDM-IGT group than in the age- and BMI-matched GDM-NGT or normal control groups. The insulin sensitivity index was lower in the GDM-IGT group than in the GDM-NGT or normal control groups. β-Cell function, as measured by the acute insulin response to glucose, was also lower in GDM-IGT.

CONCLUSIONS — High body fat content, especially visceral fat content, and a low insulin response to glucose seem to contribute simultaneously to the development of impaired glucose metabolism in Korean women with previous GDM.

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance of variable severity first recognized in pregnancy (1). GDM may complicate as many as 5–8% of all pregnancies in North America (2). Recent reports have shown that the prevalence of GDM has been increasing in multicultural populations (3,4). Although the reported prevalence of GDM seems to be slightly lower in Asian countries (5,6), adverse outcomes are similar in these two regions. Women with GDM have an increased risk of later development of type 2 diabetes (7). Studies in Western populations have found conversion rates of 3–38% within the 1st year postpartum (8–10). Although a limited number of studies have been performed in Asian countries, the prevalences of impaired glucose metabolism in the early postpartum period have been reported to be 20% in Hong Kong and 38.3% in Korea (11,12).

Several reports have identified clinical factors at antepartum testing or during pregnancy that predict the development of future diabetes in women with GDM. However, few reports have focused on postpartum metabolic characteristics as a risk factor for future diabetes. More than two decades ago, Ward et al. (13) showed that women with previous GDM had insulin-secretion defects and that only obese women with GDM had a lower insulin sensitivity index (S) and higher waist-to-hip ratio than their obese counterparts when tested postpartum. Since then, several studies have revealed that women with previous GDM have greater insulin resistance and lower insulin responses than women with no history of GDM (14–17).

Buchanan et al. tested >30 clinical parameters to discriminate between women with previous GDM who have a high and low risk for type 2 diabetes, and they found that the postpartum oral glucose tolerance test (OGTT) provides the best discrimination (18). Women who develop diabetes have a lower acute insulin response to glucose and lower disposition index than women who remain free of diabetes (19).

To our knowledge, few researchers have investigated both insulin sensitivity and body composition, especially the amount of visceral fat at the postpartum evaluation, as contributors to the development of impaired glucose metabolism after GDM. Many studies showed that Asians have a higher percentage of body fat compared with Caucasians with the same BMI (20,21). The prevalence of obesity is lower in Asian women than in their Western counterparts, but the GDM prevalence is not lower in Asian women than in Western women (11,22). We have reported previously that most women in Korea who develop GDM are not obese before pregnancy according to the usual body weight criteria for obesity (11). Increased visceral fat deposition plays an
important role in the development of type 2 diabetes in Japanese Americans (23). This finding suggests that a high amount of visceral fat could have an important role in the development of diabetes in Asian women with GDM even though they are not obese by the usual BMI criteria. Body fatness can now be evaluated by more technologically advanced methods such as bioelectrical impedance or computed tomography (CT) (24, 25), but these methods remain largely untested in women with a history of GDM. Postpartum insulin sensitivity and body composition, especially visceral fat amount, have not been assessed systematically to determine their combined role in the development of impaired glucose metabolism in women with a history of GDM.

We investigated factors that might be associated with altered glucose metabolism in women with previous GDM at 1 year postpartum. In particular, we proposed that we would find a relationship between insulin sensitivity and visceral fatness measured by CT. To accomplish these aims, we studied an age- and BMI-matched sample of Korean women with previous GDM, whose insulin sensitivity was determined by the minimal model technique.

**RESEARCH DESIGN AND METHODS** — The study subjects were recruited at Seoul National University hospitals in Seoul, Korea. Between 1999 and 2000, GDM was diagnosed in 236 women in the Seoul National University Hospital. Our protocol for screening and diagnosis of GDM has been described previously (5, 11). The diagnosis of GDM was made using the criteria of the Third International Workshop-Conference on GDM (1). To evaluate their glucose metabolism, 173 women were given the standard 75-g OGTT at 2 months postpartum, and 28 were excluded because of a diagnosis of diabetes. At 1 year postpartum, 132 of 145 women completed the 75-g OGTT, and 11 were excluded because they were taking an oral contraceptive or were positive for GAD antibody. Of the 121 remaining women at 1 year postpartum, diabetes was diagnosed in 5, 28 had impaired glucose tolerance (IGT), and 88 had normal glucose tolerance (NGT). Of the 116 women with IGT and NGT, 21 who agreed to have a frequently sampled intravenous glucose tolerance test and a CT scan to measure fat content were selected for the GDM-IGT group. Sixty age- and BMI-matched women were assigned to the GDM-NGT group by 1:3 matching to the GDM-IGT group. At the same time, we also recruited another 18 subjects with normal glucose metabolism during pregnancy and no family history of diabetes as a normal control group in the follow-up study. This control group was selected from women who had a 50-g glucose challenge test at 24–28 weeks of gestation, and their 1-h plasma glucose concentration after the glucose challenge was <7.2 mmol/l. Subjects were matched for age and BMI using ranges of ±1.0 years and ±1.0 kg/m², respectively. All subjects provided informed consent, and the study protocol was approved by the ethical committee of the institutional review board of Seoul National University Hospital.

OGTT All participating women completed a standard 75-g OGTT at 1 year postpartum. A 2-h postload plasma glucose concentration >11.1 mmol/l was used as the diagnosis of diabetes, and a 2-h postload glucose concentration of 7.8–11.1 mmol/l was used as the criterion for IGT.

**Anthropometric assessments and blood pressure measurement**

Height and body weight were measured to the nearest 0.1 cm and 0.1 kg with the patient barefoot in light clothing, respectively. BMI was calculated as body weight in kilograms divided by the square of height in meters. Blood pressure was measured after the subject had remained seated for 10 min. Measurements were made twice with a 5-min rest period between measurements. If a difference >5 mmHg was found between these two measures, blood pressure was measured one more time, and the average of two measures that showed the least difference among the three was used.

**Laboratory assessments**

After a 12-h overnight fast, venous blood samples were drawn from an antecubital vein at 0800–0900 h. Plasma was separated immediately by centrifugation (2,000 rpm, 20 min, 4°C). Serum insulin concentration was measured using insulin-specific radioimmunoassay kits (Linco Research, St. Louis, MO). Total cholesterol and triglyceride concentrations were determined by enzymatic procedures using a Beckman analyzer (Beckman Instruments, Brea, CA). HDL cholesterol concentration was determined using the Sigma direct EZ-HDL assay. Antibody levels to GAD were measured using a radioimmunoassay method (RSR, Cardiff, U.K.). Plasma glucose concentration was measured using a glucose oxidase method (YSI 2300-STAT; Yellow Springs Instrument, Yellow Springs, OH) immediately after blood was drawn. The oral and intravenous glucose tolerance tests were scheduled during the follicular phase of the menstrual cycle.

**Body composition measurement**

Body fat was measured by tetrapolar bioelectrical impedance analysis (Inbody 3.0; Biospace, Seoul, Korea). Bioelectrical impedance measures two parameters, fat and lean tissue, using empirically derived formulas that have been validated by earlier studies and that correlate well with values obtained using underwater weighing (25). For the accuracy of the body composition measurement, the interobserver variation (<0.1%) was confirmed by testing of the same subjects by different observers. Intraobserver variation was also confirmed by testing of the same subjects on different days by the same observer (<1.0%).

**Visceral fat measurement**

The abdominal adipose tissue areas were quantified by a single scout view of a CT scan (Somatom Sensation 16; Siemens, Munich, Germany). Subjects were examined in the supine position with arms outstretched overhead to decrease beam hardening or streak artifact. Scanning was performed at a 90-kV exposure. The exposure time was 0.1 s, and the scanning time was 0.5 s. A 10-mm CT slice scan was acquired at the L3–L4 level to measure the total abdominal and visceral fat areas. Adipose tissue attenuation was determined by measuring the mean value of all pixels within the range of −190 to −30 Hounsfield units. The images were converted into files compatible with a commercial software program (Rapida; 3DMED, Seoul, Korea). To assess visceral adipose tissue volume, each abdominal image was edited by erasing the image excess adipose tissue, and the resulting images were saved in separate files.

**The frequently sampled intravenous glucose tolerance test and the minimal model**

In the morning between 0700 and 0900 h, after an overnight fast of 12 h or more, an intravenous catheter was placed...
Visceral fat, insulin sensitivity, and GDM

Table 1—Anthropometric characteristics and biochemical parameters 1 year postpartum in study subjects

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GDM-NGT</th>
<th>GDM-IGT</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>17</td>
<td>60</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.6 ± 4.4</td>
<td>33.6 ± 4.2</td>
<td>34.4 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158.6 ± 6.3</td>
<td>158.1 ± 5.6</td>
<td>157.7 ± 5.5</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>54.9 ± 7.0</td>
<td>56.3 ± 8.1</td>
<td>57.9 ± 9.3</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.8 ± 2.4</td>
<td>22.5 ± 2.8</td>
<td>23.3 ± 3.3</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>108.4 ± 7.7</td>
<td>114.8 ± 10.3</td>
<td>114.4 ± 8.6</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>67.6 ± 6.2</td>
<td>70.7 ± 7.2</td>
<td>70.1 ± 8.2</td>
<td>NS</td>
</tr>
<tr>
<td>A1C (%)</td>
<td>4.6 ± 0.4</td>
<td>4.9 ± 0.3</td>
<td>5.4 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.0 ± 0.8</td>
<td>4.7 ± 0.7</td>
<td>4.9 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>0.9 ± 0.4</td>
<td>1.2 ± 0.6</td>
<td>1.7 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.5 ± 0.3</td>
<td>1.4 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.0 ± 0.7</td>
<td>2.8 ± 0.6</td>
<td>2.8 ± 0.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

75-g OGTT

| Glucose, 0 min (mmol/l) | 5.0 ± 0.4 | 5.3 ± 0.6 | 5.4 ± 0.7 | B       |
| Glucose, 30 min (mmol/l) | 7.3 ± 0.9 | 9.1 ± 1.3 | 10.6 ± 3.0 | B,C     |
| Glucose, 60 min (mmol/l) | 5.9 ± 1.8 | 8.8 ± 1.8 | 12.5 ± 3.9 | B,C     |
| Glucose, 90 min (mmol/l) | 5.8 ± 1.0 | 7.4 ± 1.5 | 11.6 ± 4.4 | B,C     |
| Glucose, 120 min (mmol/l) | 5.8 ± 1.0 | 6.5 ± 0.9 | 9.4 ± 1.2  | B,C     |
| Insulin, 0 min (pmol/l) | 56.2 ± 11.4 | 77.2 ± 35.5 | 91.5 ± 61.0 | B       |
| Insulin, 30 min (pmol/l) | 369.8 ± 183.7 | 355.9 ± 158.2 | 416.6 ± 324.0 | NS     |
| Insulin, 60 min (pmol/l) | 330.9 ± 162.3 | 496.6 ± 270.4 | 642.0 ± 498.3 | B       |
| Insulin, 90 min (pmol/l) | 225.5 ± 128.3 | 459.3 ± 283.5 | 694.1 ± 656.8 | B,C     |
| Insulin, 120 min (pmol/l) | 195.8 ± 150.7 | 377.8 ± 194.0 | 745.6 ± 591.3 | B,C     |

Data are means ± SD. *ANOVA with post hoc test was used (A, B, and C indicate significant difference between two groups: A = control vs. GDM-NGT, B = control vs. GDM-IGT, C = GDM-NGT vs. GDM-IGT; P < 0.05 in all cases).

in the subject’s forearms: one catheter for bolus injections of glucose and the other for rapid, repeated blood sampling to measure glucose and insulin concentrations. After baseline samples (−30, −15, and −1 min), a bolus of glucose (0.3 g/kg body wt) was injected over a 60-s period. Human regular insulin (0.03 units/kg) was injected 20 min later over a 30-s interval. Blood was sampled 29 times over the next 180 min at an initial frequency of one sample per minute for the first 10 min and then at longer intervals as follows: 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 160, and 180 min. Bergman’s minimal model was used to determine the S1, which measures the quantitative influence of insulin that enhances the fractional rate of glucose disappearance. A nonlinear least-squares method was used to fit the time course of plasma glucose disappearance with the plasma insulin concentration as a known input to the system (26). The acute insulin response to glucose (AIRg), the mean insulin increment in the plasma insulin concentration above the basal level in the first 10 min after the administration of glucose, was also calculated. The disposition index, a measure of acute pancreatic β-cell compensation for insulin resistance, was calculated by multiplying S1 by AIRg (27).

Statistical analysis

Statistical analyses were conducted using SPSS for Windows, version 11.0 (Chicago, IL). Data are expressed as means ± SD. Significant differences between groups were evaluated using Student’s t test and ANOVA with post hoc tests to locate the difference. Correlations between variables were analyzed by Spearman correlation because of the relatively small numbers of women in each subgroup. P < 0.05 was considered significant.

RESULTS—Table 1 shows the anthropometric characteristics, biochemical parameters, and results of the 75-g OGTT at the 1 year postpartum evaluation in the three groups: GDM-IGT, age- and BMI-matched GDM-NGT, and normal control subjects. Height, body weight, systolic and diastolic blood pressure, and the concentrations of total cholesterol, HDL cholesterol, and LDL cholesterol did not differ among the three groups. The concentrations of A1C and triglycerides were higher in the GDM-IGT group than in the GDM-NGT and normal control groups. As expected, fasting and postload glucose concentrations and plasma insulin concentration were highest in the GDM-IGT group, intermediate in the GDM-NGT group, and lowest in the control group. The increment of insulin at 30 min during the OGTT was divided by the increment of glucose for the same time using the equation:

\[ \frac{[\Delta I_{30}]}{[\Delta G_{30}]} = \text{insulin (30 min − 0 min)} / \text{glucose (30 min − 0 min)}. \]

\[ \Delta I_{30}/\Delta G_{30} \] was significantly higher in the control group (147.6 ± 93.4) than in the GDM-NGT group (83.7 ± 48.6, P < 0.01) and GDM-IGT group (71.0 ± 58.0, P < 0.01).

Table 2 shows the mean body composition measured by bioelectrical impedance in the three groups. Total body fat and lean body mass did not differ among the groups. However, in the fat amounts measured by CT, visceral fat and the visceral-to-subcutaneous fat ratio were significantly higher in the GDM-IGT group than in the GDM-NGT or normal control groups. Interestingly, total, visceral, and thigh fat were similar in the normal control and the GDM-NGT groups.
Table 2—Body composition by bioimpedance method, body fat by CT, and Sₙ, AIRg, and disposition index by minimal model

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 18)</th>
<th>GDM-NGT (n = 60)</th>
<th>GDM-IGT (n = 21)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>18</td>
<td>60</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td><strong>Bioimpedance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>28.7 ± 4.3</td>
<td>28.7 ± 5.2</td>
<td>29.7 ± 4.2</td>
<td>NS</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>36.7 ± 3.4</td>
<td>38.0 ± 4.3</td>
<td>38.5 ± 4.7</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Fat CT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fat amount (cm²)</td>
<td>306.3 ± 95.0</td>
<td>309.7 ± 108.7</td>
<td>350.1 ± 117.3</td>
<td>NS</td>
</tr>
<tr>
<td>Visceral fat amount (cm²)</td>
<td>86.9 ± 38.3</td>
<td>85.9 ± 30.8</td>
<td>113.9 ± 42.4</td>
<td>B,C</td>
</tr>
<tr>
<td>Subcutaneous fat amount (cm²)</td>
<td>223.4 ± 63.9</td>
<td>224.6 ± 80.9</td>
<td>240.1 ± 93.8</td>
<td>NS</td>
</tr>
<tr>
<td>Visceral-to-subcutaneous fat ratio</td>
<td>0.38 ± 0.13</td>
<td>0.40 ± 0.12</td>
<td>0.51 ± 0.22</td>
<td>B,C</td>
</tr>
<tr>
<td>Thigh fat amount (cm²)</td>
<td>108.9 ± 31.8</td>
<td>101.1 ± 29.8</td>
<td>95.2 ± 33.2</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Minimal model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sₙ (10⁻⁴ min⁻¹ per μU/ml)</td>
<td>5.6 ± 2.3</td>
<td>4.9 ± 2.1</td>
<td>3.6 ± 1.9</td>
<td>B,C</td>
</tr>
<tr>
<td>AIRg (μU/ml)</td>
<td>450.7 ± 240.4</td>
<td>265.5 ± 193.0</td>
<td>170.3 ± 118.3</td>
<td>B</td>
</tr>
<tr>
<td>Disposition index</td>
<td>2,420.3 ± 1,345.8</td>
<td>1,194.9 ± 840.0</td>
<td>581.2 ± 421.7</td>
<td>A,B</td>
</tr>
</tbody>
</table>

Data are means ± SD. *ANOVA with post hoc test was used (A, B, and C indicate significant difference between two groups: A = control vs. GDM-NGT, B = control vs. GDM-IGT, C = GDM-NGT vs. GDM-IGT; P < 0.05 in all cases). Sₙ, minimal model insulin sensitivity index; AIRg, incremental area under plasma insulin curve during the first 10 min after glucose injection; disposition index = Sₙ × AIRg, B-cell compensation for insulin resistance.

Minimal model parameters calculated for the frequently sampled intravenous glucose tolerance test are also summarized in Table 2. At 1 year postpartum, Sₙ and AIRg were highest in the normal control group and lowest in the GDM-IGT group. β-Cell function, as measured by AIRg, was highest in the normal control group and lowest in the GDM-IGT group. The disposition index differed significantly between the control and GDM-NGT and GDM-IGT groups. Glucose effectiveness was significantly lower in the GDM-IGT group than in the GDM-NGT or control group (data not shown).

The associations between visceral fat and insulin sensitivity, insulin secretion, and disposition index (B-cell compensation for insulin sensitivity) were examined. The Sₙ by the minimal model was significantly negatively correlated with the visceral fat amount by CT when the three groups were combined (r = −0.421, P < 0.01, Spearman correlation). Compared by subgroup, Sₙ was significantly negatively correlated in the GDM-NGT group (r = −0.352, P = 0.012) and GDM-IGT group (r = −0.644, P = 0.003) but not in the normal control group (r = −0.259, NS) (Fig. 1). After excluding two outliers with visceral fat >175 cm², the Spearman correlation between Sₙ and visceral fat decreased slightly to −0.535 in the GDM-IGT group but remained significant (P = 0.033). The absolute value of the correlation coefficient was higher in the GDM-IGT group than in the GDM-NGT group, although the slopes of the regression lines did not differ significantly.

Body weight varied substantially in all groups. Mean body weight was 3 kg greater in the GDM-IGT subgroup than in the control subjects, although this difference was not significant. We included body weight in the regression model as a covariate to investigate the association between Sₙ and visceral fat. Sₙ remained significantly associated with visceral fat if adjusted for body weight (P = 0.013). Insulin secretion (AIRg) and visceral fat amount were significantly negatively correlated in the normal control group (r = −0.520, P = 0.033), although these variables did not differ when all groups were combined or in GDM subgroups. The disposition index and visceral fat amount were significantly negatively correlated when all groups were combined (r = −0.242, P = 0.025) and in the control group (r = −0.517, P = 0.034), although these variables did not differ between the GDM subgroups.

**CONCLUSIONS**— We previously demonstrated in Korean women that impaired β-cell function during pregnancy is associated with early postpartum glucose intolerance (11). Buchanan and colleagues (28,29) showed that the plasma insulin concentration in the 75-g OGTT is lower during pregnancy in women with GDM who were proved to have diabetes or IGT than in normal control subjects.

In this age- and BMI-matched study, the visceral fat content was greater 1 year postpartum in the women with previous GDM and IGT (GDM-IGT group) than in those who had previous GDM and NGT (GDM-NGT group) or in those who had normal glucose metabolism during pregnancy (normal control group). This finding agrees with previous data in Japanese Americans by Fujimoto et al. (23). Insulin sensitivity measured by the minimal model (Sₙ) was lower in the GDM-IGT group than in the GDM-NGT or normal control groups and was significantly correlated with visceral fat content, with or without adjustments for body weight or BMI.

Like the patterns of visceral fatness and insulin sensitivity, β-cell function, as measured by the AIRg, was lower in the GDM-IGT group. Similarly, Δ₁₃₀/ΔG₃₀, which reflects β-cell function compensation for insulin resistance and is calculated as the increment of insulin relative to the increment of glucose in the first 30 min during the OGTT, was lower in the two GDM groups than in the control group. The disposition index, the index of β-cell compensation for insulin resistance, was also lower in both GDM subgroups. Interestingly, the disposition index and Δ₁₃₀/ΔG₃₀ were lower in the GDM-NGT group than in the control group. β-Cell function, expressed by AIRg, was lower in the GDM-NGT group than in the control group. Thus, the decreases in insulin sensitivity and the insulin secretory response to glucose along with the increase in visceral fat content are associated with the presence of IGT in Korean women with a history of GDM; this suggests that these factors play a role in the development of IGT, which may account for the similar prevalence of GDM and postpartum diabetes in Korean women and women in Western countries despite the lower BMI in Korean women than in Caucasian women (11).

Prepregnancy obesity is an independent risk factor for postpartum glucose intolerance (11,29,30). We focused on visceral fatness by matching BMI, because BMI is an important risk factor for the development of insulin resistance or dia-
We found that the visceral fat content was higher in the GDM-IGT group than in the GDM-NGT group after matching for age and BMI, suggesting that visceral obesity is one possible pathophysiological mechanism responsible for the development of impaired glucose metabolism in women with previous GDM. It has been suggested that enlarged fat cells in the fat depot are more responsive to the lipolytic effects of catecholamines and less sensitive to the antilipolytic action of insulin than are smaller fat cells from the gluteal-femoral region and that this difference in responsiveness may underlie the detrimental effects of visceral fat (32,33). These actions are believed to promote the release of fatty acids into the portal circulation and liver, leading to an increase in hepatic glucose output and induction of elevated insulin resistance (34). Taken together, these data suggest that, in Korean women with previous GDM, development of IGT is related to both insulin resistance (the insulin secretory response to glucose) and an increase in body fat content, especially the visceral fat content.

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References


Sex Differences in Endothelial Function Markers Before Conversion to Pre-Diabetes: Does the Clock Start Ticking Earlier Among Women?

The Western New York Study

Richard P. Donahue, PhD1
Karol Rejman, MS1
Lisa B. Rafalson, MS1

Jacek Dmochowski, PhD1,2
Saverio Stranges, MD1
Maurizio Trevisan, MD, MS1

OBJECTIVE — We examined whether biomarkers of endothelial function, fibrinolysis/thrombosis and adiponectin, predict the progression from normal to pre-diabetes more strongly among women than men over 6 years of follow-up from the Western New York Health Study.

RESEARCH DESIGN AND METHODS — In 2002–2004, 1,455 participants from the Western New York Health Study, who were free of type 2 diabetes and cardiovascular disease at baseline (1996–2001), were selected for reexamination. An incident case of pre-diabetes was defined as fasting glucose <100 mg/dl at the baseline examination and ≥100 and <126 mg/dl at the follow-up examination. Biomarkers of endothelial function (E-selectin and soluble intracellular adhesion molecule-1 [sICAM-1]), fibrinolysis/thrombosis (plasminogen activator inhibitor-1 [PAI-1]), and fasting insulin, adiponectin, and inflammation (high-sensitivity C-reactive protein) were measured in frozen (−190°C) baseline samples.

RESULTS — Multivariate analyses revealed higher adjusted mean values of biomarkers of endothelial dysfunction (E-selectin and sICAM-1) and fibrinolysis (PAI-1) and lower mean values of adiponectin only among women who developed pre-diabetes compared with control subjects. Formal tests for interaction between sex and case/control status were statistically significant for E-selectin (P = 0.042), PAI-1 (P = 0.001), sICAM-1 (P = 0.011), and frequency of hypertension (P < 0.001).

CONCLUSIONS — These results support the concept that women who progressed from normoglycemia to pre-diabetes have greater endothelial dysfunction than men as well as more hypertension and a greater degree of fibrinolysis/thrombosis. Whether this relates to the higher risk of heart disease among diabetic women awaits further study.


Death rates from coronary heart disease (CHD) in the U.S. have fallen dramatically over the last several decades; however, the rate of decline has been greater among men than among women (1). The factors underlying this sex difference are unclear (2–6). It has also been suggested that women, especially those with type 2 diabetes, are more likely to have coronary microvascular disease than men (7,8). It is well known that diabetic women have a much higher risk for CHD than their male counterparts, a risk not completely explained by traditional biological and psychosocial factors (9,10).

Previous studies have shown that the frequency of nonfatal myocardial infarction is increased before the clinical diagnosis of type 2 diabetes (11) and that women with impaired glucose tolerance tend to have a more atherogenic risk profile than their male counterparts years before the diagnosis of clinical diabetes (12). This observation has led to the “ticking clock” hypothesis (13), wherein the elevated CHD risk among diabetic individuals may be due more to their long-standing atherogenic risk profile than to hyperglycemia per se, which is more strongly associated with the risk of microvascular events (14). Recently, attention has been focused on the role of emerging risk factors including markers of endothelial dysfunction, inflammation, and fibrinolysis/thrombosis as precursors of both CHD and type 2 diabetes (15–17). In particular, elevated concentrations of E-selectin and plasminogen activator inhibitor-1 (PAI-1) have been associated with a twofold risk of CHD and diabetes (18,19). Adiponectin concentrations, which may be involved in chronic low-grade inflammation (20), have also been seen in some populations to predict CHD risk (21,22). Thus, progression from normal to impaired fasting glucose (pre-diabetes) to diabetes would appear to be associated with a worsening of CVD risk factors, although longitudinal data on this hypothesis are scant. It is not known whether the clock starts ticking earlier among women and thus might contribute proportionately more to the risk for subsequent CHD in those with type 2 diabetes.

These analyses were designed to investigate the following questions: 1) what traditional and emerging CHD risk factors
predict conversion from normoglycemia to pre-diabetes, 2) does sex modify the association between these emerging risk factors and conversion to pre-diabetes, and 3) are these sex differences independent of other covariates including adiposity and insulin resistance?

**RESEARCH DESIGN AND METHODS** — The study design and methodology of this population-based investigation have been published previously (23,24). Briefly, participants in this study were originally enrolled as healthy control subjects in the Western New York Study, an epidemiologic case-control investigation of alcohol intake patterns and risk of CVD in Erie and Niagara Counties, New York, conducted from 1996 to 2001. The initial cohort of control participants was randomly selected from driver’s license lists for those aged <65 years and from the Health Care Finance Administration rolls for those aged 65–79. In 2001–2004, we conducted the first follow-up of the apparently healthy myocardial infarction control group. Eligible participants for the follow-up study were men and women aged 39–79 years selected from the baseline examination cohort without known clinical CVD (self-reported myocardial infarction, angina, or revascularization surgery) or type 2 diabetes (measured fasting plasma glucose >125 mg/dl or self-report and taking medication) who were capable of completing the current study protocol \( n = 2,652 \). Exclusion criteria also included self-report of a medical condition that would prohibit participation (e.g., all cancers except skin cancer, type 1 diabetes, or physical or mental impairment), a permanent change in residence to out of state, death, or inability to contact and determine eligibility. This left 2,139 individuals eligible for this examination of whom 1,455 completed the full clinic protocol (68.0% response rate). The mean ± SD follow-up time was 5.9 ± 0.8 years. The protocol was approved by the State University at Buffalo Health Science Institutional Review Board, and all participants provided written informed consent before participation.

At both the baseline and 6-year follow-up examinations, all participants received a clinical examination that included resting blood pressure and measures of height, weight, and waist girth. Resting seated blood pressure was obtained by trained and certified technicians according to a standardized protocol (25). Hypertension was defined as a systolic pressure ≥140 mmHg or a diastolic pressure ≥90 mmHg or use of antihypertensive medications regardless of blood pressure level. Height was recorded to the nearest one-quarter inch, and weight was measured to the nearest one-quarter pound. BMI (weight in kilograms divided by the square of height in meters) was calculated and served as a measure of relative weight. Waist girth (centimeters) was determined with participants standing erect with the abdomen relaxed, arms at the side, and feet together. A linen tape was placed horizontally between the bottom of the last rib and the top of the iliac crest around the smallest of these two reference points. The measurement was taken at end-expiration and recorded to the nearest 0.1 cm. Study subjects also provided a fasting (at least 10 h overnight) blood sample and were asked to refrain from smoking or vigorous physical activity for 24 h. Several standardized questionnaires were administered to determine cigarette use, physical activity (Stanford 7-Day Recall), alcohol use, general health and well-being, personal and family health history, medication use, and socioeconomic status. In this analysis, no one reported a positive history for gestational diabetes. Participants were instructed to bring all medications to the clinic visits to permit identification of oral medications as well as insulin use. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting glucose \( \times \) fasting insulin/22.4 (26). A positive family history of type 2 diabetes was defined as a positive report in a first-degree relative. Data concerning family history of type 1 diabetes were not available.

Fasting glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA). The interassay coefficient of determination was <5%. Fasting triglycerides were measured with enzymatic techniques. Information on HDL cholesterol was not available. After identification of those who progressed (or not) to pre-diabetes, the baseline aliquots of serum or plasma were retrieved and sent by overnight courier to the Laboratory for Clinical Biochemistry Research, University of Vermont, Burlington, Vermont, or the Department of Endocrinology at the University of Pittsburgh, Pittsburgh, Pennsylvania, for assay. Interleukin-6, a major proinflammatory cytokine, was measured by an ultrasensitive enzyme-linked immunosorbsent assay (R&D Systems, Minneapolis, MN). Using this method, we have determined a routine coefficient of variation (CV) in the laboratory of 6.3%. Soluble E-selectin, also known as endothelial leukocyte adhesion molecule-1 and CD62E, was measured using a high-sensitivity quantitative sandwich enzyme (Parameter Human sE-Selectin Immunoassay; R&D Systems). Intra-assay and interassay CVs range from 4.7 to 5.0% and from 5.7 to 8.8%, respectively. Human soluble intercellular adhesion molecule-1 (sICAM-1) was measured by an enzyme-linked immunosorbsent assay (Parameter Human sICAM-1 Immunoassay; R&D Systems). The laboratory CV was 5.0%. High-sensitivity C-reactive protein (hsCRP) was measured using the BNII nephelometer from Dade Behring with a particle-enhanced immunonephelometric assay. Intra-assay CVs range from 2.3 to 4.4%, and interassay CVs range from 2.1 to 5.7%. Adiponectin was assayed from a kit provided by Linco Research. This kit is a radioimmunoassay using a double antibody-polyethylene glycol separation. The assay has a detection limit of 1 ng/ml. The intra-assay CV ranges from 12.2 to 14.5%, and the interassay CV ranges from 3.7 to 6.1. Fasting insulin was assayed from a kit provided by Linco Research that has minimal cross-reactivity with human proinsulin. The assay has a lower detection limit of 2 μU/ml with an interassay CV ranging from 3.6 to 8.4% and an intra-assay CV ranging from 2.2 to 4.4%. A frozen spot urine sample was also retrieved and used for the assessment of the albumin-to-creatinine (ACR) ratio. Collections were done in the morning of the baseline visit and stored on liquid nitrogen. Albumin was assayed with a nephelometric immunoassay using a monospecific antiserum to human albumin. Creatinine concentration was determined using the modified Jaffé method. The urinary ACR (milligrams of albumin/grams of creatinine) was calculated and used as a surrogate of albumin excretion rate (27). Intraclass correlation coefficients among duplicate samples exceeded 0.97. A value exceeding 300 mg/g was considered to be evidence of overt nephropathy, and one study sample was omitted from these analyses. Of the values, >94% were <30 mg/g.

**Statistical procedures** — For these analyses, we identified a “case” of pre-diabetes as an individual whose
fasting serum glucose was <100 mg/dl at baseline but was between 100 and 125 mg/dl (5.7 and 6.5 mmol/l) at follow-up. Each case patient was matched to three control subjects based upon sex, ethnicity (white or nonwhite), and year of study enrollment. Age was considered as a continuous covariate in all analyses. All control subjects had a fasting blood glucose concentration <100 mg/dl at both the baseline and follow-up examinations.

For this report, the data were analyzed using statistical techniques that incorporated the matching variables as covariates. The distributions of fasting triglycerides, ACR, and fasting insulin were log transformed (natural log) before analysis to approximate more normal distributions. General linear models (28) or logistic regression techniques (29) were used to compare the adjusted differences between case subjects and control subjects. Likelihood ratio tests were performed to determine statistical interactions between sex and case-control status by comparing the log likelihood between the two nested models, one with only the main effects and the other with both the main effects and the interaction terms in the model. All statistical tests were two-sided, and a $P$ value <0.05 was considered statistically significant.

RESULTS — During a mean ± SD follow-up of 5.9 ± 0.8 years, 52 women, and 39 men progressed from normoglycemia to pre-diabetes (Table 1). Compared with the control subjects, the pre-diabetic women were older and had a higher mean waist circumference after adjustment for age, ethnicity, and year of study enrollment. They also displayed higher age-adjusted mean levels of E-selectin, sICAM-1, and total triglycerides, and fasting glucose, a higher value for the HOMA-IR index, and a greater frequency of hypertension ($P < 0.05$). Mean concentrations of adiponectin were significantly lower among female case subjects compared with control subjects ($P = 0.004$). Among men, case subjects with pre-diabetes displayed significantly higher adjusted mean concentrations of hsCRP ($P = 0.047$) and a marginally higher mean value for the HOMA-IR index ($P = 0.08$). No significant differences were noted for any of the endothelial biomarkers or PAI-1. Statistically significant sex by case-control interactions were observed for E-selectin, sICAM-1, and PAI-1 (all $P < 0.05$). The results for adiponectin were suggestive but did not achieve a conventional level of statistical significance ($P = 0.111$), due perhaps to the limited sample size.

Table 2 presents the results adjusted further for BMI. Among women, differences in HOMA-IR and triglyceride concentrations diminished ($P = 0.149$ and 0.058, respectively). However, differences in E-selectin, sICAM-1, and adiponectin remained essentially unaltered. Among men, the results were also virtually unchanged, except for the HOMA-IR index, which was no longer significant ($P = 0.262$). The sex by case-control interactions noted in Table 1 remained significant. Additional adjustment for the HOMA-IR index (Table 3) resulted in nonsignificant differences in triglyceride concentrations among women. Formal tests for interaction between sex and case/control status were statistically significant for E-selectin ($P = 0.042$), PAI-1 ($P = 0.001$), sICAM-1 ($P = 0.011$), and the frequency of hypertension ($P < 0.001$) and were again suggestive but not statistically significant for adiponectin ($P = 0.131$). It was not possible to adjust the sex differences in risk factors for waist circumference because of the limited overlap in the distributions between men and women; i.e., adjustment for waist girth was equivalent to adjustment for sex. To examine whether differences in endothelial function were moderated by inflammation, we further adjusted for hsCRP (data not shown). The results remained virtually unchanged. Further consideration of weight change, family history of type 2 diabetes, physical inactivity, and use of lipid-lowering medications did not alter these findings.

CONCLUSIONS — It is clear from several studies that risk factors for CVD and diabetes are elevated long in advance of clinical diagnosis (9,11,13). Factors other than worsening hyperglycemia, however, are responsible for the origins of the increased risk of CHD in those with

### Table 1—Age and age-adjusted mean levels of selected risk factors according to sex and case/control status

<table>
<thead>
<tr>
<th>Risk factor at baseline</th>
<th>Women</th>
<th></th>
<th>Men</th>
<th></th>
<th>P value</th>
<th>P value for interaction*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case subjects</td>
<td>Control subjects</td>
<td></td>
<td>Case subjects</td>
<td>Control subjects</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>52</td>
<td>156</td>
<td></td>
<td>39</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>61.6 ± 10.8</td>
<td>56.5 ± 10.3</td>
<td></td>
<td>63.0 ± 11.3</td>
<td>60.9 ± 11.4</td>
<td></td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>28.8 ± 5.4</td>
<td>27.5 ± 5.3</td>
<td></td>
<td>25.7 ± 4.9</td>
<td>24.7 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>CRP (µg/ml)</td>
<td>91.3 ± 12.9</td>
<td>86.2 ± 12.3</td>
<td></td>
<td>89.7 ± 12.8</td>
<td>86.9 ± 9.2</td>
<td></td>
</tr>
<tr>
<td>Interleukin-6 (pg/ml)</td>
<td>4.7 ± 3.5</td>
<td>4.1 ± 2.9</td>
<td></td>
<td>2.5 ± 2.6</td>
<td>1.8 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>E-selectin (ng/ml)</td>
<td>51.2 ± 17.9</td>
<td>45.5 ± 18.2</td>
<td></td>
<td>40.1 ± 16.9</td>
<td>42.0 ± 16.6</td>
<td></td>
</tr>
<tr>
<td>sICAM-1 (ng/ml)</td>
<td>274.7 ± 61.4</td>
<td>252.5 ± 52.8</td>
<td></td>
<td>252.2 ± 50.7</td>
<td>255.8 ± 57.7</td>
<td></td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>37.3 ± 18.8</td>
<td>26.1 ± 16.3</td>
<td></td>
<td>27.5 ± 15.6</td>
<td>28.4 ± 17.8</td>
<td></td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>9.1 ± 5.2</td>
<td>11.4 ± 5.0</td>
<td></td>
<td>6.8 ± 4.4</td>
<td>7.2 ± 4.0</td>
<td></td>
</tr>
<tr>
<td>In triglycerides (mg/dl)</td>
<td>4.7 ± 0.5</td>
<td>4.5 ± 0.5</td>
<td></td>
<td>4.7 ± 0.6</td>
<td>4.6 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>91.9 ± 4.8</td>
<td>88.3 ± 5.5</td>
<td></td>
<td>94.0 ± 3.6</td>
<td>90.6 ± 5.1</td>
<td></td>
</tr>
<tr>
<td>In ACR (mg/g)</td>
<td>1.91 ± 0.8</td>
<td>1.79 ± 0.7</td>
<td></td>
<td>1.4 ± 0.9</td>
<td>1.4 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.3 ± 1.6</td>
<td>2.8 ± 1.3</td>
<td></td>
<td>3.7 ± 1.9</td>
<td>3.1 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)†</td>
<td>49.7</td>
<td>27.0</td>
<td></td>
<td>61.1</td>
<td>47.1</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SD. *Interaction of sex by case-control status. †Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or receiving treatment.
diabetes. How long in advance of clinical diabetes this risk can be detected and whether or not sex differences may be observed are poorly understood.

Most extant studies have documented progression from impaired glucose tolerance to diabetes and have naturally focused on the traditional CVD risk factors that predict conversion to diabetes (13,30). Our report supports and extends previous work by noting the novel role that several emerging risk factors may play in progression from normoglycemia to “pre-diabetes,” particularly among women.

Our results are clearly important as recent clinical trial evidence shows that delay in development or prevention of diabetes is possible, and preventive efforts should occur early in the pre-diabetic state (31,32). Whether certain prevention efforts or treatment should be sex specific awaits further study. However, this is among the first reports to show that women are more likely than men to manifest elevated levels of endothelial factors, fibrinolysis/thrombosis, and adiponectin during the transition from normoglycemia to pre-diabetes. Previous findings by Haffner et al. (12) demonstrated that those who progressed to type 2 diabetes were likely to have a more atherogenic cardiovascular risk profile than those who did not. Most of the study subjects had impaired glucose tolerance at baseline, however. The same pattern held true when the analyses were confined to normoglycemic individuals at the start of follow-up, consistent with our results.

Nondiabetic women are more likely to have a more favorable CVD risk profile than nondiabetic men and are less likely to develop and die from CVD at any age, findings that are not explained by sex differences in obesity or other metabolic or behavioral factors. One hypothesis to explain this “female advantage” is that women (especially younger women) are more insulin sensitive (less insulin resistant) than their male counterparts, an advantage that is diminished or abolished in the diabetic state (33). Surrogate indexes of insulin resistance including the HOMA-IR have been positively associated with E-selectin, PAI-1, and other endothelial markers (34,35). In this report, consideration of the HOMA-IR index failed to reduce or eliminate the sex differences in the risk factors of interest. Co-variate adjustment for the HOMA-IR may

Table 2—Age- and BMI-adjusted mean levels of selected risk factors according to sex and case/control status

<table>
<thead>
<tr>
<th></th>
<th>Women Case subjects</th>
<th>Women Control subjects</th>
<th>P value</th>
<th>Men Case subjects</th>
<th>Men Control subjects</th>
<th>P value for interaction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>52</td>
<td>156</td>
<td></td>
<td>39</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>87.9</td>
<td>89.6</td>
<td>0.023</td>
<td>92.9</td>
<td>92.4</td>
<td>0.627</td>
</tr>
<tr>
<td>CRP (µg/ml)</td>
<td>4.4</td>
<td>4.0</td>
<td>0.452</td>
<td>2.7</td>
<td>2.2</td>
<td>0.112</td>
</tr>
<tr>
<td>Interleukin-6 (pg/ml)</td>
<td>2.8</td>
<td>2.8</td>
<td>0.856</td>
<td>1.9</td>
<td>2.2</td>
<td>0.159</td>
</tr>
<tr>
<td>E-selectin (ng/ml)</td>
<td>51.0</td>
<td>45.5</td>
<td>0.073</td>
<td>41.1</td>
<td>43.6</td>
<td>0.424</td>
</tr>
<tr>
<td>sICAM-1 (ng/ml)</td>
<td>272.5</td>
<td>252.0</td>
<td>0.022</td>
<td>257.8</td>
<td>264.4</td>
<td>0.523</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>34.7</td>
<td>25.9</td>
<td>0.001</td>
<td>29.2</td>
<td>31.6</td>
<td>0.420</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>9.7</td>
<td>11.4</td>
<td>0.023</td>
<td>6.6</td>
<td>6.9</td>
<td>0.636</td>
</tr>
<tr>
<td>ln triglycerides (mg/dl)</td>
<td>4.7</td>
<td>4.5</td>
<td>0.058</td>
<td>4.7</td>
<td>4.6</td>
<td>0.213</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>91.6</td>
<td>88.3</td>
<td>&lt;0.001</td>
<td>94.2</td>
<td>90.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ln ACR (mg/g)</td>
<td>1.6</td>
<td>1.5</td>
<td>0.711</td>
<td>1.8</td>
<td>1.8</td>
<td>0.801</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.61</td>
<td>26.9</td>
<td>0.011</td>
<td>65.5</td>
<td>54.5</td>
<td>0.203</td>
</tr>
</tbody>
</table>

Data are means. *Interaction of sex by case-control status. †Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or receiving treatment.

Table 3—Age-, BMI-, and HOMA-IR–adjusted mean levels of selected risk factors according to sex and case/control status

<table>
<thead>
<tr>
<th></th>
<th>Women Case subjects</th>
<th>Women Control subjects</th>
<th>P value</th>
<th>Men Case subjects</th>
<th>Men Control subjects</th>
<th>P value for interaction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>52</td>
<td>156</td>
<td></td>
<td>39</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>87.7</td>
<td>85.8</td>
<td>0.056</td>
<td>93.2</td>
<td>92.5</td>
<td>0.511</td>
</tr>
<tr>
<td>CRP (µg/ml)</td>
<td>4.5</td>
<td>4.1</td>
<td>0.457</td>
<td>2.8</td>
<td>2.2</td>
<td>0.110</td>
</tr>
<tr>
<td>Interleukin-6 (pg/ml)</td>
<td>2.8</td>
<td>2.9</td>
<td>0.762</td>
<td>1.9</td>
<td>2.2</td>
<td>0.225</td>
</tr>
<tr>
<td>E-selectin (ng/ml)</td>
<td>51.2</td>
<td>45.5</td>
<td>0.062</td>
<td>40.5</td>
<td>43.2</td>
<td>0.374</td>
</tr>
<tr>
<td>sICAM-1 (ng/ml)</td>
<td>273.0</td>
<td>254.5</td>
<td>0.036</td>
<td>254.8</td>
<td>264.0</td>
<td>0.374</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>34.6</td>
<td>27.0</td>
<td>0.003</td>
<td>26.8</td>
<td>31.7</td>
<td>0.097</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>9.6</td>
<td>11.1</td>
<td>0.055</td>
<td>6.8</td>
<td>6.9</td>
<td>0.903</td>
</tr>
<tr>
<td>ln triglycerides (mg/dl)</td>
<td>4.6</td>
<td>4.5</td>
<td>0.230</td>
<td>4.7</td>
<td>4.6</td>
<td>0.363</td>
</tr>
<tr>
<td>ln ACR (mg/g)</td>
<td>1.6</td>
<td>1.5</td>
<td>0.783</td>
<td>1.7</td>
<td>1.8</td>
<td>0.523</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>44.5</td>
<td>28.8</td>
<td>0.035</td>
<td>63.6</td>
<td>54.8</td>
<td>0.306</td>
</tr>
</tbody>
</table>

Data are means. *Interaction of sex by case-control status. †Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or receiving treatment.
not completely capture the effects of insulin resistance, however, as it is only modestly correlated with insulin resistance assessed with the hyperinsulinemic-euglycemic clamp method (36).

Several studies have shown that elevated concentrations of E-selectin and PAI-1 are significant independent predictors of both CHD and type 2 diabetes (18,19). Few studies have examined these novel risk factors with respect to the development of pre-diabetes in a population-based cohort. Caballero et al. (37) found abnormalities in both microvascular and macrovascular reactivity as well as abnormalities in markers of endothelial activation that were present in individuals at risk of developing type 2 diabetes, even at a stage of normoglycemia. Retinal microvascular abnormalities have been associated with blood pressure, inflammation, and endothelial dysfunction (38). Such microvascular abnormalities predicted ischemic heart disease in women but not men (39). Proinflammatory markers are also associated with endothelial dysfunction (40). However, covariate adjustment for hsCRP levels failed to remove the sex difference in these endothelial markers in our study.

Other risk factors for diabetes include impaired fibrinolysis characterized by elevated concentrations of PAI-1. Indeed, data from the Insulin Resistance Atherosclerosis Study (IRAS) indicated that PAI-1 was among the most predictive of all biomarkers examined (19). PAI-1 is associated with insulin resistance in many studies, although consideration of HOMA-IR did not eliminate the sex difference in this report. Because PAI-1 is secreted from hepatocytes and adipose tissue in addition to endothelium, it may function through a pathway different from that for the cellular adhesion molecules (41) to effect progression risk. Whether these emerging risk factors operate more strongly among women requires further investigation.

Abnormally elevated rates of albumin excretion have been suggested to reflect endothelial dysfunction or other vascular damage in several studies (42). In the current study, the ACR was not significantly related to progression to pre-diabetes in either sex. This could be due, at least in part, to the low prevalence of microalbuminuria in this cohort.

The strengths of our study include its population-based design, the measurement of several emerging risk factors including inflammation and endothelial dysfunction, and the detailed assessment of several covariates of interest. Limitations include the single determination of fasting glucose concentrations used to define pre-diabetes, although this definition is consistent with recent recommendations from the American Diabetes Association and should affect case subjects and control subjects similarly. Although fasting glucose levels show greater correlation over time than postchallenge levels (43), the use of an oral glucose tolerance test may have identified people with type 2 diabetes at follow-up who were classified as pre-diabetic. Finally, measures of endothelial markers were obtained only once, which could have led to some misclassification, although this also should tend to affect the case subjects and the comparison group similarly. It should be noted that the samples were not subjected to repeated freeze-thaw cycles. Lastly, although observational studies cannot prove causality, our data add compelling new evidence that the clock may indeed start ticking earlier among women than among men.

In summary, in this prospective study on the role of endothelial dysfunction and progression to pre-diabetes, we found that E-selection, sICAM-1, and PAI-1 concentrations were predictive of conversion among women but not among men. These results were independent of the effects of age, BMI, and HOMA-IR. These novel and important observations support a role for endothelial dysfunction in the progression to pre-diabetes.

Acknowledgments—This study was supported by National Institutes of Health Grant DK 60587.

We acknowledge the assistance of Mya Swanson for database management and the statistical analyses.

References

13. Haffner SM, Stern MP, Hazuda HP,


An Assessment of Eligibility for Inhaled Insulin (Exubera)

The Fremantle Diabetes Study

Timothy M.E. Davis, FRACP
Wendy A. Davis, PhD

The product information (1) for human insulin inhalation powder (Exubera; Pfizer) recommends spirometry at baseline, 6 months postinitiation, and at least annually thereafter. Patients with a forced expiratory volume in the first second (FEV₁) <70% of that predicted for age, sex, and height should not start Exubera. In those exhibiting ≥20% decline in FEV₁ during follow-up, Exubera should be discontinued. Exubera is contraindicated for patients with unstable or poorly controlled lung disease, and its efficacy and safety have not been established for patients with asthma or chronic obstructive pulmonary disease. To determine the proportions and characteristics of patients predicted to fall into these categories, we analyzed spirometric and other data from the representative community-based Fremantle Diabetes Study (FDS) (2).

RESEARCH DESIGN AND METHODS — The FDS was a longitudinal observational study involving 127 type 1 and 1,294 type 2 diabetic patients from a population of 120,097 in the state of Western Australia (2). Between May 1993 and September 1994, we performed spirometry on 70 type 1 and 647 type 2 diabetic patients (55 and 50% of all type 1 and type 2 diabetic FDS subjects, respectively). We had additional data from 107 nonsmoking Europid type 2 diabetic patients without spirometric data, this group was a mean 1.3 years older, had diabetes for 0.3 years longer, and had an A1C that was 0.3% lower (P ≤ 0.039) but contained a similar proportion of male subjects (P = 0.32). Exclusion of 102 smokers and 91 nonsmokers with FEV₁ <70% would leave 70% of the original sample, and excluding the 30 remaining with chronic respiratory disease would reduce this to 65%. Of these patients, just over half (57%) were treated with insulin and/or oral hypoglycemic agents but still had an A1C above the generally accepted target of 7.0% (8).

The 107 prospectively studied type 2 diabetic patients had a mean age of 61.7 ± 8.6 years, and 48% were male. Their median diabetes duration and A1C was 2.6 years (interquartile range 0.7–7.0) and 7.3% (6.3–8.5), respectively (all P ≥ 0.10 vs. similar available patients at follow-up without spirometric data). The percentage of these patients with baseline FEV₁ >70% who subsequently developed FEV₁ ≤70% was 12.5% (1.8% per year). In a further 17.3%, there was a fall of >20% in FEV₁ during follow-up (2.5% per year). We have used these rates to estimate the percentage of patients who would become ineligible for Exubera over time (Fig. 1). Assuming stable proportions of smokers and those with chronic respiratory disease, this estimate would increase from 35 to 65% over 7 years.

RESULTS — The 70 type 1 diabetic patients were aged 43.8 ± 15.7 years, and 51% were males. They had a median (interquartile range) diabetes duration of 12.8 years (4.9–23.0) with an A1C of 8.5% (7.9–10.0) (all P ≥ 0.08 vs. the 57 type 1 diabetic patients without spirometric data). Applying Exubera contraindications (1), 16 smokers and 7 nonsmokers with FEV₁ <70% would be ineligible, which would leave 67% of the original sample. A further four patients self-reporting chronic respiratory disease might also be ineligible, which would reduce this figure to 61%.

The 647 type 2 diabetic patients were aged 63.5 ± 11.1 years, and 50% were male with a median diabetes duration and A1C of 4.0 years (interquartile range 1.4–10.0) and 7.6% (6.5–8.9), respectively. Compared with the type 2 diabetic patients without spirometric data, this increase from 35 to 65% over 7 years.
other prospective studies (1,9), a similar course seems likely. Based on eligibility and treatment modality data, as well as the proportion with A1C $\geq$7.0%, only about one-third of our type 2 diabetic patients had a reasonable indication for Exubera at baseline.

Exclusion of all patients with chronic lung conditions may mean that we have overestimated ineligibility. However, criteria for unstable or poorly controlled lung disease are not specified (1), and the lack of efficacy data would justify a cautious approach. In other respects, the present ineligibility estimates appear conservative. For example, compared with U.S. data (11), our type 2 diabetic sample had a lower proportion of smokers (17 vs. 23%). In addition, there would be a minor acute therapy-related FEV$_1$ fall (1), which would further decrease the proportion in whom Exubera could be prescribed.

The significant proportion of ineligible subjects at initial assessment, and its doubling over the ensuing 7 years, reinforces the need for regular review of Exubera-treated patients (including recommended spirometry). We did not measure gas transfer. Although access to this investigation may be limited by availability and cost, it is recommended as part of initial and continued assessment. A percentage similar to FEV$_1$ (1) of predicted cut points for carbon monoxide diffusing capacity is likely to add to the proportions of patients who should not be prescribed or continued on Exubera.

Acknowledgments — The FDS was funded by the Raine Foundation, University of Western Australia.

References
Clinical Outcomes and Cost-Effectiveness of Retinopathy Screening in Youth With Type 1 Diabetes

Betty Huo, BS
Amy T. Steffen, BA
Karena Swan, MD

Kristin Sikes, MSN, PNP
Stuart A. Weinzierl, MD
William V. Tamborlane, MD

Research Design and Methods — Charts of all type 1 diabetic patients in our Diabetes Clinic were reviewed. Patients were included if they were aged ≤21 years and had written reports in their charts from an examining ophthalmologist/optometrist. Data regarding A1C, albumin-to-creatinine ratios, blood pressure, and use of antihypertensive medications were extracted. The study was approved by the Yale Human Investigation Committee. The study population was stratified into the four categories shown in Table 1.

DR screening involved ophthalmoscopy with dilated pupils. Diagnosis of DR was based on the written reports of the examining ophthalmologists (n = 195) and optometrists (n = 2). Reports indicating the presence of DR were confirmed by a follow-up discussion with the original ophthalmologist (one patient) or by referral for a second opinion by a retinal specialist (two patients).

Microalbuminuria was defined as an albumin-to-creatinine ratio ≧30 mg/g from at least two of three consecutive spot urine collections in a 3- to 6-month period (Quest Laboratories). Hypertension was defined as at least three consecutive blood pressure readings with values >90% for age, sex, and height (7).

We also calculated the total billings for eye exams that would have accrued if patients aged ≥10 years had initiated annual DR screening at ≥3 years duration of diabetes, and we compared that sum with the cost of annual exams initiated at ≥5 years duration. A new patient visit cost $200, and follow-up visits cost $175.

Results — Of diabetic patients, 197 (104 male and 93 female subjects) met the inclusion criteria (Table 1). Of these, 67 patients (34%) were either aged <10 years or had <3 years duration of type 1 diabetes and did not require screening. Eye exam reports were available in 130 of the 281 patients in our clinic who were aged >10 years and had >3 years duration of type 1 diabetes. The mean A1C averaged <8.0% in all four age-groups.

Only three eye exams were positive for DR. In one of the three positive reports, the examining ophthalmologist acknowledged that DR was misdiagnosed based on minor tortuosity of retinal vessels. The presence of microaneurysms in one eye in each of the other two patients was not confirmed by a retinal specialist; these patients were classified as having transient DR.

In contrast, 19 patients (10%) who were aged ≥10 years had hypertension, and 7 (3%) had microalbuminuria. There were 130 subjects (66%) aged ≥10 years with ≥3 years duration of type 1 diabetes. If all of these patients had followed ADA recommendations and commenced screening after 3 or 5 years of type 1 diabetes, the total eye exam charges would have been $96,615 or $67,170, respectively.

Conclusions — The most striking finding of the study is that none of the patients who met ADA screening criteria had any verifiable or sustained evidence of early DR. At most, only two cases with possibly transient microaneurysms were identified. Since diabetes-related services impose a large economic burden, the identification and elimination of unnecessary examinations could improve the efficiency of current health care delivery. The results of this study make it very difficult to justify routine screening for DR in all youth with type 1 diabetes based solely on patient age and duration of diabetes. Although standard screening only involved ophthalmoscopy with dilated pupils, it is
very unlikely that practicing ophthalmologists and optometrists would have missed more advanced retinal lesions that would require treatment or more frequent surveillance.

Our data indicate that routine eye screening for youth with type 1 diabetes also fails the criteria of cost-effectiveness. Indeed, the $67,000–$96,000 cost in eye exams is only the tip of the iceberg, since it does not include costs for transportation and time lost from work and school. In contrast, screening for hypertension and microalbuminuria in patients aged ≥10 years were positive in 10 and 3%, respectively, all of whom were undergoing treatment with an ACE inhibitor or an angiotensin receptor blocker. Considerable evidence (1) supports early identification and treatment of hypertension and microalbuminuria to delay or prevent clinical nephropathy and macrovascular disease. The very low yield from the DR exams is due in large part to the low A1C levels achieved by our patients, representing the successful translation of the Diabetes Control and Complications Trial results (3) into clinical practice. Similarly, our 3% prevalence of microalbuminuria is lower than the 13% recently reported in a large population-based study (8) of children and adolescents with type 1 diabetes in Western Australia with mean A1C values >9.0%.

In conclusion, current ADA recommendations for DR screening are not cost-effective for pediatric type 1 diabetic patients who maintain strict glycemic control with intensive insulin therapy. These results suggest that it would be more cost-effective to limit routine eye screening to youth with type 1 diabetes who have persistent elevations in A1C levels, hypertension, or microalbuminuria, all of which involve assessments that can be carried out during regular diabetes clinic visits and do not require extra days being lost from work or school.

Acknowledgments—This study was supported by the Stephan I. Morse Pediatric Diabetes Research Fund and National Institutes of Health Grants RR-00125 and DK-063703.

References

Table 1—Clinical characteristics and prevalence of diabetes complications in patients at the date of the most recent eye exam among each age-group

<table>
<thead>
<tr>
<th></th>
<th>Prescreen (age &lt;10 years)</th>
<th>Early adolescent (age 10–14 years)</th>
<th>Late adolescent (age 14.01–18 years)</th>
<th>Young adult (age 18.01–22 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>32</td>
<td>61</td>
<td>84</td>
<td>20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>7.5 ± 2.0</td>
<td>12.2 ± 1.0</td>
<td>16.1 ± 1.3</td>
<td>19.2 ± 1.0</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>17 (53)</td>
<td>30 (49)</td>
<td>42 (50)</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Duration of type 1 diabetes (years)</td>
<td>3.7 ± 2.3</td>
<td>4.6 ± 3.0</td>
<td>7.4 ± 4.1</td>
<td>7.6 ± 3.5</td>
</tr>
<tr>
<td>A1C (%)</td>
<td>6.6 ± 0.8</td>
<td>7.3 ± 1.5</td>
<td>7.5 ± 1.2</td>
<td>7.9 ± 1.5</td>
</tr>
<tr>
<td>Retinal screen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transient positive</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>False positive</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>Retinal screen</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Transient positive</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>False positive</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>False positive</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Transient positive</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microalbuminuria &amp; hypertension</td>
<td>0 (0)</td>
<td>2 (3)</td>
<td>10 (12)</td>
<td>7 (33)</td>
</tr>
</tbody>
</table>

Data are n (%) or mean ± SD. A1C levels were measured by the DCA 2000+ Analyzer (Bayer Diagnostics, Tarrytown, NY).
Depression and Depression Care in Diabetes

Relationship to perceived discrimination in African Americans

Julie Wagner, PhD
Gina Abbott, PhD

Depression is more prevalent in both African Americans and Caucasians with diabetes (1) than in nondiabetic control subjects (2), and it is associated with worse diabetes outcomes (3, 4). Prospective studies (5) show that everyday encounters with discrimination predict subsequent depressive symptoms in nondiabetic individuals. When discrimination is perceived, specifically in health care, it may also interfere with depression care. This study investigated perceived discrimination, depressive symptoms, and depression care in diabetic African Americans.

RESEARCH DESIGN AND METHODS — Participants were African-American adults with diabetes attending 2004–2006 American Diabetes Association health fairs in northeastern U.S. cities. Attendees responded to a sign advertising “Research for African Americans with diabetes.” After informed consent, participants completed questionnaires and provided fingerprick blood samples for A1C assessment (6).

Participants were paid $5.00 and given $5.00 and given A1C results with referrals to community health centers.

Demographic questions included age, sex, insurance, primary care provider, and socioeconomic status (SES), which was assessed with income and education. A medical history questionnaire asked about physician-diagnosed disorders (including depression) and whether medication was taken for each disorder. These questions were modeled after the Centers for Disease Control’s survey questions (7, 8) for patient report of physician-diagnosed disorders.

Participants completed three additional questionnaires, as follows. The Center for Epidemiological Studies Depression (CESD) scale (9) is a 20-item measure of depressive symptoms. A score of >21 discriminates between depressed and non-depressed individuals in medical populations (10, 11). α in this sample was 0.87.

The Schedule of Racist Events (SRE) (12) is an 18-item questionnaire that measures frequency and stressfulness of racial discrimination situations (e.g., in salary, housing, by store clerks) over a lifetime and in the last year. One item was added that assessed perceived discrimination in health care settings. Lifetime frequency scores were used in analyses. α in this sample was 0.91.

The John Henryism scale (13) is a 12-item questionnaire that measures active psychological coping, which has been shown (14) to moderate physiological and psychological responses to discrimination. α in this sample was 0.91. All analyses controlled for age, sex, SES, diabetes type, and A1C using SPSS version 12.0.

RESULTS — Data were provided by 120 African Americans with diabetes. The typical participant was female, aged 56 years, had type 2 diabetes, and was in suboptimal glycemic control (Table 1). Average total lifetime frequency score on the SRE was 34.7 (mean). Item scores ranged from 1.3 to 2.6, and 6% of participants reported never experiencing discrimination. These data indicate slightly lower but comparable levels of racism relative to a large, representative sample of nondiabetic African Americans (15) (mean 42.3, item score range 2.1–2.9, and 2% answering never).

In logistic regression, SRE frequency scores and covariates as a group predicted depressive symptoms \[F(6, 99) = 3.16, P < 0.01, R^2 = 0.17, \text{adjusted } R^2 = 0.13\]. Higher SRE frequency scores independently predicted higher CESD scores \[F(5, 98) = 3.88, P < 0.01\]. Higher A1C and female sex were also independent predictors \((P < 0.05)\). Effects were not modified by coping.

In logistic regression, SRE frequency scores and covariates as a group distinguished participants with CESD scores \(>21\) from those with scores \(\leq 21\) \(\chi^2(6) = 16.19, P < 0.05\), Cox & Snell \(R^2 = 0.15\). The Hosmer & Lemeshow index was not significant, indicating acceptable model fit \((P = 0.16)\). Higher SRE frequency scores independently predicted greater likelihood of CESD scores \(>21\) \((\text{odds ratio } [OR] 1.07 [95\% CI 1.02–1.13] P < 0.05)\). Older age significantly decreased odds of elevated symptoms \((0.93 \times 0.87–0.99), P < 0.05\). Effects were not modified by coping.

In logistic regression, perceived discrimination in health care settings and covariates as a group distinguished participants who reported physician-diagnosed depression from participants who did not \(\chi^2(7) = 16.37, P < 0.05, R^2 = 0.15\), Hosmer & Lemeshow \(P = 0.64\). Higher SRE frequency scores independently predicted greater likelihood of patient-reported, physician-diagnosed depression \((OR 1.06 [95\% CI 1.00–1.18] P < 0.05)\). Higher CESD scores were also an independent predictor \((1.10 [1.02–1.18], P < 0.05)\). Effects were not modified by coping.

In logistic regression, perceived discrimination in health care settings and covariates as a group distinguished participants who had used antidepres-
of clinically significant symptoms, and likelihood of patient-reported, physician-diagnosed depression. While men and women reported similar frequency of discriminatory events, these events were experienced as more stressful to women, although it should be noted that we had few male participants.

Perceptions of discrimination within health care settings were associated with not taking antidepressants. Individuals who perceived discrimination in their health care system may have had more depressive symptoms, but those same individuals may have been less trusting of providers or the medications they recommended. Implementing pharmacotherapy for depression may be challenging in these individuals. Practitioners are encouraged to pursue cultural competence training in order to avoid behaviors that can be perceived as discriminatory to patients.

Apart from perceived discrimination, other risk factors for depression indicators in this study were consistent with past reports, including female sex, higher A1C, and younger age. Higher SES and higher A1C were marginally associated with using antidepressants.

Surprisingly, coping did not buffer the association between discrimination and depression outcomes. This was likely due to low power. Alternatively, John Henryism is a specific type of coping, and it may be that other psychological resources, such as social support or spirituality, may be important in buffering the effects of discrimination.

Several limitations should be noted. The recruitment strategy may have produced a nonrepresentative sample, as suggested by the slightly lower endorsement of racist events relative to other published reports. Diagnosis and medication data were self-reported. The direction of association could not be determined by the cross-sectional design. It is possible that depressed individuals were more likely to perceive interpersonal stimuli as noxious, and they may have been more likely to make racial attributions about the noxious stimuli. For this reason, we investigated perceived frequency (rather than stressfulness) of discriminatory events. The fact that women reported equivalent frequency but greater stressfulness of discriminatory events suggests that individuals can, to some degree, differentiate an event from their psychological response to it. Nonetheless, depression may lead to heightened perceptions of discrimination, or both may share a common precursor, such as personality characteristics. Prospective studies of representative samples should control for personality variables and investigate additional moderators.

It may not be possible to eradicate discrimination from patients’ environments. However, patients’ mental health outcomes may be improved by interventions both to decrease interactions with the health care system that could be perceived as discriminatory and to help patients enhance their psychological resources to cope with perceived discrimination.

References
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Prevalence of Hyper- and Hypoglycemia Among Inpatients With Diabetes

A national survey of 44 U.S. hospitals

DEBORAH J. WEXLER, MD1
JAMES B. MEIGS, MD, MPH1,2
ENRICO CAGLIERO, MD1

DAVID M. NATHAN, MD1
RICHARD W. GRANT, MD, MPH1,2

The recent demonstration (1–3) of the benefits of intensive glycemic control in hospitalized patients has renewed interest in inpatient management of diabetes. Poor glycemic control is a marker for poor quality of hospital care (4), as well as an important safety issue: insulin is one of five medications most associated with inpatient medication errors (5,6). Moreover, many hospitals continue to solely rely on insulin “sliding scales” despite the limitations of this approach (7,8). To gain a broader understanding of the current quality of inpatient diabetes management, we analyzed the prevalence and management of hyper- and hypoglycemia among 999 patients with known diabetes treated in 44 hospitals across the U.S.

METHODS—Data were derived from two sources: the University Health System Consortium (UHC) Diabetes Benchmarking Project and VHA, Inc. The UHC project collected inpatient and outpatient data in 2003 by standardized chart review of 274 patients aged ≥18 years with type 1 and type 2 diabetes (diagnosed by their outpatient physicians), who were admitted as inpatients to 1 of 29 academic medical centers located in 20 states. Chart reviewers identified the highest and lowest glucose values during hospital admissions and recorded the highest and lowest glucose results for the 2 days preceding and following the peak and nadir.

In 2003–2004, 15 member hospitals of VHA, Inc, an alliance that serves ~1,400 not-for-profit U.S. hospitals, performed baseline chart reviews on 725 general medical and surgical patients aged >18 years with a primary or secondary discharge diagnosis of diabetes (type not specified). Data on the admission diagnosis-related group, glucose tests (n = 18,097), and NPO status were recorded; 6 of the 15 hospitals also collected complete data on diabetes treatment (n = 296).

For both cohorts, we determined the prevalence of extreme glucose values (>200 or 250 mg/dl or <60, 50, or 40 mg/dl) and of persistent hyper- and hypoglycemia, defined as hyperglycemia >200 mg/dl or hypoglycemia <60 mg/dl for 3 consecutive days. We grouped insulin regimens into three categories: sliding-scale insulin alone, sliding scale with basal insulin, and basal alone. Basal insulin was defined as any long- or intermediate-acting or intravenous insulin. Because <5% of patients were on basal insulin without sliding-scale insulin, the latter two categories were combined into a single “treatment with any basal insulin” group. The prevalence of hyper- and hypoglycemia was compared between the two treatment groups using χ² tests. We also stratified analyses by severity of disease using the available indicators within each cohort, defined as 1) diagnosis of type 1 diabetes, type 2 diabetes on outpatient insulin with or without oral hypoglycemic agents, or type 2 diabetes not on outpatient insulin (i.e., treated with oral hypoglycemic or diet and exercise) in the UHC cohort and 2) primary admission diagnosis code of diabetes in the VHA, Inc. cohort.

RESULTS—Prevalence of hyper- and hypoglycemia and treatment patterns are shown in Table 1. Hyperglycemia was common, with the majority of patients experiencing at least one value >250 mg/dl. Extreme values were more common in patients with type 1 diabetes and patients with type 2 diabetes who were on insulin as outpatients in the UHC cohort, as well as among patients who were primarily admitted for diabetes in the VHA, Inc. cohort. Persistent hyperglycemia was present in 38% percent of the UHC cohort and 18% of the VHA, Inc. cohort. While hospitalized, 16% percent of patients with type 1 diabetes and 35% of patients with type 2 diabetes on insulin as outpatients were treated with sliding-scale insulin alone; 41% of patients in both cohorts with hyperglycemia >200 mg/dl for 3 consecutive days were treated with sliding-scale insulin alone.

Hypoglycemia to <60 mg/dl was also common, with 12% of patients in the UHC cohort and 18% in the VHA, Inc. cohort experiencing at least one episode of glucose <60 mg/dl. Severe hypoglycemia (<40 mg/dl) and recurrent hypoglycemia (<60 mg/dl for 3 days) occurred in <5% of patients in both cohorts. Hypoglycemia was more common in patients with more severe diabetes and in the subset of patients treated with basal insulin.

CONCLUSIONS—Over one-quarter of hospitalized Americans have diabetes (9). While disruptions in outpatient regimens and intercurrent illness and medication changes may cause hyper- and hypoglycemia during hospitalization, the availability of frequent monitoring, skilled nursing care, and glucose-
Inpatient diabetes management

Our survey of a broad cross section of 44 academic and community hospitals revealed that among 999 inpatients with diabetes, marked, persistent hyperglycemia was very common and often treated by sliding-scale regimens alone, while severe hypoglycemia was rare.

Hyperglycemia is associated with increased mortality (10,11); improved control has been proven to reduce mortality in several populations. Severe hyperglycemia, a complication that partially drives undertreatment of hyperglycemia, is avoidable with appropriate management (12). Since the early 1990s, it has been known (17,18) that sliding-scale insulin protocols in the absence of a basal insulin are associated with wide glycemic variations. Consensus guidelines (4,13,14) and individual experts (15,16) suggest that optimal management of inpatient glyemia should include basal insulin with prandial insulin coverage, rather than sliding scales alone.

In our analysis of data from 2003, sliding scales were prescribed as the sole treatment in 41% of the UHC cohort and 45% of the VHA, Inc. cohort. Sliding-scale insulin alone may be transiently appropriate as a dose-finding strategy or in patients with type 2 diabetes not on outpatient insulin, but it was not appropriate in the 16% of patients with type 1 diabetes and probably not for the 35% of patients with type 2 diabetes on outpatient insulin (4,19,24). Hypoglycemia <60 mg/dl was more common in patients on

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Table 1—Prevalence of hyperglycemia, hypoglycemia, and mode of insulin replacement in two large national samples of inpatients with diabetes

<table>
<thead>
<tr>
<th></th>
<th>UHC cohort, 29 hospitals</th>
<th>VHA, Inc. cohort, 15 hospitals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total, n = 274</td>
<td>Type 1, n = 37</td>
</tr>
<tr>
<td>Prevalence of hyper- and hypoglycemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevalence of hyperglycemia (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single value &gt;200 mg/dl</td>
<td>77</td>
<td>92</td>
</tr>
<tr>
<td>Single value &gt;250 mg/dl</td>
<td>60</td>
<td>76</td>
</tr>
<tr>
<td>Three consecutive days &gt;200 mg/dl</td>
<td>38</td>
<td>41</td>
</tr>
<tr>
<td>Prevalence of hypoglycemia (%)</td>
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<tr>
<td>Single value &lt;60 mg/dl</td>
<td>12</td>
<td>27</td>
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<tr>
<td>Three consecutive days &lt;60 mg/dl</td>
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<tr>
<td>Mode of insulin replacement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (%)</td>
<td></td>
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<tr>
<td>Sliding scale alone</td>
<td>41</td>
<td>16</td>
</tr>
<tr>
<td>Any basal insulin</td>
<td>32</td>
<td>70</td>
</tr>
<tr>
<td>No insulin</td>
<td>28</td>
<td>13</td>
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<td>Treatment modality, patients with persistent hyperglycemia (glucose &gt;200 mg/dl × 3 days) (%)</td>
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</tr>
<tr>
<td>Sliding scale alone</td>
<td>41</td>
<td>0.36*</td>
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<tr>
<td>Any basal insulin</td>
<td>53</td>
<td>&lt;0.0001*</td>
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<td>No insulin</td>
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<td>&lt;0.0001*</td>
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<tr>
<td>Treatment modality, patient with glucose &lt;60 mg/dl (%)</td>
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</tr>
<tr>
<td>Sliding scale alone</td>
<td>12</td>
<td>0.001*</td>
</tr>
<tr>
<td>Any basal insulin</td>
<td>70</td>
<td>&lt;0.0001*</td>
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<tr>
<td>NPO (nothing by mouth)</td>
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</tr>
<tr>
<td>Prevalence of hypoglycemia (glucose &lt;60 mg/dl) among patients on basal insulin (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>&lt;0.0001*</td>
<td>25</td>
</tr>
</tbody>
</table>

Data are percentages. The total number in each cohort is given, followed by the number in each group stratified by severity of diabetes, using available data in each cohort. UHC is stratified into patients with type 1, type 2 on insulin with or without oral hypoglycemics, and type 2 on oral or diet therapy (not on insulin, based on outpatient regimen); VHA, Inc. is stratified into those with and without a primary admission diagnosis code of diabetes by DRG. *P values are for comparisons between the patients receiving and patients not receiving the specified treatment. Data are shown for patients receiving treatment. Prevalence of hyperglycemia and hypoglycemia was greater in patients treated with basal insulin. dx, diagnosis; N/A, not available.
basal insulin, but only one-quarter of patients on basal insulin experienced hyperglycemia. It is noteworthy that hyperglycemia on 3 consecutive days was prevalent in both cohorts but was not treated with basal insulin in 30% of UHC patients and 40% of VHA, Inc. patients. Confounding by indication and underdosing may explain persistent hyperglycemia in patients who were treated with basal insulin. Persistent hyperglycemia or hypoglycemia may have been underestimated in the UHC cohort, since data were only collected for 2 days before and after the most extreme value.

This analysis of 44 U.S. hospitals reveals persistent shortcomings in inpatient diabetes management. Inpatient diabetes care delivery may require systematic changes in order to meet current standards.

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Larval Therapy: A Novel Treatment in Eliminating Methicillin-Resistant Staphylococcus aureus From Diabetic Foot Ulcers

FRANK L. BOWLING, BS, DPM1
ELEANNA V. SALGAMI, MD, PHD1
ANDREW J.M. BOULTON, MD, FRCP1,2

Emerging Treatments and Technologies

BRIEF REPORT

O veruse of antibiotics and the selection of broad- rather than narrow-spectrum agents have contributed to the high prevalence of methicillin-resistant Staphylococcus aureus (MRSA) colonization in diabetic foot wounds. Consequently, MRSA is now an endemic in both the community and hospital environments (1,2). We previously highlighted the problem (3) of MRSA colonization in our diabetic foot clinic (40% of S. aureus isolates were MRSA). A follow-up study (4) demonstrated that the number of foot wounds from which MRSA was isolated doubled in a 3-year period. Although terms such as critical colonization are not clearly defined, the risk of MRSA infection and bacteremia in patients with colonized ulcers is recognized (5,6). Furthermore, there is evidence (4) that MRSA colonization of chronic ulcers is associated with delayed healing times. Strategies to eliminate MRSA from colonized wounds are therefore essential and should include the use of simple, low-cost, effective treatments.

Larval therapy is suggested (7) to successfully remove sloughy necrotic tissue from ulcers and to facilitate healing. We hypothesized that larval therapy would eradicate MRSA colonization from diabetic foot ulcers. Here, we report the results of our preliminary observational study.

RESEARCH DESIGN AND METHODS — Consecutive patients aged 18–80 years with MRSA-colonized chronic diabetic foot ulcers for >3 weeks duration were included in the study. Subjects on antibiotic treatment specific for MRSA (vancomycin or linezolid), on anticoagulation therapy, or requiring immediate systemic antimicrobial treatment or urgent surgical management were excluded. All patients were assessed by the neuropathy disability score and vibration perception threshold (VPT) (9). Ischemia was defined as nonpalpable pedal pulses and ankle-brachial systolic blood pressure index. An ulcer was deemed to be neuropathic if VPT was >25 V, and/or neuropathy disability score was >3, and neuroischemic if VPT was >25 V with absent foot pulses/ankle-brachial systolic blood pressure index <0.7. MRSA colonization was defined as the isolation of MRSA from the ulcer in subjects without clinical and/or laboratory signs of systemic infection. MRSA status was evaluated by semiquantitative wound tissue cultures taken from the wound base after debridement at baseline and before each larval application.

Sterile free-range larvae of the green bottle fly Lucilia Sericata were applied to the MRSA-colonized ulcers for 4 days at densities determined as ~10 larvae/cm² (7). The primary end point was complete eradication of MRSA from the ulcer following a minimum of two and a maximum of eight larval applications per ulcer. Patients with MRSA-positive wound cultures were all screened for MRSA carriage at other sites (nose, perineum, or both) in accordance with the hospital MRSA screening policy. A 5-day self-treatment regime for MRSA eradication was followed in those patients with positive MRSA body screening with the use of Mupirocin nasal ointment, Aquacept body wash, and Aquacept shampoo. Ulcer size was measured with the digital planimetry system (Visitrak) (10) by the same clinician after debridement. Appropriate pressure-relieving dressings (e.g., Allevyn pads) were used to prevent damage of the larvae during treatment, in addition to off-loading modalities (DH Walker; Ossur, Aliso Viejo, CA). No topical antimicrobial agents or growth factors were used on the study ulcer.

RESULTS — In the study, 13 consecutive diabetic patients with MRSA-colonized ulcers were included (Table 1), >60% of whom had a past history of ulcers. The study ulcers were chronic (average duration 3 months), of neuroischemic etiology (87%), and were located distal to the malleoli. None of the isolated MRSA strains were multiresistant or vancomycin resistant. MRSA colonization was related to hospitalization (61%), antibiotic treatment that was current (31%) rather than prolonged (15%), and residency in a nursing home (8%). MRSA colonization was eliminated from all but 1 of the 13 ulcers (92%) after a mean of three applications with a mean duration of 19 days (range 7–45). During the treatment period, no adverse events were recorded. There was a reduction in sloughy necrotic tissue and an increase of granulation tissue on removal of the last larval application.

The mean duration of larval therapy was 3 weeks, which is far shorter than the 28-week (range 3–60) duration for the conventional treatment for MRSA decontamination in diabetic foot ulcers (4).
The mean wound area was smaller at discharge from the study, but the reduction in size was not significant compared with the baseline, probably due to the short duration of larval treatment.

CONCLUSIONS — We have demonstrated for the first time, in this preliminary study, the potential of larval therapy to eliminate MRSA colonization of diabetic foot ulcers. Although ours was an observational study, the high success rate of larval treatment in eradicating MRSA colonization from diabetic foot ulcers is promising. If confirmed in a future randomized trial, larval treatment would offer the first noninvasive and risk-free treatment of this increasing problem. There were no complaints reported during larval therapy regarding pain or tenderness around the ulcer. The advantages of larvae on eradication of MRSA colonization and the absence of systemic side effects make the larvae a safe and cost-effective treatment in contrast to expensive and potentially toxic antibiotic therapies, such as vancomycin. The efficacy and superiority of larval therapy in eradicating MRSA colonization compared with conventional treatment needs to be further investigated in larger randomized controlled trials.

Acknowledgments — This study was supported by the Central Manchester and Manchester Children’s Hospitals NHS Trust Chairman’s Research Prize Scholarship, 2005–2006.

References
Platelet Response to Clopidogrel Is Attenuated in Diabetic Patients Undergoing Coronary Stent Implantation

Tobias Geisler, MD
Nicole Anders, MD
Maria Paterok, MD
Harald Langer, MD
Konstantinos Stellos, MD
Stephan Lindemann, MD
Christian Herdeg, MD
Andreas E. May, MD
Meinrad Gawaz, MD

Type 2 diabetes is accompanied by platelet function disorders leading to an accelerated process of atherosclerosis and increased risk for atherothrombotic complications (1–4). Previous data (5,6) suggest a worse outcome for diabetic patients after acute coronary events. Recently, a high variability of response to antiplatelet therapy in diabetic patients has been reported (11,12) in small patient collectives. However, little is known about the effects of type 2 diabetes on response after a 600-mg clopidogrel loading dose in large unselected cohorts of patients with symptomatic coronary artery disease (CAD).

RESEARCH DESIGN AND METHODS — Type 2 diabetic and nondiabetic patients treated by coronary stenting for symptomatic CAD were consecutively enrolled in this study. The protocol was approved by the local ethics committee, and signed informed consent was obtained from all patients. Patients with known platelet function disorders, thrombocytopenia (<10^5 cells/mm^3), or any contraindications against clopidogrel were excluded. A loading dose of 600 mg clopidogrel was given to all patients before PCI, followed by 75 mg every day. All patients received a daily dose of 100 mg aspirin before PCI. A standard dose of heparin was given to all patients immediately before PCI unless there were no contraindications. Type 2 diabetes was defined according to the recommendations of the American Diabetes Association (13).

Patient blood was collected ≥6 h (46.6 ± 99.3 h) after administration of 600 mg clopidogrel, when maximum platelet inhibition was expected (14). Among the subgroups of diabetic and nondiabetic patients, there was no significant time difference between PCI and light transmittance aggregometry (LTA). Blood samples were collected in 3.8% citrate plasma, as described. Percentage of platelet aggregation was assessed with the turbidometric method using a Chronolog-Lumi aggregometer (Chronolog, Havertown, PA) with Aggro-Link software 5 min after addition of 20 μmol/l ADP or 5 μg/ml collagen.

We performed a χ² test for analysis of dichotomous variables. Differences between means of continuous variables with normal distribution were evaluated with Student’s t test. U test (Mann-Whitney) was applied to compare platelet aggregation between two subgroups, and a Kruskal-Wallis test was used for comparison of multiple groups. We considered P < 0.05 as statistically significant. Analyses were performed with SPSS (Version 13.0; SPSS, Chicago, IL).

RESULTS — In this study, 485 patients were consecutively enrolled. Of these, 161 (33.2%) had diabetes, 264 (54%) underwent elective procedure for stable angina, and 221 (46.0%) were treated by PCI for acute coronary syndrome (ACS) (unstable angina, non-ST elevation myocardial infarction, ST elevation myocardial infarction). For diabetic patients, mean A1C was 7.7 ± 1.6% and mean blood glucose levels 172 ± 63 mg/dl.

Posttreatment platelet reactivity to clopidogrel, measured by ADP-induced LTA, was lower in diabetic patients (40.0 vs. 31.8%, P = 0.01). There was no significant impact of diabetes on collagen-induced aggregation (42.5 vs. 36.7%, P = 0.1).

In a subgroup analysis of patients with coronary stenting for stable angina and patients with ACS, diabetic patients with ACS showed significantly higher posttreatment aggregation (ADP 46.3%, P = 0.002 and collagen 45.7%, P = 0.005) compared with nondiabetic patients with ACS (Fig. 1). A1C levels did not significantly influence platelet activity in this cohort (44.1 vs. 38.8%, P = 0.18).

ADP-induced aggregation was highest in diabetic patients with acute coronary events 6–12 h after first administration of clopidogrel. Thereafter, platelet inhibition was similar in diabetic and nondiabetic patients (Fig. 1). Diabetic patients were slightly older than nondiabetic patients, more frequently suffered from severe left ventricular dysfunction, and received less β-blockers and statins, whereas diuretics were given more frequently. In univariate analysis, there was no significantly different distribution of age, sex, comedication, or cardiovascular risk factors between time groups except for a slightly higher ratio of smokers, mea-

From the Department of Cardiology/Internal Medicine, University Hospital Tübingen, Tübingen, Germany. Address correspondence and reprint requests to Meinrad Gawaz, MD, Medizinische Klinik III, Universitätsklinikum Tübingen, Otfrid-Müllerstr 10, 72076 Tübingen, Germany. E-mail: meinrad.gawaz@med.uni-tuebingen.de.

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Abbreviations: ACS, acute coronary syndrome; ARMYDA, Antiplatelet Therapy for Reduction of Myocardial Damage During Angioplasty; CAD, coronary artery disease; EPISTENT, Evaluation of Platelet IIb/IIIa Inhibitor for Stenting Trial; LTA, light transmittance aggregometry; PCI, percutaneous coronary intervention; RPA, residual platelet activity.

A table elsewhere in this issue shows conventional and Systeme International (SI) units and conversion factors for many substances.

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sured 6–12 h after a clopidogrel loading dose.

CONCLUSIONS — The principal finding of this consecutive study is that diabetic individuals with ACS show a lower response to a 600-mg clopidogrel loading dose. We measured platelet activity by LTA (with ADP), which has been established (7,14) as a reliable method for monitoring clopidogrel effects. As an additional marker of platelet reactivity, we assessed collagen-induced aggregation. Thus, we found type 2 diabetes to be associated with a decreased platelet response in a heterogeneous patient cohort with symptomatic CAD. Although diabetic individuals frequently present with other cardiovascular risk factors (such as hypertonus and dyslipidemia) that contribute to chronic platelet activation, we could not observe a significant difference of these factors between diabetic and non-diabetic patients.

The present data suggest that diabetes has a substantial influence on residual platelet activity (RPA) observed in patients with acute coronary events. Thus, diabetic patients presenting with ACS might be at increased risk for recurrent atherothrombotic events partly due to suboptimal platelet inhibition. We did not evaluate relative inhibition by measuring pretreatment reactivity. However, at a time point of >6 h after a 600-mg clopidogrel loading dose, when maximum platelet inhibition is expected (14), RPA was enhanced in diabetic patients. Also, we did not evaluate the influence of insulin therapy on RPA. There are reports (15,16) in the literature that insulin therapy is associated with increased platelet activity in diabetic cardiovascular patients.

Although not intraindividually measured, we observed a time-dependent effect of platelet response ≤24 h. This effect was most distinctive in patients with ACS and mostly independent of other factors in univariate analysis.

In a study of 16 diabetic and 36 non-diabetic patients, Angiolillo et al. (12) found that platelet response was attenuated after a 300-mg clopidogrel loading dose. This difference was still detectable ≤24 h later. The present study suggests that a 600-mg clopidogrel loading dose is not sufficient to overcome this effect, especially in diabetic patients with ACS.

Previous data (17) indicate that platelet hyperactivity plays a major role in the development of thrombotic complications in diabetes. There are data suggesting that diabetic patients gain a higher benefit from an intensified antiplatelet regimen after PCI. In the Antiplatelet Therapy for Reduction of Myocardial Damage During Angioplasty (ARMYDA)-2 trial (18), 255 patients, including 31% diabetic patients, were randomized to receive either a 300- or 600-mg clopidogrel loading dose 4–8 h before procedure. At 30-day follow-up, the primary end point of death, myocardial infarction, or target vessel revascularization occurred in 4% of patients in the high-loading dose group versus 12% of those in the conventional-loading dose group (P = 0.041). In the Evaluation of Platelet IIb/IIIa Inhibitor for Stenting Trial (EPISTENT) (19,20), additional treatment of diabetic patients with the platelet glycoprotein IIb-IIIa blockade for coronary stenting resulted in a reduction of 6-month death and myocardial infarction rate from 12.7 to 6.2% compared with stent placebo.

It is well recognized that platelet function in diabetic patients is different from that in nondiabetic patients. Moreover, diabetic patients show a reduced response to aspirin, which is probably related to an altered megakaryopoiesis (21–23). Here, we demonstrate that platelets from diabetic individuals with ACS do not adequately respond to clopidogrel therapy in a time-dependent man-
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Diabetes Care, Volume 30, Number 2, February 2007
Preeclampsia is characterized by the development of proteinuria and hypertension after 20 weeks gestation, and it is associated with maternal and fetal morbidity. Preeclampsia affects ~5% of pregnancies, though women with preexisting diabetes are three to four times more likely to develop preeclampsia (1). Preeclampsia is associated with altered angiogenic factors, including increased levels of circulating soluble FMS-like tyrosine kinase 1 (sFlt1) and reduced levels of vascular endothelial growth factor and placental growth factor (PlGF). Hypertension and proteinuria of preeclampsia may be caused by an imbalance of these angiogenic factors (2–10). Circulating sFlt1, an antiangiogenic protein, binds to proangiogenic proteins, vascular endothelial growth factor, and PlGF, preventing their interaction with endothelial cell receptors and inducing endothelial dysfunction. Rats given sFlt1 develop proteinuria, hypertension, and glomerular endotheliosis, which are hallmarks of preeclampsia (3). Alterations in sFlt1 and PlGF are observed several weeks before symptoms (2).

It is unknown whether alterations in sFlt1 and PlGF are present in women with diabetes who then develop preeclampsia or whether a different pathway is responsible. Ultimately, it would be helpful to have a way to diagnose and predict preeclampsia in women with pregestational diabetes, as they frequently have preexisting hypertension and/or proteinuria, which make it difficult to differentiate superimposed preeclampsia.

We evaluated a small group of women with diabetes and compared levels of sFlt1 and PlGF at delivery in both those who developed preeclampsia and those who did not. We hypothesized that women with preexisting diabetes who developed preeclampsia may also have elevations in sFlt1 and decreases in PlGF.

**Definition of preeclampsia**

In women without baseline hypertension or proteinuria, preeclampsia was defined as the development of hypertension plus proteinuria or thrombocytopenia (11). Hypertension was defined as either a systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥90 mmHg on two occasions, at least 4 h apart, occurring after 20 weeks gestation. Proteinuria was defined as either >300 mg of protein in a 24-h urine collection or two positive dipstick test results ≥2+ (recorded at least 4 h apart) with no evidence of a urinary tract infection. Thrombocytopenia was defined as a platelet count <100,000 per mm³.

In women who were normotensive but proteinuric at baseline, the diagnosis of preeclampsia required the presence of thrombocytopenia, an aspartate aminotransf erase >70 units/l, or hypertension accompanied by severe headaches, epigastric pain, or sudden increase in proteinuria (five times the baseline value or twice baseline if it was >5 g per 24 h). In women who had both hypertension and proteinuria at baseline, the diagnosis of preeclampsia required any of the following: thrombocytopenia, an elevated aspartate aminotransferase ≥70 units/l, or worsening hypertension (as shown by two diastolic blood pressures...
Preeclampsia and angiogenic factors in pregnancy

Table 1—Clinical characteristics and concentrations of angiogenic factors

<table>
<thead>
<tr>
<th></th>
<th>Diabetic pregnancy</th>
<th>Diabetic pregnancy with pre eclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>33.4 ± 5.9</td>
<td>33.2 ± 2.5</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>36.57 ± 2.03</td>
<td>32.14 ± 3.84</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3,761.6 ± 1,012.52</td>
<td>2,236.4 ± 943.88</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>141.4 ± 13.4</td>
<td>154.6 ± 16.4</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>90.8 ± 5.4</td>
<td>95 ± 7.9</td>
</tr>
<tr>
<td>Urine protein/creatinine ratio</td>
<td>0.425 ± 0.39 (n = 4)</td>
<td>9.88 ± 11.02</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>33.48 ± 1.95</td>
<td>33.82 ± 4.13</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>6.0 ± 0.77 (n = 4)</td>
<td>6.9 ± 1.11</td>
</tr>
<tr>
<td>A1C (%)</td>
<td>5.72 ± 0.415</td>
<td>6.3 ± 0.917 (n = 3)</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>11.75 ± 6.29 (n = 4)</td>
<td>94.25 ± 81.68 (n = 4)</td>
</tr>
<tr>
<td>sFlt1 (ng/ml)</td>
<td>18.13 ± 10.29</td>
<td>102.99 ± 39.27</td>
</tr>
<tr>
<td>PlGF (pg/ml)</td>
<td>353.85 ± 214.41</td>
<td>119.09 ± 87.30</td>
</tr>
</tbody>
</table>

Data are mean ± SD. n = 5 unless otherwise noted. All data were collected at time of delivery, except A1C, which was collected during the third trimester.

splinted blood pressure >equal to 100 mmHg taken 4 h apart in the week before delivery) combined with either exacerbation of proteinuria (as above), severe headaches, or epigastric pain.

Statistical analysis

Statistical analysis was performed using the nonparametric Mann-Whitney U test because the distributions of sFlt1 and PlGF are highly skewed. All tests were two tailed, and P < 0.05 was considered statistically significant.

RESULTS

We identified five subjects with pregestational diabetes and clinical evidence of preeclampsia as well as five subjects with pregestational diabetes and no evidence of preeclampsia (Table 1). Of the five with preeclampsia, three had a prior history of hypertension and one had a history of nephropathy. The mean ± SD sFlt1 in women with diabetes and no evidence of preeclampsia was 18.13 ± 10.29 ng/ml, and the mean sFlt1 in women with diabetes and preeclampsia was 68.19 ± 58.98 and 1,782 ± 1,723.04, respectively (P = 0.01).

CONCLUSIONS

In this small, limited study of 10 patients, we found that women with preexisting diabetes who developed preeclampsia had elevated levels of sFlt1 and lower PlGF at time of delivery, just as women without diabetes have been shown to have in preeclampsia (2–10). Compared with levels seen in prior studies, women with diabetes who developed preeclampsia tended to have higher levels of sFlt1, in the range of severe preeclampsia. These results suggest a mechanism of preeclampsia in women with preexisting diabetes similar to that in women without preeclampsia.

A prior study (15) showed a trend toward lower PlGF levels in cord serum of diabetic pregnancies with preeclampsia, though this was cord serum and not maternal serum. There was also a study (16) in nonpregnant diabetic patients that found sFlt1 levels unaltered in patients who both did and did not have atherosclerosis. The results of these studies do not elucidate whether any alterations in sFlt1 and PlGF are present in diabetic patients who develop preeclampsia.

Larger, prospective studies of sFlt1 and PlGF levels throughout gestation in women with preexisting diabetes are needed to test the hypotheses that 1) measurement of sFlt1 and PlGF can be used to diagnose superimposed preeclampsia in diabetic pregnancies with preexisting hypertension and proteinuria and 2) these markers may be used to predict the onset of preeclampsia.

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Bacterial Load Predicts Healing Rate in Neuropathic Diabetic Foot Ulcers

Ling Xu, MD1,2
Susan V. McLennan, PhD1,3
Lisa Lo, BSc1
Anas Nattafi, MD4

Thyra Bolton3,4
Yu Liu, MD1
Stephen M. Twigg, MD1,3,4
Dennis K. Yue, MD1,3,4

Despite improved treatment, a significant number of diabetic foot ulcers do not heal and eventually lead to amputation (1–5). The important local factors determining the healing rate of ulcers are pressure at the site of the ulcer, adequacy of blood supply, and infection. It is generally accepted that for optimal healing, infection needs to be treated. However, the definition of infection is arbitrary, and, in many borderline cases, clinicians will be uncertain whether antibiotic therapy is indicated. Edmonds et al. (6) showed, in a study of clinically uninfected diabetic foot ulcers, that, even in the absence of overt infection, antibiotic therapy reduced hospitalization and amputation. Conceptually, a high bacterial load by itself can retard ulcer healing by causing a wound environment not conducive to healing. Important factors that may play a role in this regard include secretion of metalloproteinases and their tissue inhibitors from the bacteria, compounds that can cause local tissue destruction (7). Little is known, however, about the effect of bacterial load on the healing rate of neuropathic diabetic foot ulcers. In this study, instead of arbitrarily deciding clinically whether infection was present, a quantitative microbiological method was used to examine the relationship between bacterial load in the wounds of diabetic neuropathic ulcers and the subsequent ulcer healing rate.

RESEARCH DESIGN AND METHODS — Wound fluid was obtained from 32 patients (22 male and 10 female) with neuropathic ulcers at the planar surface of the foot. Patients were referred to the High Risk Diabetic Foot Clinic at the Diabetes Centre of Royal Prince Alfred Hospital in Sydney, Australia. The mean ± SD age of the cohort was 60.0 ± 9.0 years with a diabetes duration of 14.6 ± 10.1 years and an A1C of 7.9 ± 1.4% (normal <6.0%). All patients had vibration perception threshold >50 V and ankle brachial index >0.9; thus, their ulcers would be considered to be substantially neuropathic in nature. Their ulcers were relatively superficial at grade 0 or 1 and stage A or B, according to the Texas Grading System (8). All patients underwent regular ulcer care, provided by the multidisciplinary High Risk Foot Service, which included a minimum of one visit per week for debridement, dressing, and other aspects of treatment. Antibiotics had been prescribed in 83% of individuals, but not all cases would be considered infected by conventional criteria. The protocol was approved by the Sydney South West Area Health Ethics Committee.

Wound fluid collection and quantitation of bacterial load
At the initial visit, wound exudates were removed by flushing with saline, and necrotic tissues were removed by local debridement. Wound fluid was then absorbed onto a sterile 1-cm² piece of Whatman filter paper (Whatman International, Kent, U.K.) by placing the disc on the most exudative area of the ulcer. When the disc became fully saturated, it was removed from the ulcer base and placed into a tube containing 100 µl sterile PBS before storage at 4°C for 1–2 h. The sample was mixed, and 10 µl of the supernatant was then serially diluted (10⁻² to 10⁻⁷), streaked onto blood agar plates, and aerobically incubated for 24 h at 37°C. Bacterial load was quantified by counting the number of colony-forming units (CFUs) on each plate. The bacterial species were identified by standard microbiological techniques, including gram stain, microscopic examination, and, where appropriate, a coagulase test and assessment for methicillin-resistant *Staphylococcus aureus* status.

To verify the reproducibility of sampling in a wound by this method, six sequential wounds were examined, each with triplicate samples from the same wound. In each wound, the within-wound variability in the measured CFU was low at 6.8 ± 2.5%. In each of the six samples, we tested residual bacteria on the filter paper used for sampling, after fluid containing the PBS was removed, by directly applying culture of the paper onto culture plates—which, in each case, showed <50 colonies (<0.5 × 2 logCFU and <1% of the measured CFU in the wound fluid sample).

Measurement of ulcer area
At each visit to the clinic, the ulcer was debrided and the ulcer borders traced onto sterile transparent film using an acetate pen. The tracing was digitized by scanning and the area calculated using National Institutes of Health image software (9). The wound healing rate over the following 28 days was calculated as: daily change in wound area (%) = [(area at visit 1 − area at visit 4)/(area visit 1/28)] × 100 (10).

Statistical analysis was performed using the Number Cruncher Statistical System. Log-transformed data were analyzed for CFUs, as the data were normally distributed. Significance was accepted at P < 0.05.

From the 1 Discipline of Medicine, University of Sydney, Sydney, New South Wales, Australia; the 2 Department of Geriatrics, Qi Lu Hospital, Medical College, Shandong University, Jinan, China; the 3 Department of Endocrinology, Royal Prince Alfred Hospital, Sydney, Australia; and the 4 Diabetes Centre, Royal Prince Alfred Hospital, Sydney, New South Wales, Australia.

Address correspondence and reprint requests to Dr. Susan McLennan, Department of Endocrinology, Royal Prince Alfred Hospital, W407, Blackburn Building, D06, University of Sydney, Sydney, NSW, Australia.

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Abbreviations: CFU, colony-forming unit.

A table elsewhere in this issue shows conventional and Systeme International (SI) units and conversion factors for many substances.

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RESULTS — The mean initial area of the ulcers was 170.1 mm$^2$ (range 6.5–979.5). After 28 days of treatment, the ulcers had reduced in size by an average of 42% to 98.7 mm$^2$ (0–567.2). Wound healing rate showed a strong inverse relationship with logCFU ($r = 0.46$, $P = 0.008$) (Fig. 1). For each log order of CFU increase, ulcer healing was delayed by 44%. Poor wound healing, indicated by either no significant change or an increase in wound area over the 28 days, was observed in 13% of patients, and all of these had a CFU in the order of at least $10^4$ CFU.

The bacterial species present in the wound fluid were typical of those commonly found in diabetic foot ulcers. *S. aureus* and *Staphylococcus epidermidis* were present in 41 and 47% of the cases, respectively. None of the *S. aureus* isolates were methicillin-resistant *S. aureus* positive. Coiliforms, streptococci, and *Bacillus* were also present in 29, 18, and 6%, respectively. The majority of ulcers (63%) contained more than one organism type, with the most common combination being *S. aureus* and *S. epidermidis*.

Glycemic control, as assessed by A1C level at presentation, was also negatively correlated with wound healing rate over the 28 days ($r = -0.41$, $P < 0.02$). However, there was no correlation between bacterial load and A1C (data not shown). On univariate analysis, neither patient age nor diabetes duration predicted wound healing rate ($P > 0.05$).

CONCLUSIONS — In this study, a quantitative measure of bacterial load is shown to be correlated with rate of diabetic foot ulcer healing. To our knowledge, this is the first time that such a relationship has been described in diabetics. Previous research (11,12) in other disease states has indicated that reduced ulcer healing may occur as a result of the presence of certain types of bacteria (such as *S. aureus*), the presence of a complex mix of bacteria including anaerobes, or $>10^6$ CFU/mg of tissue in neuroischemic ulcers. Our method of ulcer fluid sampling, from the reproducible CFU counts from the same wound, and others (13) have also shown that postdebridement wound fluid CFU highly correlates with CFU counts derived from tissue samples in the same wound.

Obviously, frank infection of diabetic foot ulcers with its associated tissue destruction is well-known to all who look after this group of patients. However, the positive correlation between bacterial load and healing lends support to the notion that high bacterial load may contribute to impaired wound healing. Being a cross-sectional study at only one time point in the natural history of diabetic foot ulceration, the primary culprit could not be determined, nor could the question of whether the observed relationship was a causal one. However, the continuous relationship between bacterial load and healing, observed without considering whether antibiotic therapy is used, may minimize the frequently encountered dilemma of whether to use antibiotic therapy in borderline clinical cases. Conceptually, there are many explanations as to how bacteria can impair wound healing. Increased activities of metalloproteinases and disturbed pattern of inflammatory cytokines and growth factors are some of the possibilities (7). On the other hand, the natural body defense system may be in a better position to eliminate bacteria in a healthy and healing wound. Insight into these possibilities can be obtained by further intervention studies using antibiotics or other therapies to modulate bacteria load, while the patterns of metalloproteinase activities, inflammatory cytokines, and growth factors are closely monitored. These are important studies that need to be performed in our quest to overcome the serious problem of foot ulceration in diabetes. Irrespective of the underlying mechanisms, our finding suggests that CFU count in wound fluid may be a useful adjunct investigation to identify patients who may need more intensive therapy for their diabetic foot at an earlier stage.

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Bacterial load and ulcer healing rate


Diabetes Is Not Treated as a Coronary Artery Disease Risk Equivalent

Baiju R. Shah, MD, PhD
Janet E. Hux, MD, SM
Peter C. Austin, PhD

Observational studies (1–3) have suggested that the risk of mortality is equivalent for patients with myocardial infarction (MI) without previous diabetes and for diabetic patients without previous MI. Because vascular risk reduction targets are based on a patient’s future risk, clinical practice guidelines (4–9) recommend that the same or lower blood pressure and lipid targets be applied to diabetic patients as would be applied for secondary prevention following MI. Patients newly diagnosed with diabetes and those with first MIs enter a high-risk category for subsequent coronary events. Therefore, if diabetes were treated as a coronary artery disease risk equivalent, we would expect that both groups of patients should have similar increases in utilization of antihypertensive and lipid-lowering medications following their index events.

RESEARCH DESIGN AND METHODS — The study used administrative health databases from Ontario, Canada, including hospital discharge abstracts, physician service claims, and records from the government drug insurance program, which covers all prescriptions filled for individuals aged ≥65 years. Individuals are linked between databases via an anonymous identification number. The study also used the Ontario Diabetes Database, a validated registry of all individuals with diabetes, derived from these administrative databases (10).

All individuals with no history of MI or diabetes were identified, and two cohorts were assembled: those who either had a first MI or were first diagnosed with diabetes between 1 January 2000 and 31 December 2002, with a 5-year look-back window. Diabetes was determined from the Ontario Diabetes Database (11), while MI was determined from hospital discharge abstracts (12). The observation period for drug utilization for each patient was 800 days before and after the index event. Because the drug benefits program only covered individuals aged ≥65 years, those aged <65 at the start of their observation period were excluded. The very elderly (patients ≥80 years) were also excluded, as were patients who died before the end of their observation period.

In each of eight 100-day intervals before and after each patient’s index date, we determined whether the patient received at least one prescription for antihypertensive and for lipid-lowering drugs. Prescriptions were counted regardless of indication. For each interval, the proportions of patients in each cohort who received antihypertensive and lipid-lowering drugs were directly standardized for age, sex, and specialist physician care after the index event. To determine whether changes after the index event were different between cohorts, the ratio of drug utilization between each postevent interval and the first pre-event interval was compared between cohorts using bootstrap methods to establish 99% CIs. The research ethics board of Sunnybrook Health Sciences Centre approved the study.

RESULTS — There were 9,742 individuals with incident MI and 38,947 with incident diabetes. Before the index event, patients who subsequently developed diabetes had greater antihypertensive and lipid-lowering drug utilization than patients who subsequently had an MI (Fig. 1). Following the event, antihypertensive drug utilization rose to 96% of individuals with incident MI compared with 75% of those with incident diabetes, while lipid-lowering drug utilization rose to 70 vs. 41%, respectively. These changes in utilization for both drug classes were significantly different between cohorts ($P < 0.01$) and remained different through all subsequent time intervals ($P < 0.01$).

CONCLUSIONS — Although patients with MIs and with diabetes are at similarly high risk for mortality, utilization of medications to control hypertension and dyslipidemia increased more dramatically following incident MI than following incident diabetes. This difference persisted, although it narrowed over subsequent time intervals.

Several possible explanations can be postulated. The two conditions may be perceived differently: An MI may be viewed as an acute life-changing event, whereas diabetes may be seen as a manageable chronic disease. Since coronary disease risk reduction may have greater relevance for patients who have undergone a coronary event, MI patients and their physicians may be more motivated to initiate and adhere to risk reduction (13). Furthermore, in-hospital MI care is often driven by pathways that may improve prescribing practices (14,15), while diabetes care is usually delivered in the less-structured ambulatory setting. Finally, ongoing acute management issues in diabetes (like glycemic control) may distract patients and physicians from addressing longer-term risk reduction (16).

Other studies have also demonstrated that utilization of cardiovascular therapies in actual clinical practice is not proportional to future risk. For example, lower-risk patients with an acute MI were more likely to receive primary angioplasty (17). Other studies (18–20) have shown that the use of aspirin and statins in the ambulatory setting is associated with predictors of better prognosis. Seniors, who are at particularly high risk following an MI, are less likely to receive thrombolyt-
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Thiazolidinediones and Risk of Repeat Target Vessel Revascularization Following Percutaneous Coronary Intervention

A meta-analysis

DANIEL M. Riche, PharmD1
RODRIGO VALDERRAMA, MD2
NICKOLE N. HENYAN, PharmD1

OBJECTIVE — Thiazolidinediones (TZDs) (rosiglitazone and pioglitazone) are a class of antidiabetes agents that have a high affinity for peroxisome proliferator–activated receptor-γ. TZDs initiate a multitude of physiologic processes that may elicit benefits as systemic agents for the prevention of restenosis requiring revascularization following percutaneous coronary intervention (PCI). Numerous trials have evaluated the impact of TZDs on repeat target vessel revascularization (TVR) in patients following PCI; however, several limitations (small sample size, inconclusive results, and risk factor stratification) complicate definitive conclusions. A meta-analysis was performed to evaluate the impact of TZDs on repeat TVR following PCI.

RESEARCH DESIGN AND METHODS — Included trials met the following criteria: 1) prospective, randomized controlled trials evaluating available TZDs versus standards of care; 2) well-described protocol; 3) minimum of 6 months of follow-up; and 4) data provided on repeat TVR. Data are presented as relative risks (RRs) with 95% CIs.

RESULTS — Seven clinical trials (n = 608) met the inclusion criteria. Upon meta-analysis, the risk of repeat TVR was significantly reduced in patients who received TZD therapy compared with standards of care (RR 0.35 [95% CI 0.22–0.57]). In studies using rosiglitazone (0.45 [0.25–0.83]) and pioglitazone (0.24 [0.11–0.51]), risk of repeat TVR was significantly reduced. Risk of repeat TVR was also significantly reduced among patients with (0.34 [0.19–0.63]) and without (0.37 [0.18–0.77]) diabetes.

CONCLUSIONS — Results from this meta-analysis suggest that TZDs effectively reduce the risk of repeat TVR following PCI.


R

estenosis requiring revascularization is a significant limitation of percutaneous coronary intervention (PCI). Despite the advent of improved mechanics and drug-eluting stents, the cumulative restenosis rate remains 20–30% in the general PCI population and approaches 40% among patients with diabetes (1–3). It is possible that an inhibitory effect on restenosis may result from a synergistic combination of local and systemic strategies aiming at different mechanisms for reducing pathological neointimal formation (6). Several attempts have been made to reduce instant restenosis rates via systemic pharmacological agents, but, to date, these results have been disappointing (7,8).

Peroxisome proliferator–activated receptors (PPARs) are nuclear receptor iso-

From the 1University of Mississippi School of Pharmacy, Jackson, Mississippi; and the 2University of Mississippi Medical Center, Jackson, Mississippi.

Address correspondence and reprint requests to Nickole N. Henyan, PharmD, Assistant Professor, University of Mississippi School of Pharmacy, Department of Pharmacy Practice, University of Mississippi Medical Center, Office Annex Building, WW 116, 2500 North State St., Jackson, MS 39216. E-mail: nhenyan@osp.umsmed.edu.

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Abbreviations: PCI, percutaneous coronary intervention; PPAR, peroxisome proliferator–activated receptor; TVR, target vessel revascularization; TZD, thiazolidinedione; VSMC, vascular smooth muscle cell.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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<table>
<thead>
<tr>
<th>Study</th>
<th>TZD; dose (mg/day)</th>
<th>TVR rate (%)</th>
<th>Restenosis rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marx et al. (24)</td>
<td>PIO</td>
<td>42*</td>
<td>PIO: 9.7; SOC: 32.3*</td>
</tr>
<tr>
<td>Takagi et al. (17)</td>
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<td>44</td>
<td>PIO: 19; SOC: 46</td>
</tr>
<tr>
<td>Nishio et al. (13)</td>
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<tr>
<td>Cao et al. (23)</td>
<td>ROsi</td>
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<td>NR</td>
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<tr>
<td>Wang et al. (21)</td>
<td>ROsi</td>
<td>70</td>
<td>ROsi: 10; SOC: 20</td>
</tr>
<tr>
<td>Choi et al. (7)</td>
<td>ROsi</td>
<td>83</td>
<td>ROsi: 17; SOC: 38.2*</td>
</tr>
<tr>
<td>Osman et al. (22)</td>
<td>ROsi</td>
<td>16</td>
<td>ROsi: 25; SOC: 37.5</td>
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</tbody>
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*R results reported as percent of lesions or percent of stents (not percent of patients). BP, blood pressure; CAD, coronary artery disease; IDF, International Diabetes Federation; NR, not reported; PIO, pioglitazone; ROsi, rosiglitazone; SOC, standard of care.
clinical trials powered to assess restenosis are needed before TZDs can be recommended as routine oral antidiabetes drug therapy following PCI (25). To evaluate the effect of TZDs on reducing the risk of repeat TVR following revascularization in a larger patient population, we conducted a meta-analysis of randomized controlled trials published through August 2006.

RESEARCH DESIGN AND METHODS — We searched Medline, EMBASE, Cinahl, and the Cochrane Database from earliest available date through August 2006. A search strategy using the MeSH and text keywords “thiazolidinedione,” “rosiglitazone,” “pioglitazone,” “restenosis,” “coronary,” and “revascularization” was utilized (Fig. 1). All searches were limited to clinical trials of human subjects published in English. References from these trials were scrutinized to reveal additional citations. Abstracts from the American Heart Association, the American College of Cardiology, and the American Diabetes Association meetings from 2001 to 2006 were also searched. To be included in this meta-analysis, studies had to be prospective, randomized, controlled trials comparing currently available TZD therapy to standards of care in patients receiving PCI with a minimum 6-month follow-up. Data had to be provided for the number of patients receiving repeat TVR (rather than number of lesions revascularized) in each study group.

The following methodological features most relevant to the control of bias were assessed: randomization, random allocation concealment, masking of treatment allocation, blinding, and withdrawals. All studies were evaluated by three independent reviewers (D.M.R., R.V., and N.N.H.), with disagreement resolved by consensus.

RESULTS — Search strategy is described in Fig. 1. Nine studies (7,13,17,20–24,26) received full publication review with seven trials (n = 608) providing data adequate for meta-analysis (Table 1) (7,13,17,21–24). All included studies were placebo controlled and compared TZD therapy with standard pharmacological therapy (Table 1) in TZD-naive patients with (n = 5 studies) or without (n = 2 studies) diabetes. All studies reported incidence of repeat TVR at 6 months. The majority of studies were conducted in an Asian patient population (Chinese, Japanese, or Korean). The mean age of study participants did not vary largely between the individual studies (Table 1). There were approximately twice as many men than women in each group evaluated. All patients received aspirin in combination with clopidogrel, ticlopidine, or cilostazol. Average baseline A1C in patients with diabetes was 7.97% in TZD groups and averages were calculated using a random effects (DerSimonian and Laird methodology) model. Statistical heterogeneity was evaluated via the Q statistic (P < 0.1 was considered representative of significant statistical heterogeneity). Publication bias was assessed through visual inspection of funnel plots, and the Egger weighted regression method with P < 0.05 was considered representative of significant statistical publication bias. Data are presented as relative risks (RRs) with 95% CIs.
466 patients), the risk of repeat TVR was significantly reduced (0.45 [0.25–0.83]) (Table 2). Statistical heterogeneity was not significant (H = 0.22–0.57) (Fig. 2). Statistical heterogeneity was not significant (P = 0.75). In studies using pioglitazone (n = 3 studies, 140 patients), the risk of repeat TVR was significantly reduced (0.24 [0.11–0.51]) (Table 2). The risk of repeat TVR was also significantly reduced among patients with and without diabetes (Table 2). The potential for publication bias was low based on the symmetrical appearance of the funnel plots (data not shown) and Egger weighted regression P values (P = 0.533 for total).

Conclusions — This meta-analysis illustrates that TZDs significantly reduce the risk of repeat TVR following PCI. Despite >50% of the studies (four of seven) in this meta-analysis reporting nonsignificant reductions in the rate of repeat TVR, the totality of evidence demonstrated a significant benefit of TZDs. To the best of our knowledge, this is the first meta-analysis to evaluate this end point in this patient population. Reduced repeat TVR rates is a critical finding for patients with insulin resistance with or without diabetes, especially considering their risk of complications is significantly higher than insulin-sensitive populations (27). Reducing the risk of developing complications by improved insulin sensitivity is beneficial for both the patient and the health care system (27,28). Repeat TVR risk reduction appears to be consistent regardless of TZD evaluated.

A large retrospective analysis by Cho et al. (20) did not demonstrate a benefit among 325 patients with diabetes (25% of patients received a TZD) and found a lower rate of repeat TVR in patients who did not receive a TZD. One complicating factor in this analysis is that patients were taking a TZD for an unknown duration before PCI. In fact, all diet-controlled patients were excluded since there was no consideration to initiate a TZD after intervention. Also, the retrospective design of this analysis limits its findings for multiple reasons (i.e., unknown compliance rates with TZDs or other medications). In addition, this study duration was 1 year, while all but one of the prospective analyses continued for only 6 months. Repeat TVR rates after 6 months may substantially differ, though the majority of restenosis typically occurs early after stent placement (29,30).

The mechanism behind the benefit of TZD therapy on atherosclerotic plaques remains unclear and should be further investigated. Though insulin sensitization may play a significant role, TZD’s benefit in reducing repeat TVR is likely related to improved endothelial function, decreased inflammation, and reduced proliferation of VSMCs, independent of PPAR-γ activity (9,12–14). Although other insulin sensitizers (i.e., metformin) could also impact the rate of restenosis, the mechanism of TZD’s benefit is multifaceted and substantially different from simple sensitization (31). In accordance with this hypothesis, only three studies in our analysis included patients on other insulin sensitizers (7,21,22). None of these studies demonstrated significant reductions in repeat TVR. Though TZDs were well tolerated throughout, studies did report mild increases in incidence of weight gain, edema, and heart failure (17,21,23).

Several limitations to this meta-analysis should be noted. Although the results of one rosiglitazone study seemed to drive the overall effect in the rosiglitazone subgroup, the existence of a power-related phenomenon is more likely than a drug failure or study design–related issue. Recently, restenosis rates have been directly correlated to the type of stent used in PCI (32). Specifically, the use of drug-eluting stents can provide additional benefit at the local site of action (8). The type of stent used was neither well documented nor uniform across all studies. In fact, some studies enrolled patients receiving up to four different brands of stents without mention of drug-eluting agent. The combination of TZDs with newer drug-eluting stents may more dramatically reduce the risk of restenosis requiring revascularization; however, it should be evaluated further.

Other pharmacologic prophylaxis (including anticoagulation and antiplatelet therapy) was not consistent among studies. Cilostazol, an agent with less-convincing evidence for use following PCI, was used in a group of patients in one study (33), rather than a thienopyridine (i.e., clopidgrel or ticlopidine). In this study, more patients received cilostazol in the pioglitazone group than in the standard-of-care group, and the rate of TVR in the pioglitazone group remained significantly less (17).

The trials evaluated in our meta-analysis predominately enrolled Asian male subjects. As such, the application of these results to the more diverse patient population with diabetes and insulin resistance would not be appropriate. The potential benefit of TZD prophylaxis in other ethnic patient populations should be evaluated. Also, all doses evaluated in the constituent trials were moderate (4 mg rosiglitazone and 30 mg pioglitazone). Speculation on the effect of higher or lower doses is difficult. Based on baseline A1C values, these patients would not be considered poorly controlled. The magnitude of TZD effect on TVR may be different in patients with well-controlled versus uncontrolled diabetes.

Results from this meta-analysis suggest that TZDs are an effective strategy to reduce repeat TVR following percutaneous coronary intervention, especially in TZD-naïve patients with some degree of insulin resistance.
Thiazolidinediones and vessel revascularization

References


A Systematic Review and Meta-Analysis of Hypoglycemia and Cardiovascular Events

A comparison of glyburide with other secretagogues and with insulin

OBJECTIVE — Glyburide is the most widely used sulfonylurea but has unique pharmacodynamic properties that may increase harm. We hypothesized that glyburide causes more hypoglycemia and cardiovascular events than other secretagogues or insulin.

RESEARCH DESIGN AND METHODS — Data sources were Medline, Embase, Cochrane, and three other web-based clinical trial registers (1966–2005). Parallel, randomized, controlled trials in people with type 2 diabetes comparing glyburide monotherapy with monotherapy using secretagogues or insulin were selected. Outcomes were hypoglycemia, glycemic control, cardiovascular events, body weight, and death. Titles and abstracts of 1,806 publications were reviewed in duplicate and 21 relevant articles identified. Data on patient characteristics, interventions, outcomes, and validity were extracted in duplicate using predefined criteria.

RESULTS — Glyburide was associated with a 52% greater risk of experiencing at least one episode of hypoglycemia compared with other secretagogues (relative risk 1.52 [95% CI 1.21–1.92]) and with 83% greater risk compared with other sulfonylureas (1.83 [1.35–2.49]). Glyburide was not associated with an increased risk of cardiovascular events (0.84 [0.56–1.26]), death (0.87 [0.70–1.07]), or end-of-trial weight (weighted mean difference 1.69 kg [95% CI −0.41 to 3.80]) compared with other secretagogues. Limitations included suboptimal reporting of original trials. Loss to follow-up exceeded 20% in some studies, and major hypoglycemia was infrequently reported.

CONCLUSIONS — Glyburide caused more hypoglycemia than other secretagogues and other sulfonylureas. Glyburide was not associated with an increased risk of cardiovascular events, death, or weight gain.

From the 1Division of Nephrology, McMaster University and St. Joseph’s Healthcare, Hamilton, Ontario, Canada; the 2Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, Ontario, Canada; and the 3Division of Endocrinology & Metabolism and Population Health Research Institute, McMaster University and Hamilton Health Sciences, Hamilton, Ontario, Canada. E-mail: clase@mcmaster.ca.

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Address correspondence and reprint requests to Catherine M. Clase, 708-25 Charlton Ave. East, Hamilton, Ontario L8P 3P7, Canada. E-mail: clase@mcmaster.ca.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/dc06-1789.

Abbreviations: FPG, fasting plasma glucose; RCT, randomized controlled trial; UKPDS, UK Prospective Diabetes Study; WMD, weighted mean difference.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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whether people taking glyburide are at increased risk for hypoglycemia or cardiovascular events compared with those taking other secretagogues (other sulfonylureas and meglitinides) or those taking insulin. For completeness of our analysis of important harms, we also examined weight gain.

**RESEARCH DESIGN AND METHODS** — We followed the recommendations of the Quality of Reporting of Meta-Analyses (QUOROM) conference (online appendix 1 [available at http://dx.doi.org/10.2337/dc06-1789]) (11).

**Search strategy**

We searched biomedical databases (Medline, Embase, the Cochrane library, clinicaltrials.gov, controlled-trials.com, and the U.K. national register of RCTs) and the bibliographies of relevant and review articles for reports of RCTs comparing glyburide with other secretagogues or with insulin. In Medline and Embase the searches combined generic and brand names of glyburide with key words specifying RCTs according to the strategy recommended by the Cochrane collaboration (online appendix 2) (12).

Two authors (A.S.G. and T.C.) independently reviewed this initial list (Fig. 1). Full text was obtained for all poten-

tially appropriate articles and each was reviewed independently for eligibility.

**Study selection**

Eligible studies 1) described people with type 2 diabetes; 2) compared glyburide monotherapy with monotherapy using other sulfonylureas, meglitinides, or insulin; 3) reported one or more of the following outcomes: hypoglycemia (major, minor, or all), cardiovascular events, or weight change; 4) described a parallel design RCT; and 5) were written in English. For hypoglycemia and cardiovascular events, we accepted the definition or outcome cluster reported in the original manuscript. Cardiovascular events included incident myocardial infarction, stroke, amputation, episodes of congestive heart failure, or cardiovascular death. Where multiple outcomes were reported, we selected the cluster that most closely matched the definition above. If no cluster was reported, we selected the single outcome we thought best represented cardiovascular outcomes. We excluded studies with <20 participants in each arm or a follow-up of <4 weeks. If studies were reported in more than one publication, we extracted data from the most recent article that met the inclusion criteria using data from related publications when necessary.

We used $k$-statistics to express the extent of agreement between reviewers. Disagreements were resolved by consensus.

**Validity assessment**

We assessed validity in duplicate using the following criteria: 1) method of randomization, 2) presence of allocation concealment, 3) blinding, 4) loss to follow-up, and 5) reporting of an intention-to-treat analysis.

**Data abstraction**

For each study, we abstracted, in duplicate: 1) inclusion and exclusion criteria; 2) baseline characteristics for the different treatment arms, including the number of participants at the start of the study; 3) the intervention and comparator (including dose, frequency, target A1C, and A1C achieved); 4) follow-up period and number of participants at study completion; and 5) the definitions used to report hypoglycemia and cardiovascular events. For each treatment arm we abstracted: 1) all episodes of hypoglycemia (number of participants with one or more episodes and number of episodes per unit of person-time), 2) number of episodes of major and minor hypoglycemia, 3) number of cardiovascular events, 4) number of deaths from any cause, and 5) weight change and end-of-trial weight. When the study included more than two arms, we chose the comparator with the largest number of people.

**Data analysis**

We summarized studies that compared glyburide with other secretagogues separately from studies that compared glyburide with insulin. Because of the unique pharmacokinetic and pharmacodynamic properties of glyburide, we prespecified a subgroup analysis comparing glyburide with other sulfonylureas.

We assessed patient characteristics, interventions, and outcomes for clinical heterogeneity and used the I² statistic to quantify the proportion of total variation that was due to statistical heterogeneity. We calculated relative risk (RR) and 95% CIs to summarize the effect size for dichotomous outcomes (number of participants with at least one hypoglycemic event, number of major and all hypoglycemic events, cardiovascular events, and overall mortality), and rate ratios and 95% CIs were calculated for event rates. For continuous data, we calculated the weighted mean difference (WMD) for each study and summarized this as an
overall WMD and 95% CI. We used random effects assumptions throughout.

To assess for publication bias, we constructed a funnel plot of the SE of the log of the RR plotted against the RR for experiencing at least one episode of hypoglycemia.

We used MetaView 4.2 in Cochrane Review Manager 4.2 (Cochrane Collaboration, Oxford, U.K.) and Comprehensive Meta-Analysis 2.2 (Biostat, Englewood, NJ). *P < 0.05 was considered statistically significant, and an I² value of >50% indicated excess statistical heterogeneity.

**RESULTS**

**Search**

We identified 1,806 publications, of which 21 articles describing 20 studies were relevant (Fig. 1) (2,13–32). Estimated *k* for agreement on relevance was 0.86 (95% CI 0.81–0.91). Of the 21 articles, 12 compared glyburide with an oral hypoglycemic agent and reported this as patients experiencing at least one episode of hypoglycemia (13, 15–17, 20, 21, 23, 25–27, 29, 30); an additional 3 articles only reported total number of hypoglycemic episodes (14, 18, 19); 3 articles compared glyburide with insulin (22, 27, 31); and 3 studies only reported a change in weight (24, 28, 32).

**Validity assessment**

Five of the 21 studies described the method of randomization (2, 14, 22, 30, 33); 3 of these used a computer generated method (2, 22, 27). The method of allocation concealment was described only in the UKPDS trial, which used consecutive opaque envelopes. Seven studies reported blinding of participants and caregivers (13, 15, 16, 19–21, 25). The UKPDS study reported that there was blinding of outcome assessors and data analyzers (2).

Twelve of the 21 studies reported the use of an intent-to-treat analysis (13, 15–17, 20, 21, 23, 25–27, 29, 30). Loss to follow-up was reported in 19 studies (2, 14–23, 25–32). There was a large amount of variability (0–37%) in the percentage of patients lost to follow-up. Reasons for loss to follow-up included inadequate glyemic control, hypoglycemia, other adverse events, noncompliance, and moving out of the study area.

**Study characteristics**

Included studies reported on 7,047 people with follow-up periods from 1 month to 10 years. Some of the studies specified the target A1C or fasting plasma glucose (FPG) to be achieved. Though this value varied widely between studies, the target level was always identical for the two arms within each study (2, 14–16, 18, 20–23, 25, 27, 28). Hypoglycemia was defined as symptoms (without a threshold glucose level) in some studies and in others as symptoms coexisting with low capillary blood glucose levels (minimum threshold 48 mg/dl [2.7 mmol/l], maximum 63 mg/dl [3.5 mmol/l]). Major hypoglycemia was defined as an episode requiring assistance or hospital admission. Details of study characteristics and study validity are available in the online appendix (Tables A and B).

**Quantitative data synthesis**

Table 1 provides a summary of effect sizes, 95% CIs, and I² values for the meta-analyses of harms.

**Hypoglycemia and glycemic control**

Figure 2 shows a 52% greater risk of experiencing at least one episode of hypoglycemia for participants receiving glyburide compared with those receiving other secretagogues (RR 1.52 [95% CI 1.21–1.92]). In the planned subgroup analysis comparing glyburide with other sulfonylureas, glyburide was associated with an 83% higher risk of causing at least one episode of hypoglycemia (1.83 [1.35–2.49]).

Five studies compared glyburide with other secretagogues and reported their results as total number of hypoglycemic episodes (14, 15, 19, 20, 26) (Table 1). These studies were heterogeneous (I² 76.8%). There was an 80% higher rate of hypoglycemic episodes with glyburide (rate ratio 1.80 [95% CI 1.06–3.09]) compared with other secretagogues. Limiting the analysis to studies comparing glyburide with other sulfonylureas led to a decrease in heterogeneity to within acceptable limits (I² 17.6%); the increased risk associated with glyburide compared with other sulfonylureas was 44% (1.44 [1.13–1.85]) (14, 15). Two studies, both using a sulfonylurea as a comparator (14, 15), reported major hypoglycemic episodes. The risk of major hypoglycemic events was over four times higher for glyburide compared with other sulfonylureas (4.69 [0.78–28.08]); however, this was not statistically significant.

Studies reporting A1C were all comparisons of glyburide with sulfonylureas: no significant difference was identified
Hypoglycemia: glyburide vs. secretagogues/insulin

<table>
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<tr>
<th>Study</th>
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<th>Secretagogue n/N</th>
<th>RR (random) 95% CI</th>
<th>RR (random) 95% CI</th>
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<tr>
<td>Baba 1983</td>
<td>20/131</td>
<td>10/146 Glic</td>
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<tr>
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<tr>
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<td>2/76</td>
<td>0/80 Chlp</td>
<td></td>
<td>5.26 [0.26, 107.81]</td>
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<tr>
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<td>7/50</td>
<td>2/47 Chlp</td>
<td></td>
<td>3.29 [0.72, 15.05]</td>
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<tr>
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<tr>
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<td>9/101</td>
<td>9/94 Repg</td>
<td></td>
<td>0.93 [0.39, 2.24]</td>
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<tr>
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<td>15/116 Repg</td>
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<td>Rosenstock 1993</td>
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<td>1/69 Glic</td>
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<td>2.96 [0.32, 27.74]</td>
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<td>Wolffenbuttel 1999</td>
<td>13/139</td>
<td>26/286 Repg</td>
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<td>Total (95% CI)</td>
<td>2199</td>
<td>2513</td>
<td></td>
<td>1.52 [1.21, 1.92]</td>
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</table>

Test for heterogeneity: \( I^2 = 42.1\% \)

Figure 2—RR for experiencing at least one hypoglycemic episode: glyburide versus other secretagogues. Chlp, chlorpropamide; Glic, glicazide; Glim, glimiperide; Glip, glipizide; Repg, repaglinide.

(A1C WMD –0.13% [95% CI −0.52 to 0.26; \( I^2 43.7\% \)). Reports of FPG comparing glyburide with other secretagogues were heterogeneous (WMD –0.49 mmol/l [95% CI –1.15 to 0.18; \( I^2 97.9\% \)). Heterogeneity was not present in the analysis comparing FPG for glyburide with other sulfonylureas. There was a small effect in the direction of improved FPG with glyburide (WMD –0.34 mmol/l [95% CI −0.40 to −0.27; \( I^2 0\% \)).

Three studies (1,339 participants) showed that the risk of hypoglycemia was similar for people treated with glyburide and those treated with insulin, though CIs were wide (RR 0.88 [95% CI 0.25–3.06]) (22,27,31).

**Weight change and end-of-trial weight**

End-of-trial weight was reported in three studies of 498 people comparing glyburide with other secretagogues (25,27,30). Overall, glyburide did not cause an increase in weight compared with other secretagogues (WMD 1.69 kg [95% CI −0.41 to 3.80]). However, in the three studies of 1,840 people comparing glyburide with insulin, body weight increased by 2.28 kg more in people treated with insulin than in those treated with glyburide (WMD –2.28 kg [−2.42 to −2.14]) (2,18,22).

**Cardiovascular events and overall mortality**

Cardiovascular events were reported in three studies including 2,822 participants (2,15,21). There was no significant difference between glyburide and secretagogues (RR 0.84 [95% CI 0.56–1.26]). The same three studies reported no significant difference in overall mortality (0.87 [0.70–1.07]). There were no studies that reported a cardiovascular outcome cluster for glyburide compared with insulin. However, the UKPDS 33 (2) study reported data from which the RR of myocardial infarction for glyburide compared with insulin could be calculated: RR 0.89 (95% CI 0.70–1.14). One study (UKPDS 33) reported data from which mortality for glyburide compared with insulin could be calculated: 0.97 (0.79–1.20).

**Assessment of publication bias**

Visual inspection of the funnel plot of the outcome “number of participants experiencing at least one hypoglycemic episode” demonstrated a paucity of studies with large SEs to the left of the overall estimate (available from the authors upon request).

**CONCLUSIONS** — The main findings of this meta-analysis are that glyburide caused more hypoglycemia than other secretagogues and more hypoglycemia than other sulfonylureas. In the meta-analysis of the two studies that reported major hypoglycemia, there was a trend toward a greater number of events in patients treated with glyburide than with other sulfonylureas. The direction of effect was consistent in all analyses (Table 1). UKPDS 33 reported the percentage of patients per year with one or more episodes and with one or more major episodes of hypoglycemia. Although we were unable to include these results in our meta-analysis because of the method of reporting, our results (glyburide vs. other sulfonylureas RR 1.83 [95% CI 1.35–2.49]) are consistent with the findings of UKPDS 33, in which the mean percentage of patients per year with one or more episodes of hypoglycemia was 17.7% for glyburide and 11.0% for chlorpropamide (RR 1.61), and the mean percentage of patients per year with one or more major hypoglycemic episodes was 0.6% for glyburide and 0.4% for chlorpropamide (RR 1.50).

We did not find a difference in A1C between patients treated with glyburide and those treated with other sulfonyl-
ureas; however, there was a small, statistically significant difference of questionable clinical importance in the comparison of FPG between these two groups. On the evidence of the A1C results, it seems unlikely that improved glycemic control accounts for the increase in hypoglycemia observed.

We did not find any difference in risk for hypoglycemia of glyburide compared with insulin. CIs for this estimate are wide, so a difference cannot be excluded. Other reasons for finding no difference include: 1) inadequate titration of insulin toward achieving glucose control (in one of the studies included in this review, the achieved end-of-trial A1C in the insulin arm was 8.5% [22]), 2) the small dose adjustments possible with insulin that are not possible with an oral agent, or 3) that the difference is minimized in patients with newly diagnosed disease who predominated in our analysis.

Though data from animal and human studies suggest that glyburide might exacerbate coronary ischemia more than other secretagogues and specifically more than other sulfonylureas (10), the meta-analysis of cardiovascular events and deaths provided no support for the hypothesis that these effects lead to adverse cardiovascular outcomes. In addition, weight gain with glyburide was similar to that observed with other sulfonylureas and less than that observed with insulin.

Methodological limitations

Limitations of the included studies. The method of randomization and allocation were seldom described in the studies reported here. Lack of allocation concealment may significantly influence observed treatment effects (34).

There was great variability between studies in the loss to follow-up, from 0 to 37%. Since hypoglycemia and loss to follow-up have been shown to be associated (19,26), differential follow-up of patients prone to hypoglycemia would lead to underestimation of the absolute rates of hypoglycemia in all studies and might also change the differential effect between groups in those studies with a large percentage of patients lost to follow-up. Follow-up time was short in the majority of studies, limiting the power to detect differences in cardiovascular event rates.

Limitations of overall review. We did not include studies published in languages other than English in our review. The lack of inclusion of non-English arti-

cles has been identified as a source of bias in some circumstances (35). However, a recent retrospective analysis suggests that excluding trials published in languages other than English has generally little effect on summary treatment effect estimates (36).

There was a paucity of studies with larger SEs to the left of the point estimate in the funnel plot. Larger SEs can be due to either smaller sample size trials, studies with more variability, or both. This may suggest publication bias or a systematic error introduced by the loss to follow-up.

Statistically significant and clinically important results were obtained for the meta-analyses of all episodes of hypoglycemia, most of which would likely have been minor. Though the clinical importance of minor hypoglycemia can be questioned, minor hypoglycemia has been shown to predict major hypoglycemia (37), and minor episodes lead to disruptions in glycemic control (38,39) that are thought to have long-term consequences (40). Although power to detect a difference in the analysis of major hypoglycemia was limited by the low number of studies reporting this outcome, seven of the eight major hypoglycemic episodes reported occurred in glyburide-treated patients (14,15). UKPDS 33 results, which were not reported in a format that enabled us to include them in the meta-analysis, also show consistency between major episodes and all episodes in the direction and magnitude of the RR when glyburide is compared with chlorpropamide (see above), lending weight to the hypothesis that minor episodes may be a useful surrogate for more clinically important major episodes.

Because all of our comparisons are with glyburide, we are unable to draw any conclusions about the properties of other drugs compared with one another.

Finally, statistical heterogeneity was noted between the studies comparing the risk of at least one hypoglycemic episode in people taking glyburide compared with those taking other secretagogues. Despite this statistical heterogeneity, visual inspection shows that glyburide consistently caused more hypoglycemia than other secretagogues. This statistical heterogeneity most likely results from the relatively tight CI around the UKPDS 13. Indeed, when the UKPDS data were removed from the analysis, the studies were deemed homogenous without a significant change in the overall estimate (P value increased from 0.06 to 0.64 and I² decreased from 42.1 to 0% with the overall RR estimate changing from 1.52 to 1.33, both statistically significant).

Implications for practice

In 2003, it was estimated that 13.8 million people in the U.S. had established type 2 diabetes; of these, 7.8 million people used at least one oral antidiabetes medication (41). Glyburide, available as a generic, is relatively inexpensive and widely used. Our results suggest that risk of hypoglycemia and rates of hypoglycemia for millions of patients are likely ~50% higher in those taking glyburide than they would be if they were taking an alternative sulfonylurea or nonsulfonylurea secretagogue.

Implications for research

Our review highlights the importance of minimizing loss to follow-up in RCTs of long duration, as our overall estimates included some clinical trials in which loss to follow-up exceeded 20%. The clinical consequences of hypoglycemia, its effects on patient compliance, and the direct health care costs of hypoglycemia are all important issues that warrant inclusion in an economic evaluation of the relative cost-effectiveness of glyburide compared with other secretagogues.

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UK Prospective Diabetes Study (UKPDS) 13: Relative efficacy of randomly allocated diet, sulphonylurea, insulin, or metformin in patients with newly diagnosed non-insulin dependent diabetes followed for three years. BMJ 310:83–88, 1995


Pharmacotherapy of Childhood Obesity

An evidence-based, conceptual approach

MICHAEL FREEMARK, MD

This review provides a comparative analysis of the benefits of lifestyle intervention and pharmacotherapy in adults and children using previously published meta-analyses, as well as new data published within the past 2 years. The manuscript critically summarizes the potential risks of various established (orlistat, sibutramine, and metformin) and new (rimonabant) pharmacologic agents and presents a conceptual approach to selection of patients for pharmacotherapy, tailored drug selection, and timing of intervention.

Perils and promise of pharmacotherapy

Forty-five years after an amphetamine was approved for the treatment of obesity in adults, an expert in the field characterized a new therapeutic formulation as being effective and long-lasting, posing “little risk” (1). Four years later, others (2) “confirmed the weight-reducing efficacy and good tolerability” of the drug and noted that adverse effects were “generally mild and transient.” The drug in question was dexfenfluramine, which was removed from the commercial market 18 months after its subsequent U.S. Food and Drug Administration approval owing to the development of valvular heart disease and primary pulmonary hypertension in a subset of patients (3,4).

This experience and many others (5) have forced us to think long and hard before making sweeping recommendations about the use of behavior-modifying drugs for the treatment of obesity.

Yet, the pediatric community confronts a serious problem: the surge of metabolic complications in obese adolescents, including impaired glucose tolerance (IGT) and type 2 diabetes, hypertension, dyslipidemia, ovarian hyperandrogenism, hepatic steatosis, and sleep apnea (6). Two recent studies highlight the concern. First (7), despite regular lifestyle counseling in a university-based clinic, one-third of obese teenagers with profound insulin resistance and IGT developed type 2 diabetes during a follow-up period of 21 months. Second (8), among Pima-Indian children and adolescents with type 2 diabetes, the rate of development of end-stage renal disease was proportional to the duration of diabetes, but not to the age of onset of glucose intolerance. It is clear that we must effectively intervene to prevent long-term complications in obese insulin-resistant children, and, given the progressive nature of these conditions, we cannot dally.

Lifestyle intervention can reduce rates of weight gain and fat deposition in children (9 and refs. cited below) and delay or prevent the development of type 2 diabetes in obese adults during trial periods lasting as long as 4 years (see below). However, lifestyle intervention is effective only if applied intensively and continuously in highly motivated subjects. Figure 1 summarizes data from seven major randomized multicenter studies that assessed the effects of lifestyle intervention in obese adults (10–16). The data are representative, but comparisons among the groups must be interpreted with caution because of variations in patient populations and study design. In general, “intensive” lifestyle intervention, with obligatory caloric restriction, multiple individual and/or group counseling sessions, daily exercise, and numerous clinic visits, reduces body weight by an average of 6 kg (~5.5–6.5% of body weight in most studies) during the 1st year. “Moderate” intervention, with specified caloric guidelines and exercise counseling, is less effective, while the standard lifestyle approaches delivered to nearly all obese people, namely dietary recommendations and regular clinic visits, have little or no effect. Also of note is the rebound weight gain in both the intensive and moderate groups, though some weight loss can be maintained for 3–4 years if the patient remains vigilant (10,11,13).

The story in children is similar, at least in the short run (Fig. 1). Intensive lifestyle intervention can reduce body weight by 4.3–7 kg (~4.5–6.5% of body weight) during the 1st year, while standard lifestyle intervention has little benefit for the majority of kids (17–22). Most studies demonstrating clinical benefit of lifestyle intervention in children have been short-term (6 months to 2 years) investigations, and rebound weight gain has in some cases obliterated prior weight loss (9). Nevertheless, several controlled trials provide evidence for long-term (5–10 years) weight maintenance in children who received intensive intervention, including dietary, exercise, and family counseling (9).

Why do obese people have difficulty losing weight or sustaining weight loss? Time commitments and costs of lifestyle changes may play important roles (23), and some people may simply tire of living with, or may rebel against, dietary restrictions. However, there are also important biological considerations (Fig. 2): weight loss is accompanied by reductions in plasma leptin, insulin, and tri-iodo thyronine and increases in insulin sensitivity and plasma ghrelin (6). These changes stimulate appetite, reduce sympathetic tone and energy expenditure, and promote lipogenesis, thereby facilitating rebound weight gain (6). Consequently, short-term weight loss cannot be sustained without considerable effort. Many will fail.

Mechanisms of action of pharmacologic agents and metabolic benefits

Can pharmacologic agents complement the effects of lifestyle intervention and re-
duce the risks of complications in those who fail to respond adequately to lifestyle change? This review focuses on four major classes of medications used to treat obesity and/or its complications. The drugs have differential mechanisms of action (Fig. 3) and, as will be seen, differential benefits and adverse effects.

Sibutramine acts centrally to inhibit reuptake of serotonin, norepinephrine, and, to a lesser extent, dopamine. It reduces hunger and increases satiety, and in brown adipose tissue, promotes thermogenesis, which increases energy expenditure (24). Rimonabant is a specific inhibitor of cannabinoid receptor 1. It reduces food intake through actions on the hypothalamus, mesolimbic system, and vagus nerve and directly stimulates the expression of adiponectin in white adipose tissue (25). Orlistat inhibits intestinal lipases and reduces the gastrointestinal absorption of fat by 30% (24). Finally, through activation of AMP-activated protein kinase (AMPK), metformin reduces hepatic glucose production and plasma insulin concentrations and inhibits fat cell lipogenesis. It can increase peripheral insulin sensitivity and may reduce food intake by raising levels of glucagon-like peptide 1 (26,27).

How effective are these agents in promoting weight loss and reversing comorbidities?

Figure 4 summarizes the findings of placebo-controlled studies performed in several thousand obese adults (11,13–16,24,28–30). The figure compares mean values calculated from data obtained in three studies of rimonabant (14–16), with the results of published meta-analyses of the effects of sibutramine (24,28–30) and orlistat (24,28–30). Only the effects of the highest dose of rimonabant (20 mg/day) are depicted, since lower doses of the medication (5 mg/day) were far less effective. The results of the XENDOS Study (orlistat) (13) and the Diabetes Prevention Program (DPP) (metformin) (11), each of which involved >1,000 subjects, are illustrated separately. Figure 4 shows the benefits of each drug in excess of that achieved by lifestyle intervention alone. Comprising the findings of a multitude of investigators, the data do not account for differences in study design such as the nature of dietary restriction or the sex or ethnicity of the patients. Thus, group comparisons must be interpreted with caution. Moreover, the data assess only the benefits achieved during the period in which subjects actually took the drugs. In other words, Fig. 4 (and the majority of published studies) show optimal benefits for those who tolerate and accept the medications.

Four general conclusions appear warranted. First, few studies have lasted >1 year. Second, all of the agents promote weight loss, although the magnitude of the effect varies considerably among individuals. Third, the short-term benefits of sibutramine and rimonabant (20 mg/day) exceed those of orlistat and metformin. Finally, some weight regain occurs after 1 year, and the final absolute weight loss is modest. Nevertheless, the combination of lifestyle intervention plus medication can promote as much as 10–12 kg of weight loss, amounting to 7.5–10% of overall body weight (13,31).

It is interesting that striking reductions in body weight are not always associated with reductions in blood pressure or improvements in glucose tolerance (11,13–16,24,26–30) (Table 1). For example, sibutramine appears to have little or no effect on fasting glucose or insulin levels in adults, and the effects of metformin on glucose and insulin greatly exceed those of either rimonabant or orlistat. On the other hand, rimonabant may increase plasma adiponectin (15), a marker of insulin sensitivity, while metformin (in contrast to the thiazolidinediones) may have little or no effect (32). Metformin and orlistat cause variable and small reductions in blood pressure, while rimonabant has no effect. Sibutramine causes 1- to 3-mm increases in mean systolic and diastolic pressure.

A major goal of pharmacotherapy is reduction in long-term cardiovascular
risk. In adults, orlistat is most effective in reducing serum cholesterol and LDL levels and slightly lowers the LDL-to-HDL ratio (11,13–16,24,16–30) (Table 2). Sibutramine has variable and small effects on HDL and triglycerides, while rimonabant at 20 mg/day robustly increases HDL and reduces serum triglycerides. Lower doses of rimonabant cause small and variable increases in plasma HDL and have no consistent effects on plasma triglycerides. The effects of metformin on plasma lipids are variable. The drug reduced LDL in women with polycystic ovary syndrome (PCOS) (37) and increased HDL in insulin-resistant adults in the DPP (12).

How do the effects of the medications in obese children compare with those in adults?

The literature comprises eight randomized placebo-controlled studies in obese adolescents: one major study with orlistat (21), four with sibutramine (17,19,22,33), three with metformin (34–36), and none with rimonabant (Table 3). Certain outcomes in obese adolescents appear similar to those in adults: for example, weight loss with sibutramine exceeds that with orlistat or metformin, and metformin reduces plasma insulin levels (mean decrease 8 μU/ml) and, to a lesser extent, plasma glucose concentrations in glucose-tolerant subjects. In contrast to its effects in adults with IGT, orlistat had no significant effects on glucose or insulin levels in glucose-tolerant children (21). The effects of sibutramine on glucose metabolism were highly variable. A major multicenter study (33) demonstrated that weight loss with sibutramine is accompanied by reductions in plasma insulin (7 μU/ml) but not glucose. In contrast, three smaller studies of sibutramine (17,19,22) demonstrated no effects of the drug on plasma glucose or insulin levels.

The effects of the medications on plasma lipids in adolescents appear to be highly variable. Sibutramine reduced plasma triglycerides (17–25 mg/dl) in two studies (19,33) and increased plasma HDL (3.1 mg/dl) in one study (33) but had no effect on plasma lipids in two other studies (17,22). Metformin reduced serum cholesterol (−14 mg/dl), triglycerides (−47 mg/dl), and free fatty acids (−0.07 mmol/l) in one investigation (36) but had no significant effect on plasma lipids in the remaining two studies (34,35). In contrast to its effects on plasma lipids in adults, orlistat had no effect on plasma lipids in a multicenter study of obese adolescents (21).

Do medications act in concert with lifestyle change to facilitate weight loss?

The demonstration that medications in combination with lifestyle change reduce weight more than lifestyle intervention alone is indirect evidence for synergism or additivity of the effects. A 12-month randomized study in obese adults (31) showed that sibutramine alone was as effective as intensive lifestyle intervention in reducing weight (mean ± SD weight loss for sibutramine alone 5.0 ± 7.4 kg, amounting to 4.6% of body weight; intensive lifestyle 6.7 ± 7.9 kg, 6.4% of body weight); the addition of so-called brief lifestyle intervention to sibutramine therapy provided no additional benefit, while the effects of sibutramine plus intensive lifestyle intervention (12.1 ± 9.8 kg, 11.4% of body weight) exceeded the benefits of either intervention alone. These findings suggest that lifestyle change plus pharmacotherapy may act in concert when lifestyle intervention is pursued with resolve.

Do pharmacologic agents prevent the development of long-term complications?

In theory, this is the most important question regarding any intervention for obe-
Pharmacotherapy of childhood obesity

Figure 4—Effects of pharmacologic agents on body weight in obese adults. Data represent placebo-subtracted mean values compiled from meta-analyses of studies of sibutramine (24,28–30) and orlistat (24,28–30) and from the results of the DPP (metformin) (11), the XENDOS Study (orlistat) (13), and three multicenter studies of rimonabant (14–16). The effects of only the higher dose of rimonabant (20 mg/day) are depicted. A lower dose (5 mg/day) caused mean weight loss of 1.5 kg at 1 year and 0.6 kg at 2 years. SEs of the means were ~10–15% of the mean values.

Study attrition rates and adverse effects of pharmacologic agents

It is notable that the magnitude of weight loss achieved with these various medications appears to positively correlate with the rate of attrition or drop out from experimental studies (11,13–16,24,26–30,33) (Table 4). This finding suggests that the more potent weight-reducing agents may also be the least well tolerated. This raises an important question: are the drugs safe (Table 5)?

Orlistat is considered safe because it is minimally absorbed. It can, however, cause flatulence, diarrhea, and malabsorptive stools and may reduce vitamin D levels and increase bone turnover in some patients (24,45); a multivitamin may help to prevent osteopenia. Of possible concern was the development of seven new cases of gall bladder disease among the 357 children who took orlistat for a single year (21). One of these children required cholecystectomy. Among the placebo-treated patients, only 1 of 182 developed new gall bladder disease. Since cholecystitis occurs more commonly even in untreated obese individuals (6), it is unclear whether orlistat increases the risk of gall bladder disease or whether long-term use of the drug should be discouraged for patients with preexisting gall stones.

Meta-analyses show that sibutramine increases pulse rate by 4–8 bpm and increases blood pressure 1–3 mmHg in adult subjects (24,28–30). In a major placebo-controlled study of sibutramine in obese adolescents (19), hypertension forced 19 of 43 subjects to reduce the dose of the drug and 5 of 43 (11.6%) to discontinue the medication altogether. The follow-up multicenter study (33) excluded subjects with baseline systolic and/or diastolic blood pressures exceeding 130 and 85 mmHg, respectively. Nevertheless, sibutramine increased mean systolic and diastolic blood pressure by 1 mm and 1.7 mmHg, respectively, and 2.1% of patients developed hypertension

<table>
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Data represent placebo-subtracted mean values compiled from meta-analyses of studies of sibutramine (24,28–30) and orlistat (24,28–30) and from the results of the DPP (metformin) (11) and three multicenter studies of rimonabant (14–16). Only the effects of the higher dose (20 mg/day) of rimonabant are shown. A lower dose (5 mg/day) had no consistent effect on plasma cholesterol, LDL, or triglycerides and caused small and variable increases (~0.5 mg/dl) in plasma HDL at 1 year, with no change at 2 years. SEs of the means are ~10–20% of the mean values. None, no significant effect detected.
during treatment. A total of 6.3% of patients became tachycardic, and mean pulse rate increased by 2.5 bpm. No patients developed arrhythmias, but there are reports of ventricular ectopy and prolonged QT syndrome in a few patients treated with sibutramine (47). Sibutramine can also cause insomnia (3.2% of adolescents), dizziness (4%), dry mouth, and constipation and must not be used with monoamine oxidase inhibitors or a variety of other medications that can cause the serotonin syndrome (24).

In the three major studies of rimonabant in adults (14–16), the drug (at the most effective dose of 20 mg/day) caused an excess (5.6%) of “psychiatric and nervous system disorders” including anxiety, depression, and insomnia. At the less-effective dose of 5 mg/day, there were lesser increases in the incidence of anxiety, insomnia, and dizziness. Whether such problems would occur in young patients is unclear; however, we must be vigilant given the prevalence of eating and mood disorders in severely obese children (48).

Finally, both rimonabant and metformin can cause abdominal discomfort, nausea, and even vomiting. The great majority of adolescents tolerate metformin, and gastrointestinal problems are often transient and dose related. No cases of lactic acidosis have been described in children; indeed, lactic acidosis appears to be extremely rare even in adults in the absence of chronic cardiopulmonary, renal, or hepatic disease. Long-term studies demonstrate the overall tolerability and relative safety of the drug (49).

Summary of the benefits and risks of pharmacologic agents
In summary, pharmacologic agents provide modest to moderate, short-term reduction in body weight and (in some cases) cardiovascular risk factors. The effects of the drugs appear to be facilitated by lifestyle change. Their efficacy appears highly variable among individuals, which may reflect genetic influences, perinatal programming, parental motivation, and past and current behavior. The medications have differential effects on weight and metabolic function. Adverse effects are concerning in a subset of patients, and attrition rates from experimental studies are high. The length of time required for treatment is unclear, and the long-term risks of anorectic agents are unknown. Importantly, certain agents (metformin and orlistat) delay the development of type 2 diabetes in high-risk adults, but the long-term benefits for cardiovascular disease or malignancy are unclear.

**Approach to pharmacotherapy in pediatric patients**
Can we identify pediatric candidates for pharmacological therapy? The major goals of any intervention or treatment for childhood obesity are: 1) to prevent or reverse metabolic comorbidities, 2) to reduce the risk of long-term complications including cardiovascular disease and malignancy, and 3) to improve psychosocial function and quality of life. The risk of metabolic complications correlates with the severity of obesity and insulin resistance (6,50) and with the presence of abdominal adiposity and/or ovarian hyperandrogenism/PCOS, which predispose to glucose intolerance. A family history of maternal gestational diabetes or of early-onset glucose intolerance or cardiovascular disease also bodes poorly (50). Consequently, the author believes that peripubertal children and adolescents with severe insulin resistance, IGT,
hepatic steatosis, and/or ovarian hyperandrogenism are potential candidates for pharmacotherapy, particularly if there is marked abdominal adiposity and/or a strong family history of gestational diabetes, early-onset type 2 diabetes, myocardial infarction, or stroke. No absolute guidelines can be provided for the selection of pediatric patients for pharmacologic therapy; the decision to begin medication(s) should be undertaken only after a comprehensive evaluation of the child’s metabolic status and family history and after an assessment of the current and previous responses to lifestyle intervention. An open and sympathetic discussion with the parents or caretakers is obligatory.

When should we intervene? Lifestyle intervention represents the core treatment for obese and insulin-resistant children and adults (9,11,13,19,31). In the opinion of the author and many other clinicians, lifestyle changes should be undertaken before pharmacotherapy and maintained during pharmacotherapy (Fig. 6). The addition of a pharmacologic agent may be considered if diet and exercise fail to achieve the medical objectives established by the health care professional and family. The use of medication early in the course of adiposity (Fig. 6) might in theory prevent the progression to severe obesity and metabolic complications; nevertheless, such an approach would likely treat many children without due cause or benefit, raise the rate of “unwarranted” side effects, and increase the costs to individuals and to society. On the other hand, initiation of medication very late in the course of obesity may run the risk, by delaying treatment, of “runaway” or irreversible weight gain and long-term morbidity. One approach that reconciles these difficulties is to begin pharmacotherapy when the risk of comorbidities is very high or soon after complications emerge (denoted by the dotted vertical line in Fig. 6). Such complications include IGT, hepatic steatosis, dyslipidemia, and severe menstrual dysfunction. The timing of pharmaco-intervention could in theory be moved to the left (in other words slightly sooner) if the family history for a major comorbidity such as type 2 diabetes is particularly strong.

Which medication should be used? The available evidence suggests that drug selection should be tailored to the individual patient, with strong attention paid to the family history and potential adverse effects.

The author considers metformin, which reduces the rates of type 2 diabetes in high-risk adults (11,37), a valuable adjunct to the treatment of obese patients.

Information compiled from studies of sibutramine, orlistat, and metformin in children and adults (11,13,19,24,28–30,33–36) and from the results of the three multicenter studies of rimonabant (14–16) in adults. ADEK, vitamins A, D, E, K.

### Lifestyle intervention

- **BMI z**
- **Insulin resistance**
- **IGT/co-morbidities**

**Intervene EARLY**
- Prevent severe obesity
- Prevent complications
- Excess patients treated
- Unwarranted side effects
- Individual/societal costs

**Intervene LATE**
- Treat emergent complications
- Progressive untreated obesity
- Risk of long-term morbidity

**Figure 6**—The author’s conceptual approach to balancing lifestyle intervention and pharmacotherapy in the management of obese children.
with severe insulin resistance, IGT, or PCOS. Orlistat also reduces rates of adult-onset diabetes (13) and might prove beneficial in glucose-intolerant children. Dyslipidemic patients may benefit from orlistat or metformin, which reduce LDL levels and the LDL-to-HDL ratio in adults (12,13,38). Metformin or orlistat may also prove useful for obese patients with hepatic steatosis, although additional study is clearly required.

Of the medications tested thus far in children, sibutramine is most effective at reducing body weight, at least in the short term. However, its tendencies to raise blood pressure and pulse are concerning, given the high rates of systolic hypertension among obese adolescents (6). Sibutramine should not be used in children with poorly controlled hypertension or cardiovascular disease and is contraindicated in adolescents with preexisting psychiatric disorders. The long-term safety of anorectic agents in children has not been established, and, in the author’s opinion, sibutramine remains an experimental approach for the treatment of pediatric obesity, requiring long-term study in carefully controlled clinical trials.

Whether rimonabant will prove effective and safe in children is unclear. Given its propensity for inducing behavioral problems in adults, and the relatively high prevalence of eating and mood disorders among severely obese children (48), the author believes that rimonabant should not be used in young individuals without extensive additional investigation. Rimonabant should not be administered to children with a history of psychiatric disease or severe mood disorders.

In the future, other classes of pharmacologic agents (e.g., centrally/vagally active incretin mimetics, melanocortin 4 receptor agonists, ghrelin antagonists, etc.) may be used for the treatment of obesity or maintenance of weight loss in adolescents or adults. Other medications, including the thiazolidinediones, target one or more components of the metabolic syndrome and reduce the risk of type 2 diabetes in adults with IGT (51); however, their tendency to cause weight gain, edema, and, rarely, heart failure (51,52) may be problematic in obese subjects. All of these drugs will require systematic investigation and careful consideration of their potential risks, as well as benefits, before they can be used in the general pediatric population.

How long do we need to treat? Obesity is a chronic, and in many cases life-long, condition. Yet, pharmacotherapy might be discontinued or the dose of medication reduced significantly if short-term objectives of treatment are achieved, i.e., reduction in BMI z score and normalization of blood pressure, plasma lipids, and hepatic and renal function and in girls with PCOS, reduction in hirsutism scores and restoration of ovulatory menses.

Nevertheless, the Rimonabant North America Study (16) showed that discontinuation of drugs was associated with nearly complete regain of lost weight within 1 year. Thus, adults may require long-term pharmacotherapy for long-lasting benefit. We don’t know if this is the case for children. Still, if an anti-obesity medication is discontinued or its dose reduced, it is essential that lifestyle intervention be maintained throughout; this may limit rebound weight gain and might prevent relapse of comorbidities.

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References
Pharmacotherapy of childhood obesity


Glucose Measurement: Confounding Issues in Setting Targets for Inpatient Management

Kathleen Dungan, MD1
John Chapman, PhD2
Susan S. Braithwaite, MD3
John Buse, MD, PhD3

Van den Berghe et al. (1) reported a significant reduction in mortality with normoglycemia (target value 80–110 mg/dl) in patients whose medical intensive care unit (ICU) stay was >72 h and reduced morbidity in all patients, regardless of the duration of ICU stay. Although severe hypoglycemia did not occur in the Van den Berghe et al. study, 18.7% of patients in the intensive treatment group compared with 3.1% of those who received conventional therapy did experience hypoglycemia (defined as glucose <40 mg/dl), albeit with no adverse consequences reported. However, altered consciousness is common in the ICU, and even severe hypoglycemia may be unrecognized. Other studies (2,3) examining intensive insulin protocols in various inpatient settings have suggested benefits in clinical outcomes associated with improved glycemic control. In a mixed ICU population, Van den Berghe et al. (2) previously demonstrated reduced morbidity and mortality with three- to fourfold less hypoglycemia than the medical ICU population (2). Thus, careful assessment of glucose measurement and how it may impact the targets selected in the hospital are critical safety issues in intensive management of hyperglycemia. As a result of increasing evidence that tight glycemic control is beneficial in the management of inpatients with diabetes, the American Diabetes Association (ADA) currently recommends a glucose target “as close to 110 mg/dl as possible and generally <180 mg/dl” for critically ill patients (4). The American Association of Clinical Endocrinologists recommends the “upper limits for glycemic targets” of 110 mg/dl in critically ill patients (5).

In practice, it may be difficult to obtain the level of glycemic control (average glucose 111 mg/dl in the intensively managed group) achieved by Van den Berghe et al. Though a wider range of glucose values has been targeted, rarely have mean glucose values between 80 and 110 mg/dl been achieved, particularly in those studies involving patients with diabetes (6). In many hospitals, samples for laboratory glucose determination are obtained from either venous or arterial sites to determine serum or plasma glucose. These laboratory values are generally obtained less frequently than bedside capillary glucose values using point-of-care (POC) systems that report whole-blood glucose or plasma glucose values. In the Van den Berghe et al. study, a HemoCue B glucose analyzer was used to report the values of arterial whole-blood glucose.

Variability is introduced into the reporting of glucose values because of patient variables and also because of differences between assays (Table 1). Patient variables may include issues of physiology and interfering substances. These variables may be of importance when there are unexpected laboratory results. Among institutional variables, there are differences between assay characteristics, performance of commercial products, the source of the sample, and specimen matrix (i.e., plasma versus whole blood). This study will review assay principles, patient variables, and systematic variables and then encourage clinicians to carefully consider how standard recommendations regarding glycemic targets, particularly in the ICU, should be implemented in their individual health care facilities.

ASSAY PRINCIPLES — In this review, we will signify reference laboratory methods with the term “central laboratory method.” “POC” refers to hand-held devices or portable ward-based analyzers. We recognize that some of these devices are also used in the ambulatory setting. “Plasma correlated” refers to glucose concentrations measured in samples of whole blood but are converted to values that would be expected of plasma measurements.

Table 1—Confounding variables in glucose measurement

<table>
<thead>
<tr>
<th>Methodology affected*</th>
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<tbody>
<tr>
<td>GO</td>
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<tr>
<td>Whole blood</td>
</tr>
<tr>
<td>Arterial</td>
</tr>
<tr>
<td>Capillary</td>
</tr>
<tr>
<td>Postprandial state</td>
</tr>
<tr>
<td>Hematocrit</td>
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<tr>
<td>Anemia</td>
</tr>
<tr>
<td>Polycythemia</td>
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<tr>
<td>Oxygen concentration</td>
</tr>
<tr>
<td>Hypoxia</td>
</tr>
<tr>
<td>Oxygen therapy</td>
</tr>
<tr>
<td>pH (6.8–7.55)</td>
</tr>
<tr>
<td>Low pH</td>
</tr>
<tr>
<td>High pH</td>
</tr>
<tr>
<td>Hypothermia</td>
</tr>
<tr>
<td>Hypotension</td>
</tr>
<tr>
<td>Drugs</td>
</tr>
<tr>
<td>Ascorbic acid</td>
</tr>
<tr>
<td>Acetaminophen</td>
</tr>
<tr>
<td>Dopamine</td>
</tr>
<tr>
<td>Icodextrin</td>
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<tr>
<td>Mannitol</td>
</tr>
</tbody>
</table>

*Change relative to venous plasma measured at central laboratory. GO, glucose oxidase.

From the 1Division of Endocrinology, Ohio State University School of Medicine, Columbus, Ohio; the 2Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina; and the 3Division of Endocrinology, University of North Carolina School of Medicine, Chapel Hill, North Carolina.

Address correspondence and reprint requests to John Buse, MD, PhD, CB# 7110, Old Clinic 5039, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7110. E-mail: j.buse@med.unc.edu.

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Abbreviations: ADA, American Diabetes Association; CAP, College of American Pathologists; GD, glucose-1-dehydrogenase; ICU, intensive care unit; POC, point of care.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Enzymatic reaction

Glucose measurements are based on one of three enzymes: glucose oxidase, glucose-1-dehydrogenase (GD), or hexokinase (7). For POC devices, glucose oxidase is the classic methodology. Glucose oxidase requires oxygen and water and is therefore susceptible to extremes of hydration or oxygenation. Glucose oxidase–catalyzed reactions result in the production of gluconic acid and hydrogen peroxide, the latter of which is detected by various means. GD, like glucose oxidase, is specific for β-D-glucose but may have less interference than glucose oxidase–based techniques. Hexokinase, the basis for many central laboratory methods, phosphorylates D-glucose to form glucose-6-phosphate, which is then oxidized with concurrent reduction of NADH.

Detection method

The enzymatic reaction is either colorimetrically or amperometrically detected. Colorimetric detection is available for techniques using glucose oxidase, in which \( \text{H}_2\text{O}_2 \) reacts with various hydrogen donors to produce a color change that is proportional to the glucose concentration. Most POC colorimetric reactions are measured using a reflectance photometer that converts the reflected light to an electronic signal for digital display. Amperometric detection is available for either glucose oxidase– or GD-based POC devices, in which the electrical current produced from the reaction is directly measured. In the case of hexokinase, NADH reacts with the dye to produce the color change.

POC techniques

POC devices typically use test strips (biosensors) with a porous layer that separates blood cells from the enzyme-impregnated reagent layer (7). In general, biosensor technology is less precise and less accurate than the wet chemistry methods used in most central laboratory methods. Blood gas analyzers are often used at the bedside and generally use wet chemistry techniques that more closely approximate central laboratory methods (8).

A notable exception to this biosensor technology is the HemoCue B Analyzer used in the Van den Berghe et al. studies, a POC method that measures glucose via GD using a disposable microuvette instead of a traditional biosensor (9). The HemoCue B Glucose Analyzer (HemoCue AB, Angelholm, Sweden) measures glucose via absorbance of reaction products at unique wavelengths. The method allows colorimetric measurement from a whole-blood sample.

Interstitial fluid glucose monitoring

Other investigators have focused on continuous interstitial fluid glucose measurements in order to simplify the need for frequent capillary sampling (10). However, the measurement of glucose in interstitial fluid is complex and affected by tissue perfusion, temperature, and local humoral factors (11). A detailed discussion of this technology is beyond the scope of this review.

PATIENT VARIABLES WITHIN A POPULATION

Patient factors

Hypotension. In the ICU, multiple variables that may affect bedside glucose measurements may be present all at once. In particular, hypotension may result in a reduction of perfusion and an increase in glucose utilization, potentially obscuring the true result for capillary whole-blood samples. A GD-based POC device demonstrated that in 31 hypotensive patients (systolic blood pressure <90 mg/dl), capillary whole-blood values differed from the central laboratory venous plasma glucose to a greater extent than those of normal control subjects (−61.7 ± 12.4 vs. −14.1 ± 2.0 mg/dl, \( P < 0.001 \)) (12). Sixty-four percent of values fell outside the acceptable range of 20% compared with 10% of the control group. On the other hand, venous samples measured with the POC meter correlated well with the central laboratory method. A glucose oxidase methodology fared no better in 38 patients with shock (13). Capillary whole-blood glucose was significantly higher than the venous plasma glucose determined by the central laboratory method (mean difference 77 mg/dl, \( P = 0.04 \)), but venous whole-blood glucose on the POC device was no different (13). In addition, 31.6% of the capillary glucose measurements were outside of the allowable 20% variance. Other studies (8,14) that did not show an effect were limited by sample size. More recently, Kulkarni et al. (15) reported that in cases of hypoperfusion, the accuracy of agreement between an arterial blood gas POC method and GD-based POC capillary glucose readings may still result in undetected hypoglycemia when a lower limit of 80 mg/dl is targeted. This occurs despite what would otherwise be considered low bias (4.0 mg/dl) and imprecision (16.2 mg/dl).

Hematocrit. In general, increases in hematocrit are known to decrease glucose measurements and vice versa. Although manufacturers set acceptable testing limits for hematocrit, POC devices do not exclude samples by hematocrit, and hematocrit is not always known at the time of testing. Proposed mechanisms include mechanical impedance of plasma diffusion into the reagent layer of the strip at higher hematocrit and increased relative plasma volume at higher viscosity, resulting in slower diffusion of glucose (16). The net result would potentially mask hypoglycemia in patients with anemia and underestimate glucose in patients with polycythemia. A POC glucose meter that measures and automatically corrects for hematocrit was recently described and had less error than other devices (17).

A n vitro study examined the effects of hematocrit on six different POC glucose meters (18). At low hematocrit, most POC systems yielded a higher glucose result (5–15%) relative to venous plasma, and the opposite was true at higher hematocrit (−10 to 30%), with the exception of amperometric glucose oxidase methods, which yielded lower values at all three hematocrit levels.

Differences have been observed in clinical studies as well (19). Surgical patients may be most at risk for errors in glucose measurement as a result of fluctuations in hematocrit (20–22).

The HemoCue system, which determines glucose concentration on lysed whole blood instead of measurement based on membrane separation of plasma from red cells, does not show significant hematocrit dependence (23). However, this GD-based POC system has been shown to falsely produce decreased glucose values in patients with methemoglobin values ≥10% (24).

Oxygenation. High oxygen tension, i.e., \( pO_2 >100 \) mmHg, can falsely lower glucose readings on some glucose oxidase–based POC instruments, particularly in patients on oxygen therapy. Oxygen levels as high as 400 mmHg may be seen with surgical patients, particularly those undergoing cardiopulmonary bypass (25). Conversely, higher altitudes overestimate glucose readings by 15% with glucose oxidase methods (26). As might be expected, the effect is largest in arterial blood and smallest in venous blood, but there is little data on the effect of \( pO_2 \) on capillary whole blood (27).
Tang et al. (28) evaluated six POC glucose meter systems with respect to effects of oxygenation using venous whole blood and venous plasma. Measurements at $pO_2 > 100$ mmHg were outside of error tolerances (15 mg/dl for glucose $< 100$ mg/dl or 15% for glucose $> 100$ mg/dl) 14.3–31.6% of the time. Overall, lower oxygen tension (40 mmHg) had a negligible effect. An older study reported errors at lower $pO_2$ (29).

Kurahashi et al. (30) found that arterial whole blood from surgical patients using an amperometric glucose oxidase–based POC meter underestimated glucose by 39 mg/dl. Similar results were reported elsewhere with some glucose oxidase–but not GD-based POC devices in mixed hospital patients (19,31).

**pH.** As with any enzymatic reaction, changes in pH may affect the performance of the POC meter. This has not been shown to be a major source of error at a pH range of 6.97–7.84 (32) or at lower pH (6.8–7.55) (31). However, Kilpatrick et al. (29) found significant deviation in glucose measurement at pH <6.95 and >7.85, with >15% from the central laboratory whole-blood method using an older POC method. Nonetheless, this may be cause for concern in cases of severe acidosis (e.g., diabetic ketoacidosis), or where other factors may contribute, leading to clinically significant interpretation errors.

**Temperature.** Some data suggest that cold temperatures may produce discrepant results (26,33). Active warming may improve measurements; conversely, the effects of fever are unknown.

**Interfering substances**
The majority of substances that interfere with glucose oxidase–based POC devices do so at the peroxide reduction detection step and not at the level of the enzyme itself (which is very specific for $\beta$-d-glucose). Table 1 lists some examples. In the case of the photometric strips, reducing agents such as ascoraminopen and ascorbic acid may consume peroxide and diminish its reaction with the dye, thus resulting in lower readings (34). Newer amperometric POC devices have attempted to compensate for this by introducing a third electrode that reduces background current (34). Devices that use GD as the catalytic tend to have less interference but may occasionally falsely increase POC readings through direct oxidation at the electrode (34). Blood gas analyzers may also give more accurate POC results in patients with possible drug interferences (35).

**Drugs.** Tang et al. (34) examined the effects of therapeutic and toxic concentrations of 30 different drugs on glucose readings from six different POC glucose meters. In this study, a comparatively low error threshold of $\pm 2$ mg/dl was used. Interferences were found for ascorbic acid, acetaminophen, dopamine, and mannitol. At high doses, ascorbic acid increased GD-based POC readings but decreased those that used glucose oxidase (34). False low glucose readings were reported with other glucose oxidase–based POC devices (36,37) but not with testing based on hexokinase or other GD-based methods (36).

Acetaminophen increased POC glucose readings with GD meters but decreased readings with some, but not all, glucose oxidase–based meters at therapeutic drug levels (34). This may be particularly problematic in overdose patients, in whom hypoglycemia may develop in the presence of hepatic failure. Other reports (36,38) had similar findings, and there may be a reduction in glucose measurements in patients given only 1.5–2 g acetaminophen (39).

Dopamine increased glucose values on GD-based POC systems, primarily at high drug concentrations (34,40). Mannitol increased glucose oxidase–based POC readings, possibly through detection by the analyzer or by a nonspecific osmotic effect (34,35). Finally, interferences with salicylates (36) and nitroprusside (+1) have been described in past literature but not more recently (34).

**Other substances.** Most GD-based POC devices display large overestimations of glucose in patients undergoing peritoneal dialysis using icodextrin as an osmotic agent (42–44). Icodextrin is metabolized to maltose and is indistinguishable from glucose on GD-based POC devices. A similar mechanism of interference prompted U.S. Food and Drug Administration warnings for intravenous immunoglobulin solutions (45). Skin preparations have been reported to interfere (46). Other patient factors, such as bilirubin (9,47), triglycerides (9,47), and paraproteinemias (48–51), may also cause “pseudohypoglycemia.”

**Sources of systematic difference between institutions**
— When the method of measurement of circulating glucose differs between institutions, the absolute values and variability of glucose measurements will systematically differ. These systematic differences have implications for the appropriate glucose targets and algorithms of care developed on the basis of demonstrated risks and benefits of interventions in published studies; appropriate targets in one site with one methodology may not be generalizable.

**Standards for comparison**
Much of the difficulty with assessing the performance of POC glucose measuring devices lies in the lack of consensus among professional and regulatory groups regarding allowable error (52–55). As a result, published studies are often difficult to directly compare. Of these, the ADA guidelines established in 1996 are the most stringent, calling for total error (bias plus imprecision) of $<10\%$ for current devices and $<5\%$ for future devices (55). Error grids have been used in an attempt to predict clinically important errors; however, they are comparatively inaccurate (56).

Standards do not specify differences for POC devices that are intended for hospital use versus those meters intended for home use. Despite a strong correlation between capillary whole-blood glucose and central laboratory methods in an ICU population as a whole, bedside POC devices may be unreliable for use in the individual patient in the ICU (15). A simulation modeling study showed that for glucose meters that achieve both coefficient of variation (CV) and bias $\leq 5\% – 6\%$ (total $<14\%$), major errors in insulin dosing are rare, but up to 23% of measurements would result in small errors (57).

Therefore, it would seem that the ADA guidelines should serve as the minimum proficiency standard in the hospital.

**Performance of POC devices**
Over the past decade, POC devices for measuring glucose have become more user friendly, resulting in greater accuracy (58). In hospital patients, recent studies report 91–100% accuracy of various POC devices (59,31). Although the accuracy may have significantly improved in published studies under controlled conditions, this may not be the case in the typical clinical setting, particularly among hospitalized patients. The latest College of American Pathologists (CAP) proficiency results demonstrate large CVs for mean glucose values obtained from all POC instruments at all institutions combined (59). At glucose levels of 120–170 mg/dl
Glucose measurement: confounding issues

(mean 143.8 mg/dl), the overall inter-
laboratory CV is 15.1%; in the hypogly-
cemic range, the CV is 31.9% (26.3–66.6
mg/dl, mean 45.7 mg/dl). This variability
is at least in part due to differences be-
tween instruments because CVs for indi-
vidual instruments are lower, ranging
from 3.9 to 10.9% in the mid-100 range
and 6.2 to 13.3% in the hypoglycemic
range. Depending on the type of device
used, the mean glucose measurement for
a particular unknown test sample re-
ported by an institution varies by >30%
at glucose levels >150 mg/dl and by 60%
in the hypoglycemic range. In compari-
son, interlaboratory CVs for various cen-
tral laboratory methods are uniformly
<5%. The variability among POC devices
may be due to analytical differences in
struments or due to user interfaces that
are more susceptible to operator error.
For an institution to be considered profi-
cient, results should deviate by no more
d than 12 mg/dl or 20% from the peer
goup mean, but this may be inadequate
as institutions aim to establish tighter gly-
cemic control using recent standardized
guidelines of inpatient management.

Operator error

Unfortunately, operator error is incom-
pletely captured with CAP data, as well as
with studies that evaluate POC devices
based on aqueous controls, venous sam-
iples, or prepared blood samples (60,61).
However, the potential for operator error
still exists and remains the largest source
of error (up to 91–97%) overall (46,62–
64). Sources of error such as differences
between lots of test strips (up to 14.5 mg/
dl) in some (28,65) but not all (19,31)
studies may be unrecognized. It is advis-
able to regularly test split-sample controls
referenced to the central laboratory
method to detect both performer error
and instrument accuracy (62). Quality
control may be particularly challenging in
ICU and surgical patients (62,63). Pro-
grams that use training, quality control
procedures combined with national inter-
hospital proficiency surveys, and newer
technology have produced significant im-
provements in precision (62,66,67).

Source of sample

Differences in measurements among
blood sources (i.e., arterial, capillary, or
venous) may be attributable to variations
in glucose extraction by tissues, perfu-
sion, oxygenation, pH, feeding, and tem-
perature (see PATIENT FACTORS above), as
well as theoretically neurovascular func-
tion (68). It has been suggested that on
average, arterial glucose concentrations at
normal pO2 are 5 mg/dl higher than capi-
illary blood and ~10 mg/dl greater than
venous concentrations (69). In recent
studies, assessments are limited due to a
lack of data comparing all sources of
blood, particularly arterial versus venous
blood.

Arterial samples compared with capil-
ary samples. Some ICU studies using
arterial samples measured with the POC
device show acceptable agreement with
capillary blood (70,71). A recent abstract
found that with newer POC devices in
ICU patients, arterial samples had greater
accuracy than capillary whole-blood
compared with the central whole-blood
method (72). However, an older GD-
based POC device reported no greater ac-
curacy with arterial whole blood than
with capillary whole blood in 50 post-
cardiothoracic surgery patients, resulting
in potential errors of insulin dosing in 31
of 50 patients (20). Using a plasma-
correlated glucose oxidase method in 30
critically ill patients, arterial measure-
ments were 8.8 ± 17.8% higher, and capi-
llary measurements were 3.6 ± 15% higher
on the POC meter than on the ar-
terial plasma central laboratory method
(14). On error grid analysis, only 88% of
arterial and 73% of capillary readings fell
within target range using the POC meter.
Arterial blood gas analysis performed bet-
ter than the POC device (14).

Venous samples compared with capil-
ary samples. A POC GD device in 31
patients with diabetes reported venous
whole-blood measurements exceeding
capillary whole blood by 9.6% (72). In
mixed hospital patients (31) and hypo-
tensive patients (12,13), venous whole
blood measured on POC devices was
found to be superior to capillary whole
blood on the same device, with the excep-
tion of one study (73). However, in a re-
cent study (74) using a POC GD-based
method, glucose measured from the same
site showed better agreement with the
central laboratory (POC venous whole
blood vs. central laboratory venous
plasma, R² = 0.83) than glucose meas-
ured from different sites (POC capillary
whole blood vs. central laboratory venous
plasma, R² = 0.55). The authors argue
that anatomical site is more important in
determining glucose values than speci-
men matrix.

Postprandial state. Differences between
sources of blood may be amplified in the
postprandial state (72,75–77). During
periods of fasting, capillary glucose may
be only slightly (2–5 mg/dl) higher than
venous plasma glucose. After a glucose
load, however, capillary glucose values
may be 20–25% higher than venous
plasma values (75). Conversely, hyper-
glycemia may be misdiagnosed in blood
samples drawn from intravenous lines
carrying dextrose.

Differences between plasma and whole
blood (specimen matrix)
The difference between plasma and whole
blood is the most important variable that
clinicians must consider when setting tar-
gets for inpatient glucose measurement.
These differences are a consequence of
variables in specimen matrix, including
water content, lipid and protein concen-
trations, and cellular elements (see PATIENT
FACTORS). Although the glucose concentra-
tion in the water that makes up plasma is
equal to that of erythrocytes, plasma has
greater water content than erythrocytes
and therefore exhibits higher glucose lev-
els than whole blood (78). The World
Health Organization uses a conversion
factor of 1.12 that has been mathemati-
cally derived assuming a hematocrit of
45% and a red cell-to-plasma water ratio
of ~0.80 (79). The conversion factor is
less appropriate in patients with severe
perturbations in hydration, osmolarity, or
hemoglobin. In general, manufacturer
specifications describe limitations in
methodologies under these conditions,
but the clinician must be aware that POC
devices are not capable of excluding such
samples. Furthermore, based on simple
regression analyses, the conversion be-
tween plasma and whole blood is depen-
dent on the glucose level itself and may
vary considerably at extremes of glucose
measurement (76,80). Whole blood may
be tested with the POC meter but con-
verted to equivalent plasma glucose val-
ues obtained from donor blood samples
supplemented with glucose; therefore,
measurements of plasma samples are in-
accurate on such devices (81). On the
other hand, meters may attempt to ap-
proximate plasma glucose directly via ul-
tрафильтрации of erythrocytes from samples
with the use of a specialized porous mem-
brane (74). Finally, some POC devices
have the capability of reporting values as
whole-blood or plasma equivalents, and
this is not always specified in studies (82).
Arterial whole blood compared with ar-
terial plasma. Limited data exists for
this important comparison. The conver-
sion of arterial whole-blood glucose to
plasma-correlated results may not be valid using POC measurements in cardiothoracic surgery patients (20). A glucose oxidase–based device in 10 ICU patients found only a small difference (0.76 mg/dl) between POC arterial whole-blood values compared with the arterial plasma central laboratory method, but wide CIs negate this finding (14).

**Venous whole blood compared with venous plasma.** Using four amperometric and two colorimetric glucose oxidase–based devices in 31 patients with diabetes, Kuwa et al. (72) found that venous whole blood measured with the central laboratory method was 11.3% less than venous plasma measured with the central laboratory method. A 13% difference was reported in 126 healthy volunteers (81).

**Capillary whole blood compared with venous plasma.** In recent studies, variable results from POC devices are in part attributable to manufacturers’ efforts to convert results of measurements made on samples of whole blood to plasma-correlated values (72). In the Kuwa et al. (72) study, the mean capillary whole-blood glucose measurements from several POC devices combined was actually 3.2% higher than venous plasma glucose determined by the central laboratory method (contrary to the expected relationship that would be created by the difference in matrix but consistent with the difference that would be created by site of sampling). Other studies using plasma-correlated POC devices in ICU (83) and mixed hospital (80) patients also showed similar results. Therefore, the site of sampling may outweigh the importance of matrix in determining systematic differences. Conversely, the HemoCue B glucose meter (which reports whole-blood glucose) produced results that were contrary to expectation based on site of sampling but were consistent with expectation based on the matrix (77).

**Ramifications for the clinician**

Unfortunately, studies that directly compare plasma and whole-blood glucose measurements from all sources (arterial, capillary, and venous) are lacking. However, it should be assumed that under physiologic conditions, glucose measurement determined from arterial sites generally exceeds that of capillary sites, which, in turn, is greater than venous sites. Glucose from plasma generally exceeds that of whole blood. In 2001, the International Federation of Clinical Chemistry recommended that glucose meters be calibrated to plasma glucose, using a constant factor of 1.11 (78). In fact, most, but not all, meters today are calibrated to report plasma glucose values. A notable exception is the HemoCue B glucose analyzer used in the Van den Bergh et al. studies, which reports whole-blood values. Based on CAP data, most hospitals do use plasma-correlated methods. Therefore, it is imperative that hospitals using these devices set targets that reflect plasma glucose rather than whole-blood glucose. Failure to do so may result in more significant hypoglycemia than was reported in the Van den Bergh et al. data.

**CONCLUSIONS** — Manufacturers have improved the accuracy of glucose measurement with many (84) but not all (85) newer generation devices, mainly through improvements in user interfaces that reduce operator error. However, for individuals in the hospital, variables that are unique to the patient must be considered, particularly in situations where discrepancies arise between the bedside measurement and the clinical scenario. Nowhere else is there greater potential for multiple confounding factors to be present at once than in the hospital setting. Furthermore, the accuracy of POC devices may not be sufficient to achieve tight glycemic control in hospital patients, and studies are not standardized in methods of glucose measurement, despite well-characterized differences in specimen source and matrix. Unfortunately, the unacceptable time delay imposed by central reference laboratory measurements mandates the use of POC in the ICU. Accurate, well-validated blood sensors, particularly those that provide continuous readings, are sorely needed. In the meantime, providers should use caution when selecting patients for monitoring glucose with the use of bedside monitors. If the whole-blood glucose targets of the Van den Bergh et al. study (80–110 mg/dl) are to be applied to venous plasma-correlated values used in many hospitals, a more appropriate target range might be 90–120 mg/dl. Targets should be individualized in each institution and in each setting based on the methodology of glucose testing and the needs of a given patient population to reflect, at a minimum, the 1.11 whole-blood-to-plasma glucose conversion factor recommended by the International Federation of Clinical Chemistry.

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Prediction of Clinical Outcome in Islet Allotransplantation

Federico Bertuzzi, MD1
Camillo Ricordi, MD2

Recent progresses in the pancreas' enzymatic digestion process along with novel immunosuppression strategies have led to successful clinical trials of islet transplantation in humans. On the other hand, clinical outcome remains variable and unpredictable in centers with limited experience. The possibility of predicting in vivo islet graft function should allow the selection of preparations on the basis of their potential success, thus improving the overall results and making the processes more consistent and reproducible.

Graft function prediction is a work in progress. Initially, the best parameters representative of engrafted islet mass in recipients should be defined. Fasting C-peptide and exogenous insulin requirements are commonly used, although other methods for a more complete characterization of graft function (i.e., β-score) have recently been described but not validated in a large number of patients. In addition, some of these data were shown to predict long-term graft function and might be used to establish whether recipients require further islet infusions.

Many pretransplant parameters representative of islet preparations and predictive for in vivo function have been proposed. C-peptide values as well as the exogenous insulin requirements of recipients were shown to be directly correlated with the number of transplanted islets, but there are many exceptions to this association. Other methods to define the quality of an islet preparation include analyses of islet morphology, cell composition, response to glucose, and viability and production of proinflammatory molecules. The most promising appear to be those that simultaneously analyze more than one aspect of islet physiology.

Islet transplantation has great potential for the normalization of the main parameters of glucose metabolism in diabetic patients. A sufficient number of islets can now be obtained from good quality pancreata more frequently than in the past, and diabetes can generally be reversed, normalizing A1C levels and eliminating severe hypoglycemic episodes (1). Clinical data produced by some centers on patients >1 year after the transplant show that the percentage of normal C-peptide secretion is 100% and that of insulin independence 80%, with an improvement of glycemic compensation (1,2).

In fact, data from the Islet Transplant Registry (Giessen, Germany) and the Collaborative Islet Transplant Center and the conclusions of a multicenter trial sponsored by the National Institutes of Health, produced to verify the reproducibility of the Edmonton Protocol, report that the comprehensive percentage of success (in terms of insulin independence) is only ~50% (3,4). This means that results are not reproducible to the same extent among different centers (5). Even in the centers with the highest percentage of transplant success, insulin independence is reached in only a few cases by a single transplant; in most cases it is necessary to repeat two or even three infusions (6,7). Therefore, it is clear that the efficacy of a transplant preparation is variable and not always predictable.

The possibility of predicting whether a preparation can work in vivo represents a difficult goal. There are many factors that interfere with islet function in vivo, including quality and number of transplanted islets and their engraftment, pre- and posttransplant immunological conditions (both in terms of autoimmunity and alloimmunity), recipient immunological condition, and toxicity of administered drugs (1,8–10). The prediction of transplant success, however, represents an important objective to be reached. The possibility of predicting in vivo islet graft function should allow the selection of preparations on the basis of their potential success, thus improving overall results. Furthermore, it should result in more consistent, reproducible procedures and permit a proper evaluation of costs and benefits. Altogether, these represent prerequisites for the evolution of islet transplantation from a research procedure to a therapeutic option available to diabetologists. To define the relation between in vivo parameters and transplant function, it is necessary to define evaluation criteria for in vivo functionality.

**Representative Parameters of Engrafted β-Cell Mass** — The function of transplanted islets has often been defined on the basis of C-peptide values, the presence of which in before-transplant C-peptide-negative patients seems to be the best function marker for engrafted islets (6,11–15). C-peptide is also correlated with the reduction of exogenous insulin requirement (12), confirming that it may be representative of engrafted β-cell mass and function. This parameter is, however, influenced by several factors, e.g., inappropriate kidney functionality and increase of insulin resistance, which could overestimate the real engrafted β-cell mass. On the other hand, the exogenous insulin administration, which reduces the insulin and C-peptide secretion through a feedback mechanism, makes transplant functionality seem lower. The normalization of C-peptide on corresponding glycemia values (16) and creatinine should solve this problem.

From 1The Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT), Palermo, Italy, and the 2Diabetes Research Institute, Miller School of Medicine, University of Miami, Miami, Florida.

Address correspondence and reprint requests to Camillo Ricordi, MD, Diabetes Research Institute, Miller School of Medicine, University of Miami, 1450 NW 10 Ave., Miami, FL 33136 or Federico Bertuzzi, MD, ISMETT, via Tricomi 1, Palermo, Italy. E-mail: ricordi@miami.edu or fbertuzzi@ismett.edu.

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**Abbreviations:** IL, interleukin; MCP-1, monocyte chemoattractant protein-1; OCR, oxygen consumption rate.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Considering that the true expectation of recipients of islet transplantation is independence from exogenous insulin administration, the transplant outcome has often been quantified through reaching insulin independence (1,2,17,18) or, when this is impossible, through the decrease of insulin requirement as percentage of initial need or reduction of absolute values of insulin units (12,19). However, this parameter too may not adequately represent the transplanted β-cell mass, especially in the case of multiple infusions, where it is necessary to discriminate the effects of various preparations.

According to interests of diabetologists for the real impact of the transplant on the overall metabolic recipient homeostasis, other parameters should be considered as representative of grafted β-cell mass. Glycemic normalization (basal or postprandial glucose levels), obtained both with and without the administration of exogenous insulin, has recently been proposed as a function parameter (20,21). The choice of this parameter is particularly justified for type 1 diabetic recipients proposed for transplant due to a serious problem of glycemic instability (brittle diabetes).

Records of glycemic levels could also include the frequency of hypoglycemic episodes that have been described as greatly decreased after islet transplantation (20) as a further indicator of the quality of clinical care. As an indicator of glycemic instability, glycosilated hemoglobin (which could not adequately express postprandial glycemic excursions) or the mean amplitude of glycemic excursions (2,6,7,22) have been proposed. The former is commonly used by diabetologists, and the latter is more complex but, including the evaluation of the postprandial glycemic excursion, provides more complete information in a short time. The new parameter proposed by the Edmonton group (β-score) considers different criteria of function including A1C (23). Indeed, the β-score evaluates the following data from points 0 to 2: fasting glycemia (<100, 100–126, >126 mg/dl), A1C (≪6.1, 6.2–6.8, >6.9%), insulin requirement (0, 0.01–0.24, >0.24 units/kg), and stimulated C-peptide (>0.8, 0.3–0.8, <0.3 ng/mL). Although this score must be validated through further studies, it currently appears to be very interesting, as it provides a simple scoring system that encompasses glycemic control, diabetes therapy, and endogenous insulin secretion.

The analyses of glycemic levels post-transplant as markers of graft function should avoid recipient exposure to prolonged hyperglycemia that may be responsible for glucotoxicity. In fact, the maintenance of normoglycemia in recipients early after transplantation is critical for graft function: Hyperglycemia was described to desensitize human islets to further glucose stimuli (24) and might be deleterious for islet survival early after transplantation (25).

At present, there are no univocal data on the need to use other tests to measure graft function, such as oral or intravenous glucose test, arginine test, glucagon test, hyperglycemic clamp, disposition index, or mixed-meal tolerance test. Indeed, data in this regard are contrasting and inconclusive (3,26,27), even though the first step of insulin response to an arginine test or the first and the second step to a test for intravenous charge of glucose have been described to correlate with the functioning β-cell mass in several studies (17,19,28,29). In particular, the first phase and the area under the curve for insulin in response to intravenous glucose administration were closely related to glycemic control (29), but inversely correlated with the posttransplant insulin requirement and proinsulin levels (17), thus representing a useful test for islet graft function follow-up.

It is known that islet transplant function is variable with the passing of time, with a slow and progressive decrease of secretive reserve (17,30,31). Therefore, it is very important to decide at which time of follow-up islet function should be evaluated. It is reported in the literature that the first representative data on the engrafted mass are at 1 week after transplantation (32), although data at 1 month seem more reliable (19). In fact, the definitive evaluation should be carried out at least 1 year after the transplant, due to the clinical importance for patients.

At this point, C-peptide currently represents the best method, although with some limitation, representative of the functioning transplanted β-cell mass in a pretransplant C-peptide–negative patient. The other three parameters, A1C, basal glycemic levels, and frequency of hypoglycemic episodes, should also be considered with the aim of understanding whether islet graft may or may not normalize metabolic control in transplanted patients. All together they are clear, basic, and recognized parameters in the hands of diabetologist to measure the quality of the care for diabetic patients. The values of other methods have been proposed but need to be further assessed or mainly have only academic value.

### IN VITRO PREDICTIVE PARAMETERS OF GRAFT FUNCTION: ISLET PREPARATION QUALITY

#### β-Cell mass

Transplant function is in direct relation to the number of transplanted islets. It has been demonstrated that increasing the number of transplanted islets leads to better in vivo function in terms of C-peptide and decreased insulin requirement, with a higher probability of insulin independence (12,19,28). However, there is a significant dispersion of data concerning this correlation showing that transplanting many islets is not sufficient to obtain good transplant function. The reevaluation of islet number after a brief (at least overnight) culture period provides a better evaluation of the real number of islets available for transplant. Furthermore, the relative decrease in their number may be an indirect parameter of their viability, although it was shown to not be correlated with graft function (33).

The number of islet β-cells rather than the number of islets was proposed to be more representative of the transplanted tissue and hence correlated with grafted tissue (34). A group from Minnesota University in particular has observed that human islet β-cells seem to be a better predictor than the number of human islets of sustained insulin independence in both mouse and human recipients (B. Hering, personal communication).

However, also in this case, a large variability between transplanted islet cell mass and graft function remains (34). This data could be partially explained by a difference in quality of islets. At the moment, there are no absolute criteria to define whether an islet is suitable for transplantation in diabetic patients; thus, there are no means of predicting in vivo function. Islet quality can be defined not only as a level of viability or a three-dimensional structure conservation, but also as secretive reserve in response to glucicid stimulation (35,36).

#### Islet viability

Islet viability is often difficult to assess. Several approaches have been tested to study islet viability: vital probes or stains
on fixed tissue for apoptotic/necrotic/viable cells, oxygen consumption rate, or ATP-to-ADP assessment as measurement of mitochondrial activity.

There are many vital stains available for human islets (37,38), and SYTO-13/ethidium bromide and calcein AM/ethidium homodimer seem more sensitive to islet cell damage than fluorescein diacetate/propidium iodide (37). Also, the assessment of intracellular calcium concentration in human islets was proposed as an indirect marker of islet cell viability (17). Indeed an islet is a cluster of cells where the most internal cells are not easily reached by the colorants designed to stain singular cells; therefore, common optical analysis instruments are not adequate. The possibility of using a confocal to study islet viability has been proposed by some authors (39–41), but none of them have correlated the information collected by this method with in vitro or in vivo islet functional data, yet. Therefore, the use of a confocal to assess islet viability remains interesting, potentially feasible, and requires further study. The dispersion of islets into singular cells should optimize their coloration and study but would be damaging because of further enzymatic digestion and could result in an underestimated integrity. Recently, the method for cell dispersion proposed by the University of Miami, calculates fractional β-cell viability in addition to cellular composition of the final islet cell products (42), but its true standardization has yet to be proven.

Islet cell viability is largely tested on DNA-binding dyes. While these tests identify cells that have lost selective membrane permeability, they do not allow us to recognize apoptotic cells, which do not yet stain with DNA-binding dyes. The simultaneous cell staining with probes for apoptosis and necrosis (i.e., tetramethylrhodamine ethyl ester and 7-aminoactinomycin D, ref. 42) is a practical and complete method for the assessment of cell conditions. Alternatively, the mitochondrial oxygen consumption rate (OCR) has been proposed as a dynamic indicator of cellular viability (43–45). The central hypothesis is that the OCR of cells is directly proportional to viable tissue volume and that its normalization to DNA content is a measure of fractional viability. In addition, the increase of OCR by glucose administration might be considered a parameter representative of the fractional β-cell mass of the analyzed tissue. At this time such tests are still research tools and have not yet been applied as product release criteria in clinical islet transplantation. Finally, the ATP-to-ADP ratio in β-cells has been shown to represent the metabolic condition of islets and therefore to be an indirect marker of islet viability (33,46). In addition, although biochemical markers of islet cell function do not really reflect actual metabolic function of the islet β-cells, especially upon their graft, the increasing evidence of their correlation (in particular ATP-to-ADP ratio) with graft function deserves some attention (33).

Islet integrity
It has been demonstrated that the preservation of islet morphology as a representative parameter of morphostructural integrity is important for a prediction of islet function subsequent to transplantation (12,47). Morphostructural integrity is defined as the right interactions and rapport of three-dimensional architecture among various citotypes into islets (47).

A new method for the assessment of islet quality, recently proposed by the University of Miami, calculates fractional β-cell viability in addition to cellular composition of the final islet cell products (42). It is therefore possible to obtain information concerning not only the characterization of the percentage of necrotic and apoptotic cells, but also of cellular components in the final preparation (β- and α-cells, nonendocrine tissue); selective information on viable, nonapoptotic β-cell mass is also obtainable. This method appears to be correlated not only with graft function in model animals of islet allotransplantation, but also in allograft transplantation in type 1 diabetic patients (48). In particular, the number of equivalent islets × (% β-cell content) × (% nonapoptotic β-cell)/kg of recipient correlates with the reduction rate (>60% or not) after the first infusion or with insulin independence.

Islet preparation composition
The preparation composition for transplants is another variable that could play a role in the success of islet allotransplantation. The contamination of preparations by exocrine and ductal tissue is assessed to define the preparation purity. The contamination by exocrine tissue may be like a mantle around the islets (embedded islets) due to incomplete pancreas digestion or, like free tissue, inefficacy of the purification procedure.

The presence of embedded islets does not seem to interfere with the transplant; moreover, it has been reported that it may, in some way, be a sign of insulin integrity and can protect islets during the initial phase of transplant (49).

The level of purification of a successful islet preparation is controversial. It is believed that the large volume of intraportal distribution of preparation during the transplant permits the infusion of islets and exocrine tissue together without interfering with islet engraftment. In addition, the production of chemo-attractive chemokines, which attract macrophages, seems for the most part to be produced by β-cells rather than exocrine tissue (50). Therefore, also in this case, the presence of exocrine tissue would not amplify the transplant inflammatory response. But partial thrombosis of portal vein branches (51) and, in the long-term, tissue remodeling and morphological alteration into the liver (13,52) have been described as complications of large tissue volume transplantation. In addition, in a univariate analysis, the islet purity level was directly correlated with the C-peptide value of recipients a month after transplant (12), although this was not confirmed by the multivariate analysis or other studies (12,53).

Even more controversial is the role of ductal contaminants in an islet preparation for transplantation. The ductal contaminants of preparations have been observed to be the only variable that correlates with the transplant function over a long period of time (54). This suggests a possible role of ductal cells in the process of β-cell regeneration (55) being able to prevent a functional exhaustion of transplanted islets. On the other hand, it has been observed that ductal cells have a strong proinflammatory connotation, both because they produce tissue factor and CD40 (the former able to activate the coagulation cascade, the latter to contribute in triggering rejection) and because they produce NO and tumor necrosis factor-α, which damage islets (56–59).

Therefore, highly purified islet preparation should be transplanted not only because there are few reports on the harmlessness of contaminating tissue but also because the eventual benefits of contaminant cells have not yet been proven.

Islet insulin secretion
The secretive capacity of isolated islets has long been considered a useful criterion for the selection for transplants expressed as absolute value after glucose stimulation or as secretion index, i.e., the ratio between basal and stimulated insulin levels both in static (static incubation) and dynamic perfusion (35,60). In any case, islets with
a well-preserved morphology, or constituted by viable cells, often present a deficit in their insulin responsiveness to stimuli (61), whereas in vitro lightly responsive islets may be capable of restoring the normoglycemia after transplantation in a diabetic patient (62). In addition, secretory defects should be reversible; therefore, it is not a strong parameter of islet quality. At this stage, the evaluation of insulin responsiveness to glucose in isolated islets is not justified as a control quality test predictive of graft function.

A final observation concerns the proposal to use animal models, such as immune-deficient mice, to evaluate human islet quality (63–65). This consists of transplanting human islets in nude mice as quality control for islets that are designed for transplant. This, in any case, does not permit the selection of preparations for transplant, as results would be obtained too late relative to the time available to keep islet preparations in culture without losing islet function. Moreover, it has been reported in a recent study (64) that the animal model has low sensitivity for the prediction of islet function in humans. Indeed, it has been observed that in animals, human islet function is strictly correlated with purification level and insulin content (63). The role of these parameters could be valid for the animal model only. The lack of purification worsens the oxygenation level and the inflammatory state under the renal capsule of mice but is different in humans because the preparation is dispersed in an ample vascular bed. Furthermore, in mice, the low insulin content could be responsible for an increase of glycemia immediately posttransplant, causing glucose toxicity in the freshly implanted islets, whereas in humans, the insulin treatment maintains a condition of rigorous normoglycemia. To overcome hyperglycemia, in animal models of transplantation a peritransplant maintenance of normoglycemia by exogenous insulin was shown to improve islet graft function (66), thus preserving islet by glucotoxicity. Also, in this case, the animal model remains an unsuitable method to assess islet quality before transplantation.

Finally, among the obstacles to defining a function predictive quality control for isolated islets, there is the lack of standardization of the before-mentioned procedures, rendering a comparison of results obtained from different laboratories difficult.

Among the parameters for an evaluation of the purified islets in order to use them in recipients, it seems that donor age could represent a determinant of quality. This emerges from the experience of the group from the University of Minnesota (2), who include age among the criteria for the selection of preparations, resulting in one of the most successful clinical protocols. It is known that young age is associated with better islet insulin responsiveness to glucose and graft function in transplanted patients (67). Therefore, young age of donors should be considered an additional parameter predictive of islet transplant success.

In all, several are the methods that have been proposed to assess islet and many are the parameters used to describe islet quality. Data on islet cell viability and composition appear to be the most important parameters, not exclusive but complementary to one another.

**IN VITRO PREDICTIVE PARAMETERS OF GRAFT FUNCTION: ISLET PROINFLAMMATORY CONDITION** — It has been demonstrated without a doubt that pancreatic islets can produce several molecules with proinflammatory activity. It has been observed that isolated islets present high mRNA expression of monocyte chemoattractant protein-1 (MCP-1), migration inhibitory factor, vascular endothelial growth factor, tissue factor, and thymosine β-10 (68). In addition, interleukin (IL)-8, IL-1β, IL-5R, and interferon-γ antagonist were expressed in islets that had been cultured for 2 days. IL-2R was expressed in islets that had been cultured for >6 days. The production of these molecules seems to be associated with the donor's clinical conditions, mainly concerning cerebral death, or procedures of isolation, due to the exposition of the tissue to lack of oxygen and free radicals (69–71). The possibility that islets produce proinflammatory factors has stimulated the search for their effects upon the early steps after transplant (engraftment of islets) and upon transplant function in the short term. Graft function depends on several variables, such as donor condition, digestion, purification characteristics, and especially immunosuppression therapy, not only on molecules released by islets. Therefore, any attempts to evaluate the effects of the chemokines/cytokines released by islets on the fate of their transplant in patients should be carefully considered.

Some of these molecules may stimulate islet engraftment (e.g., vascular endothelial growth factor), whereas others have been considered responsible for causing coagulation cascade (tissue factor) or for amplifying the posttransplant inflammatory response (MCP-1). It has been demonstrated that the production of tissue factor activates a coagulation cascade in recipients (72) with negative effects on transplant function in terms of C-peptide 1 week after transplant (32), as well as on hepatocytes, as demonstrated by a correlation between tissue factor in islets and increase of transaminases during the 1st week posttransplant (73). In any case, a truly predictive role of tissue factor for in vivo islet function has yet to be confirmed, due to lack of data on its effect over the medium and long term. Furthermore, data concerning the consequences of MCP-1 production by islets on transplant function are more complex. In islets after kidney recipients were treated with cyclosporine and mycophenolate, a negative correlation has been observed between high MCP-1 level in transplanted islets and clinical success of the transplantation in the 1st year of follow-up (30). This correlation has not yet been confirmed in recipients transplanted using the Edmonton protocol (73). This suggests that the different immunosuppressive therapies might modulate the inflammation pathogenesis with consequent damage to β-cells caused by MCP-1. A further element that renders an understanding this phenomenon difficult is the possibility that recipients may receive more than one pharmacological treatment capable of modulating the inflammatory response. On the other hand, the variability of cellular culture conditions, as well, could interfere with a correct in vitro evaluation of the proinflammatory activity of islets.

Considering the above-mentioned data, although the role of proinflammatory molecules secreted by islets on their engraftment on the hepatic location appears undisputable, it is only possible to consider secretion as a predictive factor of the in vivo function of transplanted islets for MCP-1 and only in the case of recipients who receive the traditional immunosuppressive therapy with cyclosporine and mycophenolate, but studies on these aspects are ongoing.
IN VIVO PREDICTIVE PARAMETERS OF GRAFT FUNCTION — The possibility of transplanting twice in the same recipient permits a repetition of the procedure in case the first infusion of islets proves insufficient for insulin independence. The decision to repeat the transplant should be made as soon as possible in order to avoid rendering the immunosuppressive therapy induction step too long. It is therefore important to define which parameters of in vivo function of transplanted islets could predict the transplant function in the long term in order to indicate the patients who need a new infusion. The benefits of islet transplant in recipients emerge progressively during the early months after transplantation. One of the peculiar characteristics of the recipients with the Edmonton protocol is the rapidity of action of transplanted islets, such that within 2 months it is already possible to reach the maximum reduction of insulin requirement (18). Conversely, the experience of islet after kidney transplant recipients treated with an immunosuppressive therapy with cyclosporine and mycophenolate presents a progressive reduction over the successive months (17). The most extreme example is a patient who became insulin independent 11 months after the transplant (17). In this situation the identification of precocious parameters of insufficient transplant function becomes extremely important in case a further transplant is required.

One month after transplant of islets in kidney recipients, the glycemia at fasting (measured suspending exogenous insulin from the previous evening) and proinsulin values correlated with the insulin requirement checked 1 year after the transplant (17). In islet recipients isolated according to the Edmonton protocol, the values of the area under the insulin curve in response to an endogenous charge of glucose or the acute insulin response to glucose were lower in patients who once again began to experience the need for exogenous insulin (19).

In a more general context, these parameters can be considered part of a metabolic condition that predicts a progressive functional exhaustion and includes a progressive increase of glycemic value and consequently of glycosilated hemoglobin, a reduction of the insulin responsiveness to a glucose charge, and perhaps later to an arginine charge and increase of proinsulin values; these parameters may therefore need to be evaluated together (17,31,32).

Finally, the prediction of graft failure could be obtained by analyses of immunological parameters anti-GAD and anti-IA2 or, better, their increase after transplantation, which has been shown to be associated with poor graft function (74–76).

PREDICTION OF CLINICAL OUTCOME IN ISLET ALLOTRANSPLANTATION: THE “INTEGRATED APPROACH” — Many are the parameters, formula, and methods proposed to predict graft function, and a final decision on the best method has not yet been established. In particular, it appears evident that single in vitro parameters are scarcely representative of the whole preparation and that single recipient values may not be representative of graft function.

The most experienced centers are therefore integrating data from various types of analyses. This method, we call the “integrated approach,” allows more detailed characterization of the quality of the islets available for the transplant and also the new metabolic condition of islet-transplanted patients.

Cell viability and composition appeared to be the most critical information of an islet preparation. This is why some of the most experienced laboratories integrate data on cell viability (apoptosis/necrosis for the University of Miami and fractional viability assessed by ATP-to-DNA and OCR-to-DNA ratios for the University of Minnesota) with those on islet cell composition (β-cell mass, assessed by laser scan cytometer for the University of Miami and by immunostaining for the University of Minnesota). Preliminary results in these laboratories provide evidence that this approach is predictive not only in animal models of transplantation but also in human islet recipients (B. Hering, personal communication). Our point of view is that the analysis of MCP-1 in the case of islet after kidney transplantation should also be evaluated, since islet graft function is lower in the case of transplantation of islets releasing a huge amount of this chemokine.

In recipients, an integrated evaluation of their metabolic conditions appears to better describe the effects of islet transplantation than single parameters such as C-peptide or exogenous insulin requirement. The β-score proposed by the University of Alberta is a clear example of this new approach, though not yet validated. We believe that C-peptide values should be recorded together with fasting glycemia and A1C values and the frequency of hypoglycemic episodes for a complete assessment of recipient medical care quality. Other parameters are informative but not essential at this stage.

PREDICTION OF CLINICAL OUTCOME IN ISLET ALLOTRANSPLANTATION: AN ON-GOING SCIENCE — Only a multicentric study in a large number of patients could be conclusive toward the prediction of clinical outcome in islet allotransplantation. Due to the limited number of transplants feasible per year, the possibility to soon have some final guidelines on this topic is not realistic. In addition, even with the large number of proposals for standardization of these procedures, the experience of the center remains the key factor in determining the success of the islet transplant (5), thus sometimes complicating comparisons between centers. To overcome these problems, the islet centers both in Europe and in North America have organized important meetings to share protocols and experiences specific to the unsolved matter related to in vitro and in vivo islet function characterization. In Europe the workshop of the Network for Islet Centre of Europe (NICE, in its 5th year) and in North America the Human Islet Isolation and Transplantation Techniques Training (HIITT, in its 6th year) are fruitful meetings aimed at updating training researchers on islet transplantation problems as well as favoring discussion and sharing of protocols and ideas between experienced researchers. An additional meeting that shares these aims is the Islet Cell Resource Center’s (ICR) Consortium Annual Islet Workshop (in its 2nd year). These workshops should be an opportunity for the proposal of common protocols aimed at defining guidelines on parameters and strategies to predict clinical outcomes of islet allotransplantation.

CONCLUSIONS — The prediction of graft function in islet allotransplantation remains a challenging objective, although recently proposed parameters could be of assistance in standardizing reproducible procedures for an assessment of islet cell products before transplantation and also in providing useful product.
release criteria for the prediction of post-transplant function.

The prediction of graft function must take into account many variables, from islet mass, viability, function, and proinflammatory conditions to the type of the immunosuppression therapy. In addition to these variables, recipient conditions and treatments may seriously interfere with any attempt to predict in vivo graft function. The identification of integrated with any attempt to predict in vivo graft and treatments may seriously interfere to these variables, recipient conditions immnosuppression therapy. In addition islet mass, viability, function, and proinflammatory profile of islet cell products are considered highly desirable.

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Clinical Implications of the DREAM Study

In response to an article (1) published in 2003 demonstrating that both diet and exercise as well as pioglitazone reduced insulin resistance in upper-body obese, sedentary, nondiabetic individuals, I wrote an editorial (2) discussing whether the treatment of insulin resistance independent of any effect on glycemia could be beneficial for reducing the risk of cardiovascular disease (CVD). At that time, evidence for a beneficial effect rested on surrogate end points and intermediate outcomes of CVD. The final sentence in the editorial was, “If the ongoing clinical trials demonstrate a reduction in hard clinical events, difficult decisions will need to be made.” Although thiazolidinediones (TZDs) continued to lower many of the surrogate risk factors associated with and early manifestations of CVD (e.g., endothelial dysfunction, intima medial thickness of carotid arteries) in subsequent studies, the effect on preventing hard clinical outcomes in the first clinical trial reported was less robust than many had anticipated (3). Now the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) study (4) has been published, raising the question of treating nondiabetic individuals with a TZD, to reduce the risk of developing type 2 diabetes rather than CVD.

The DREAM study (4) randomized over 9,000 individuals with impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG) to receive either 8 mg rosiglitazone or placebo over a median of 3 years. There was an ~60% less chance of those receiving the TZD to develop diabetes compared with those receiving the placebo. For every 1,000 subjects with IFG and/or IGT given rosiglitazone, 144 would be prevented from developing diabetes. There would be, however, four to five excess cases (i.e., over what would have occurred if a TZD had not been given) of heart failure. In addition to the small increase in heart failure, the cost of the TZD (approximately $2,000 per year) must also be factored in when deciding how to incorporate these findings into clinical practice. Thus, for an outlay of $2 million per year or $6 million for 3 years, 144 individuals will avoid diabetes over that period and 856 will ostensibly not have benefited. The latter may not entirely be true because the resultant decrease in insulin resistance may be beneficial by helping to preserve β-cell function (5).

If one were to use a TZD to delay or prevent the development of type 2 diabetes, it would be most efficient to target a population that is at highest risk. Individuals with IGT are certainly at increased risk. In subjects in the control groups of the Finnish Diabetes Prevention Study (6), the Diabetes Prevention Program (7), the STOP-NIDDM trial (8), and the DREAM study (4), 14–26% developed diabetes after 2 years, 21–37% after 3 years, and 23–46% after 4 years. It should be noted that most of the subjects in the Finnish study (6) had first-degree relatives with type 2 diabetes and that the inclusion criteria in the Diabetes Prevention Program (7) and the STOP-NIDDM (8) studies required fasting plasma glucose (FPG) concentrations ≥95 or ≥100 mg/dl, respectively, thus increasing the risk beyond simply IGT alone.

Be that as it may, diagnosing IGT for the purpose of identifying individuals who may benefit from a TZD is problematic. The oral glucose tolerance test (OGTT) is inconvenient and not ordered by many physicians to diagnose diabetes in those felt to be at risk (9). Moreover, nearly 50% of individuals with IGT on an OGTT will have normal glucose tolerance if the OGTT is repeated within 2–6 weeks (10–12). Thus, almost half of these individuals who would seem eligible to receive a TZD might not be at that high of a risk for developing diabetes. This comes from the San Antonio Heart Study, in which the sensitivity of simply using the IGT alone to predict incident diabetes was only 51% with a false-positive rate of 10% (13).

Might measuring an FPG concentration be helpful? Although certainly more convenient than an OGTT, FPG concentrations also suffer from some imprecision. Using the 1997 American Diabetes Association criterion of 110–125 mg/dl to diagnose IFG, one-third of individuals were normal on repeat testing (14). Furthermore, other risk factors greatly influence the risk of an elevated FPG concentration (13). For instance, Table 1 shows the progressive increase in the risk of developing type 2 diabetes from obesity, a positive family history, a low HDL cholesterol, and hypertension. Therefore, other clinical factors must be taken into account in deciding whether an FPG concentration places the individual in a high enough risk category to warrant a TZD.

The distribution of glucose concentrations in most populations is unimodal, which makes the choice of what cut points to use to designate various abnormalities of carbohydrate metabolism somewhat arbitrary (15). The National Diabetes Data Group (NDDG) (16) in 1979 decided that the level to diagnose diabetes should predict the development of its specific complication, i.e., retinopathy. They chose a 2-h value on the OGTT of ≥200 mg/dl based on the results of three studies (17) in which 1,213 subjects were followed for 3–8 years during which period 77 of them developed retinopathy. There was no reason given for defining IGT as 2-h glucose values on the OGTT of 140–199 mg/dl. (One suspects that it was because clinical observations suggested that normal individuals would have glucose concentrations <140 mg/dl 2 h after eating.)

Since A1C data, reflecting 3–4 months of glycemia, were not available at that time, the NDDG’s decision was based on one glycemic point in time. Subsequent studies following over 2,000 diabetic patients for 6–9 years have evaluated the association between A1C levels and the development or progression of diabetic retinopathy (18,19) and nephropathy (20–22). All five studies showed that if the average A1C level was <7%, there was virtually no development or progression of these microvascular complications.

Although A1C assays differ somewhat, it is generally accepted that the normal range for a Diabetes Control and Complications Trial (DCCT) standardized assay is 4–6%. Therefore, following the reasoning of the NDDG of diagnosing diabetes at a glycemic level that is associated with its microvascular complications and utilizing A1C levels, values between 6 and 7% would reflect pre-diabetes. This contention is supported by two studies that have evaluated A1C levels and incident diabetes. One (23) utilized an assay with a normal range of 4.0–6.0%, and followed 1,253 veterans between the ages of 45–64 years for 3 years. The diagnosis of diabetes was made by an FPG ≥126 mg/dl, an A1C level >7.0%, or by self-report. The annual incidence of diabetes for patients with baseline A1C levels
<5.5, 5.6–6.0%, and 6.1–6.9% was 0.8, 2.5, and 7.8%, respectively. After adjusting for baseline A1C levels, only baseline BMI, but not age, race, family history, or hypertension, was associated with an increased risk of developing diabetes. In a French study (24), incident diabetes over 6 years was evaluated after measuring a baseline A1C level in a DCCT standardized assay in 2,820 subjects, aged 30–65 years. Diabetes was defined as an FPG concentration ≥126 mg/dl or treatment with an oral antihyperglycemia drug or insulin. Baseline A1C levels were divided into deciles. The A1C levels in the last three deciles were 5.7, 5.8, and 5.8–7.1%, respectively. The proportion of individuals who developed diabetes in these deciles was 3, 5, and 12%, respectively. After adjustment for age, A1C levels predicted diabetes at 6 years independent of sex, blood pressure, smoking, and physical inactivity. Unlike the prediction of diabetes by FPG concentrations, which is influenced by other risk factors (Table 1), prediction by A1C levels is largely independent of these other risk factors. Thus, society would get a big “bang for the buck” if individuals with A1C levels between 6 and 7% were to receive a TZD.

In the DREAM study, rosiglitazone increased the likelihood of regression to normoglycemia by ~70–80% suggesting that the drug was treating dysglycemia as well as decreasing the frequency of developing diabetes (4). Therefore, if the TZD were given to individuals with A1C levels between 6 and 7%, many of these values would no doubt return to within the normal range. Regardless of whether one believes that some of these individuals, if given an OGTT, might already have diabetes by the rather arbitrary, but apparently sacrosanct, criterion of a 2-h value of ≥200 mg/dl rather than by a glycemic level associated with the microvascular complications, restoring euglycemia can only be beneficial. Of 819 people diagnosed with diabetes by an OGTT, 42% had a normal A1C level and another 26% had a value which corresponded to one between 6 and 7% in a DCCT standardized assay (15).

Based on the positive results of the DREAM study, the time for decisions concerning under what circumstances TZDs should be used in people without documented diabetes is now upon us. They won’t be easy decisions.

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MAYER B. DAVIDSON, MD

From the Clinical Center for Research Excellence, Charles R. Drew University, Los Angeles, California. Address correspondence to Mayer B. Davidson, MD, Clinical Center for Research Excellence, Charles R. Drew University, 1731 East 120th St, Los Angeles, CA 90059. E-mail: mayerdavidson@cdrewu.edu.

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Table 1—Influence of other clinical risk factors on the effect of an increased FPG concentration on incident diabetes

<table>
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<th>FPG (mg/dl)</th>
<th>BMI (kg/m²)</th>
<th>Family history</th>
<th>HDL cholesterol</th>
<th>Blood pressure</th>
<th>10-year incidence rate of diabetes (%)</th>
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<td>Low</td>
<td>High</td>
<td>95</td>
</tr>
</tbody>
</table>

*Calculated from the Cardiometabolic Risk Calculator provided by Michael Stern, MD, based on the data in reference 13.
Surgical Decompression for Diabetic Sensorimotor Polyneuropathy

Diabetic neuropathy remains an unmet medical need. While scientific advances (1,2) have been made in understanding pathophysiology, the impact on the clinical care of patients has been minimal, aside from symptomatic treatments for the pain that may accompany diabetic sensorimotor polyneuropathy (DPN) (3). Improved glucose control is still the main recommendation for the prevention and treatment of DPN, based on studies conducted over 10 years ago. Recently, two evidence-based reviews (4,5) for the treatment of diabetic neuropathy have been published, which form the basis of the subsequent American Diabetes Association position statement (6) on the topic.

Into the apparent void of therapy for DPN, surgical decompression of multiple lower or upper limb nerves is being advocated as the treatment (7). The procedure is being utilized to treat symptomatic and generalized DPN. This approach is based on a series of hypotheses. First, the signs and symptoms of DPN are due to multiple nerve entrapments. In the lower limb, foot numbness is ascribed to “entrapment” of the peroneal nerve at both the fibular head and the anterior tarsal tunnel, the tibial nerve in the tarsal tunnel, and the sural nerve in the distal posterior calf. In the upper limb, hand numbness is ascribed to entrapment of the ulnar nerve at both the wrist and elbow, the radial nerve in the radial tunnel, and the median nerve at the wrist. Second, these entrapments can be diagnosed by a trained examiner whose sole tool is the Tinel sign. Third, surgical “release” of these nerves will correct DPN by decompressing the “compressed” nerves. Fourth, special surgical training is needed to be able to identify these patients and operate on them. This series of hypotheses has spawned an entire industry.

There is much that is wrong with this thinking. First, the distal neuropathy that characterizes DPN is due to progressive distal axonal loss (8–10). The proposed pathophysiological mechanism of entrapment cannot explain sensory or motor symptoms or signs above the anatomic levels of the “entrapped” nerves. Despite this, patients have undergone these operations with neuropathy above the level of the foot and hand. Additionally, the actual frequency of peripheral nerve entrapment in diabetic individuals is small.

While some patients with DPN have superimposed nerve entrapment syndromes, these are the well-known sites of classic entrapments: the median nerve at the wrist causing classic carpal tunnel syndrome, the ulnar nerve at the elbow causing ulnar neuropathy at the elbow, and the peroneal nerve at the fibular head causing foot drop. Before this recent “epidemic” of nerve entrapments, entrapments at the other postulated sites have been considered rare or even nonexistent (11–13).

Second, the Tinel sign (14), which was originally described in the setting of nerve regeneration and not entrapment, is poorly standardized and lacks sensitivity and specificity. The proponents of the subjective Tinel sign ignore the proven value of electrodiagnostic studies, an objective test of nerve function.

Third, the American Academy of Neurology (15) used an evidence-based criteria review for decompression surgery for generalized DPN. Using standard procedures to assess evidence, there was only one prospective trial. The utility of surgical decompression for symptomatic diabetic neuropathy received a grade IV rating; i.e., based on evidence from uncontrolled studies, case reports, or expert opinion. It was assigned a U grading, which is defined as “data inadequate or conflicting given current knowledge, treatment is unproven.” Given that conclusion, we believe that the treatment cannot be recommended at this point in time. A report on this topic by the Cochrane Collaboration will shortly follow.

In the unblinded series of these procedures, pain relief as assessed by the operating surgeon occurred in 80–92% of patients, some even occurring on the operating table while recovering from the anesthetic. Even more impressive are patients reporting bilateral improvement from unilateral procedures or patients with numbness or pain beyond the anatomic distribution of the released nerves who improve after these procedures. If only symptoms are being reported, the results may be no better than a number of other noninvasive and less expensive interventions (15–18), all of which have been claimed to achieve symptomatic short-term improvement.

Fourth, numerous centers have sprung up around the U.S. and the world promoting their specially trained surgeons and touting the benefits of these procedures (7). One can only guess the medical costs of these unproven procedures.

Unfortunately, medicine has been here before. For >50 years, surgical procedures have been advocated for all sorts of diseases. In the 1950s, there were a number of procedures for angina with many others to follow (19). While there are many explanations for the results from these types of surgeries, most important are the placebo effect and the natural history of the disorder. Only well-controlled, randomized, double-masked, sham-procedure, controlled clinical trials will allow us to know whether these surgeries are safe and effective for this indication—the same standard that any drug for DPN would have to meet.

What are we to do now? First, we believe the findings of the American Academy of Neurology’s evidence-based review (15) should be strong evidence that the procedures should not be considered care but, rather, subjected to further research until proven beneficial. Second, we strongly support trials to determine whether these surgical procedures are beneficial. At this point, pilot trials should be conducted to see whether there is reason to mount large phase 3 studies. The Centers for Medicare and Medicaid Services (CMS), which supported the recent Lung Volume Reduction Surgery trial (20), is in the best position to support such trials and should have a great interest in doing so, given the widespread application of these unproven surgical procedures among Medicare patients.

Third, we support further research into the causes and treatment of DPN, an unmet medical need. In conclusion, until such time as definitive randomized trials are conducted and the supporting evidence is stronger, surgical decompression should not be recommended for patients with diabetic sensorimotor polyneuropathy.
References
Prevention of Cardiovascular Disease

ZACHARY T. BLOOMGARDEN, MD

Perspectives on the News commentaries are now part of a new, free monthly CME activity. The Mount Sinai School of Medicine, New York, New York, is designating this activity for 2.0 AMA PRA Category 1 credits. If you wish to participate, review this article and visit www.diabetes.procampus.net to complete a posttest and receive a certificate. The Mount Sinai School of Medicine is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians.

This is the fifth in a series of articles on presentations at the American Diabetes Association’s 66th Scientific Sessions, Washington, DC, 9–13 June 2006, reviewing aspects of the interrelationships between cardiovascular disease (CVD) and diabetes, returning to the theme of obesity and further addressing benefits and adverse consequences of peroxisome proliferator–activated receptor (PPAR)α and -γ agonists.

Robert H. Eckel (Denver, CO) gave the Edwin Bierman Award Lecture on the topic of prevention of CVD, beginning by remembering training with Bierman, whose career focused on the relationship of diabetes and CVD. Bierman’s “rules” for trainees comprised “the principles of academic medicine at its best.” He recommended a simple ethical test, never performing an experiment on others that one would not have performed on oneself. He told his fellows to always plot data before statistical analysis and to consider adjustment for basal values when a biological response was proportional to the basal. He encouraged trainees to choose both innovative projects and less novel but surer projects. Finally, he stressed the intellectual challenge of research, with every experimental question producing more questions.

Eckel reviewed projections that obesity would dramatically increase over the next two decades (1), leading to increasing rates of development of diabetes and, as a consequence, CVD. It is relatively straightforward to make recommendations to reduce these outcomes: not smoking cigarettes, increasing physical activity, and following a balanced diet—not with “the concept of good foods and bad foods” but rather following “overall healthy eating patterns”—including fruits, vegetables, and whole grains and limiting cholesterol, saturated fats, and trans fats (vegetable oils processed by hydrogenation to increase solidity and shelf-life), as well as substituting unsaturated oils and fish, maintaining normal blood pressure by limiting salt and alcohol, and eating fruits, vegetables, and low-fat dairy products. Eckel pointed out that these are evidence-based recommendations from surveys, rather than from randomized controlled clinical trials, and reviewed some of the supporting data. In the prospective Breast Cancer Detection Demonstration Project, 42,254 women completed a food frequency questionnaire and were followed for a median of 5.6 years, with those in the top quartile of recommended diet having one-third lower all-cause mortality than those in the lowest quartile, and similar benefits were seen in reduced rates of cancer and of CVD (2).

These findings were extended by the Nurses’ Health Study of 84,129 women with 14 years of follow-up, showing that those with normal weight, having at least a one-half portion of an alcoholic beverage daily, with at least a half hour of physical activity daily, and following a healthy diet—although comprising only 3% of the studied population—had >80% reduction in coronary events (3).

Physical activity, Eckel pointed out, has anti-atherosclerotic effects on lipids, blood pressure, adiposity, insulin sensitivity, inflammation, myocardial oxygen demand, endothelial dysfunction, arrhythmia, platelet adhesiveness, and fibrinolysis. A study of 19,125 men, between the ages of 20 and 79 years followed for more than a decade, compared those whose lipid levels were at the National Cholesterol Education Program Adult Treatment Panel (ATP) III goal with 4,573 subjects who were classified as requiring pharmacologic lipid-lowering treatment, who had a sixfold increase in CVD, and with 3,420 subjects in the ATP III lifestyle intervention group, with a doubling of CVD. In each group, those who were unfit based on maximal exercise time had at least a doubling of mortality (4), leading Eckel to conclude “being fit ultimately trumps a lot of other factors.” In women, meta-analysis similarly suggests benefit both of vigorous exercise and of walking (5).

The prevention and treatment of obesity should be straightforward, Eckel suggested. “To lose weight you need to eat less than you burn,” although the inaccuracy of self-reported calorie intake has obscured this simple relationship. Relatively modest weight reduction is needed, with studies suggesting that blood pressure improves with loss of 5% of body weight, glucose tolerance with 5–10%, lipids with 10%, left ventricular function with 5%, and obstructive sleep apnea with 5%, although benefits may be particularly evident during active weight reduction rather than when the patient is weight stable at a lower level. There currently is no evidence that weight reduction prevents CVD, but there is evidence of reduced diabetes risk in individuals with impaired glucose tolerance in the Diabetes Prevention Program (6) and in the Finnish Diabetes Prevention Study (7). An important question is how long weight loss will last following intervention, although the Finnish Study recently reported that benefits were sustained 3 years after completion of the 4-year active lifestyle modification program (8). Considering obesity not to be a disease, but rather an evolved survival mechanism addressing circumstances of food lack, Eckel pointed...
out that limited data suggests only 6% of individuals who lose weight maintain this over 15 years of follow-up (9). The Look AHEAD (Action for HEalth in Diabetes) Study will assess 11.5 years’ effects of a 4-year weight loss program in 5,000 overweight and obese individuals with type 2 diabetes (10).

Eckel suggested that prevention of obesity and additional weight gain and more aggressive treatment of risk factors must be emphasized and that there is “no better place to begin than with children.” There are 8–9 million overweight children in the U.S. The American Heart Association Alliance for a Healthier Generation is working to limit portion sizes and sodas for high schools, to advertise to children healthy lifestyle approaches, and “to get kids more engaged and more involved in their own health.” Physicians need to be engaged in assessing their patients’ lifestyles.

Addressing the identification of individuals at risk of weight gain, Eckel reviewed his study of the strong correlation between more positive carbohydrate balance on a high-carbohydrate diet and a lesser degree of increase in fat mass over 4 years (11), suggesting that carbohydrate balance may in some fashion feed back to the brain. Eckel concluded that lifestyle is of great importance for CVD prevention. When asked about factors determining who loses weight and maintains weight loss, Eckel answered, “Don’t be a pessimist, be a realist.” Those who do maintain weight loss follow a healthy diet, he stated, noting that “overall, the dietary recommendations are pretty clear,” as well as exercising at least 60 min daily. He pointed out, however, that “reimbursement for those types of efforts [in encouraging lifestyle practice change] is not there.” He also suggested the need to consider “intervening earlier with blood pressure, and lipids, and diabetes.”

Many studies presented at the meeting addressed themes of Eckel’s presentation. Analyzing the treatment of risk factors among individuals with diabetes, Piatt and Zgibor (abstract 9) compared 59 male and 59 female diabetic patients, finding that 90 and 97%, respectively, had hypertension and 81 and 86% dyslipidemia, with similar frequencies of treatment, but that approximately twice as many male as female subjects achieved blood pressure $\leq 130/85$ mmHg and that approximately three times as many achieved non-HDL cholesterol $\leq 130$ mg/dl (abstract numbers refer to the American Diabetes Association Scientific Sessions, Diabetes 55 [Suppl. 1], 2006). Only 25% of male and 31% of female subjects were treated with aspirin in this population. Ngo-Metzger et al. (abstract 1,162) reported a larger study of 22,510 type 2 diabetic individuals, finding that A1C and insulin use patterns were similar across sex but that 68% of male and 60% of female subjects had LDL cholesterol $<130$ mg/dl, suggesting that there may be sex differences in quality of diabetes care.

**Metabolic syndrome/pre-diabetes**

Metabolic syndrome has been an important, although somewhat controversial, concept in the assessment of CVD risk. Saely et al. (abstract 692) followed 241 women for 4 years following coronary angiography. Metabolic syndrome was present in 84 subjects based on the ATP III definition and in 115 based on the International Diabetes Federation 2005 definition, with only moderate concordance between the definitions; the former was associated with a significant 2.1-fold increase, while the latter showed an insignificant 21% increase in vascular events. Koehler et al. (abstract 6) studied 4,020 type 2 diabetic individuals aged 35–80 years to compare the predictive power of the diagnosis of metabolic syndrome, present in 74%, with that of its component traits for CVD outcomes, which had occurred in 16%. Metabolic syndrome was predictive of CVD, but stepwise regression analysis of the component traits showed that hypertension was most strongly predictive, increasing the likelihood of CVD 4.2- and 7.7-fold in men and women, respectively, with the various metabolic syndrome components quite heterogeneous in strength of association with CVD. Other insulin resistance–associated characteristics may also play a role in CVD. Ioachimescu et al. (abstract 914) reported that each 1 mg/dl higher serum uric acid was associated with a 25% greater likelihood of mortality among 535 type 2 diabetic individuals, adjusted for age, sex, smoking, alcohol use, diuretic use, weight, BMI, waist circumference, blood pressure, CVD, glomerular filtration rate, LDL cholesterol, HDL cholesterol, triglycerides, A1C, and fasting glucose, leading to the suggestion that intervention studies might be appropriate in determining whether uric acid is a potential therapeutic target rather than solely a marker of risk.

Misra et al. (abstract 947) performed a randomized, population-based study of 1,038 Asian-Indian immigrants in seven U.S. sites, finding that 18% had diabetes, an additional 31% had impaired fasting glucose (IFG), and 32% had ATP III–defined metabolic syndrome, confirming this to be a high-risk ethnic group. Chowdhury et al. (abstract 888) analyzed data from the 1999–2002 National Health and Nutrition Examination Survey, finding that, of 3,030 participants aged 20–75 years without diagnosed diabetes, the 95% with IFG (100–125 mg/dl) compared with those having fasting glucose $<100$ mg/dl were aged 49 vs. 41 years, female sex in 38 vs. 36%, blood pressure $\geq 130/85$ mmHg or antihypertensive medication use in 48 vs. 31%, waist circumference $>102/88$ cm (male/female) in 56 vs. 37%, obesity in 38 vs. 23%, HDL cholesterol $<40/50$ mg/dl (male/female) in 44 vs. 33%, LDL cholesterol $\geq 130$ mg/dl in 48 vs. 37%, and triglycerides $\geq 150$ mg/dl in 44 vs. 26%, suggesting the importance of IFG as a marker of high prevalence of modifiable CVD risk factors. The association between the risks of developing diabetes and CVD may be related to insulin deficiency as well as to insulin resistance. Curtis et al. (abstract 241) reviewed findings of the National Heart, Lung, and Blood Institute Cardiovascular Health Study of 4,553 type 2 diabetic individuals followed for 6 years, calculating from fasting insulin and glucose values the homeostasis model assessment (HOMA)-B and HOMA-S indexes of $\beta$-cell function and insulin sensitivity, respectively. Controlling for HOMA-S, for each 20% decrease in HOMA-B, the likelihood of developing coronary heart disease (CHD) and mortality increased 9 and 10%, respectively. Yeung et al. (abstract 84) analyzed diabetes risk among 11,297 participants in the Atherosclerosis Risk in Communities Study, finding that among those with a parental history of diabetes, the highest and intermediate tertiles of familial risk of CHD were associated with 76 and 28% increased risks of developing type 2 diabetes, respectively. With a negative parental history of diabetes history, there was no significant association between familial coronary disease risk and diabetes development.

Levitzky et al. (abstract 1) studied the relationship between CVD risk and fasting glucose, adjusting for traditional CVD risk factors, in 4,058 Framingham offspring, with mean age 49 years. A total of 53% were women, with 78 CHD and 128 CVD events. There was no increased risk
at glucose levels 100–109 mg/dl, while CHD and CVD increased 2.5- and 2.1-fold, respectively, at glucose 110–125 mg/dl, comparable with the increases in risk for glucose ≥126 mg/dl. Among men, 213 CHD and 295 CVD events occurred, without increases in adjusted risk among those in either IFG category. Of course, the "adjustment" performed for traditional CVD risk factors makes the authors' conclusion that IFG is not a risk factor somewhat problematic. Suruliram et al. (abstract 702) found that, among 106 consecutive individuals hospitalized with acute coronary syndrome with a mean age of 67 years without known diabetes or glucose intolerance and with creatinine <1.7 mg/dl, an oral glucose tolerance test on day 7 showed 26% with diabetes, only 18% of whom would be identified from the fasting glucose. A total of 36% had impaired glucose tolerance, and 3% had IFG. Abnormal glucose tolerance was associated with elevated troponin T, and those with diabetes were more likely to have dyslipidemia, suggesting the importance of glycaemia assessment. In an interesting extension of the association between pre-diabetes and diabetes complications, Jia et al. (abstract 916) reported prevalences of retinopathy among 260 diabetic and 169 impaired glucose tolerance individuals in Shanghai, China, diagnosed using digital fundus photography, of 21 and 7%, respectively, and of urine albumin-to-creatinine ratio ≥30 μg/mg in 22 and 11%; the latter associated with abdominal obesity, systolic blood pressure, and history of CVD, suggesting that microvascular, as well as macrovascular, complications may precede the diagnosis of diabetes.

Several studies presented at the meeting addressed the topic of whether individuals with diabetes are resistant to the therapeutic effect of aspirin. Pitocco et al. (abstract 311) compared platelet sensitivity in 42 type 2 diabetic versus 53 nondiabetic individuals receiving aspirin, showing 16 vs. 7% maximal platelet aggregation in response to arachidonic acid with a similarly enhanced response to collagen, although not to ADP. Collagen-induced platelet thromboxane A2 was 8.3-fold higher in samples from the diabetic individuals and was decreased to levels of the nondiabetic group with the administration of aspirin in vitro, with administration of a cytoxygenase-2 further reducing thromboxane A2 in the diabetic samples, and with cytoxygenase-2 detectable in platelets from all the diabetic but just two of the nondiabetic patients, suggesting that orally administered aspirin does not effectively decrease platelet function in individuals with type 2 diabetes. Cohen et al. (abstract 889) measured platelet function with the PFA-100 platelet function analyzer in 48 type 2 diabetic individuals who had taken aspirin during the prior 24 h, finding aspirin resistance in 23% of individuals, associated with higher A1C, higher BMI, and higher depression scores, which they speculate might reflect the role of abnormal serotonin uptake both in abnormality of platelet function and in mood disorders.

**Thiazolidinediones and dyslipidemia**

Ronald Goldberg (Miami, FL) discussed the question of whether thiazolidinediones (TZDs) should be used for lipid management. In the Strong Heart Study of >4,000 American Indians, there was a strong relationship between the number of risk factors present at baseline and the 10-year CVD risk. In the UK Prospective Diabetes Study (UKPDS), when newly diagnosed type 2 diabetic individuals were compared with nondiabetic individuals, there was low HDL (39 vs. 43 mg/dl in men and 43 vs. 55 mg/dl in women) and high triglyceride (159 vs. 103 and 159 vs. 95, respectively). These abnormalities develop in the setting of insulin resistance, with consequent increase in circulating free fatty acids (FFAs), promoting hepatic synthesis of large VLDL particles having increased apolipoprotein C-III, increasing the plasma triglyceride pool. This in turn enhances the rate of exchange from triglyceride- to cholesterol-rich particles via cholesterol ester transfer protein (CETP), leading to triglyceride-rich LDL particles, which are better substrates for hepatic lipase leading to accumulation of small dense LDL particles, with hepatic lipase also upregulated by insulin resistance. A similar pathway leads to accumulation of small dense HDL particles via CETP.

Insulin sensitizers may then be particularly beneficial. Studies using NMR lipoprotein subclass analysis comparing insulin resistant and insulin sensitive individuals without diabetes, as well as type 2 diabetic individuals, show that insulin resistance leads to decreased LDL and HDL size with an increased number of LDL particles, with increased VLDL size and particle numbers, which is associated with hypertriglyceridemia (12). In the UKPDS, LDL and HDL cholesterol were the first- and second-ranked CVD predictors, respectively, while triglyceride levels were not significantly associated with CVD in multivariate analysis. Goldberg reviewed ATP III and American Diabetes Association (ADA) recommendations. ATP III suggests that all diabetic individuals should achieve LDL cholesterol <100 mg/dl, with the goal for higher-risk individuals that LDL cholesterol be <70 mg/dl, while the ADA recommends at least a 30–40% LDL cholesterol reduction for individuals with CVD. The ATP III recommends triglyceride lowering to be the first priority for levels >500 mg/dl, with fibrates recommended as first-line drugs, while for triglyceride levels, 200–500 mg/dl the non-HDL cholesterol becomes the goal at levels <130 or 100 mg/dl, depending on the degree of risk, and the ATP III recommends that for low levels of HDL cholesterol, consideration be given to use of niacin or fibrates. The ADA guidelines suggest a triglyceride goal <150 mg/dl, recommending that measures be taken to raise HDL cholesterol to levels >40 and 50 mg/dl in men and women, respectively, and suggests using apolipoprotein B rather than cholesterol as a risk predictor.

The National Health and Nutrition Examination Survey 1999–2002 data show that 70% of individuals with diabetes have LDL cholesterol >100 mg/dl and that 30–40% have triglyceride levels >200 mg/dl, while 70% have HDL below average and many well below average (13). Thus, there is need to more widely use the powerful LDL-lowering agents currently available, but Goldberg commented that "perhaps most important is the challenge raised by low HDL," with "urgent need for the development of HDL-raising drugs." In this context, he reviewed potential lipid benefits of the TZDs. With the first available agent, troglitazone, LDL cholesterol increased up to 15%, without change in apolipoprotein B levels, suggesting increase in LDL size (14). Meta-analyses suggest 15–20 vs. 0–5 mg/dl increase in LDL cholesterol with rosiglitazone versus pioglitazone (15,16), leading to Goldberg's "direct head-to-head comparison," in which 45 mg pioglitazone daily versus rosiglitazone decreases in A1C and fasting insulin levels and significantly increased LDL size, with significant increases in large and decreases in small LDL particle masses, although the
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quantitative effects differed between the two agents. Goldberg pointed out that similar shifts in particle sizes are seen with fibrates.

Little change in triglyceride level was seen with troglitazone or, in meta-analysis, with rosiglitazone, while a decrease in triglyceride was seen with pioglitazone. In the head-to-head study, triglycerides decreased 12% with pioglitazone, while increasing 14% with rosiglitazone, with VLDL particle concentration not changing versus increasing. HDL cholesterol showed a trend to increase with troglitazone and increased with both pioglitazone and rosiglitazone in the meta-analysis and in the head-to-head study, with particular effect on large HDL particles, an effect opposite of that reported in the Veterans Affairs High-Density Lipoprotein Intervention Trial (VAHIT) with gemfibrozil, which increased the number of small particles (18). Goldberg noted that non-diabetic individuals with metabolic syndrome treated with pioglitazone show increased HDL cholesterol but that it is not known whether this occurs with rosiglitazone.

Thus, Goldberg summarized, TZDs increase LDL cholesterol levels, noting that there may be a relationship between this phenomenon and higher baseline triglycerides, as well as differences between specific agents, and that increases in LDL particle size may offset the apparent adverse effect. He further noted that TZD effects on triglycerides “are modest” and unlikely to have major impact on CVD and that the rise in HDL of 5–15% represents redistribution from small to larger particle sizes. The mechanisms of these changes are uncertain, with the reduction in triglyceride potentially decreasing CETP effects on HDL and LDL particles, enhancing the efflux of cholesterol, or with TZDs perhaps reducing hepatic lipase, another potentially cardioprotective action. There is no evidence that TZDs influence the effects of statins, with studies of rosiglitazone plus atorvastatin suggesting that the benefit of statins is preserved with this agent (19).

“Perhaps the HDL story is the more compelling one,” Goldberg noted, pointing out that although its mechanism is not understood, there is a great deal of information suggesting this to be an important CVD risk factor. In the VAHIT study, the rise in HDL cholesterol with gemfibrozil was the only significant predictor of CVD prevention, despite the quantitatively greater fall in triglycerides, but the increase in HDL cholesterol itself explained only 20% of the gemfibrozil benefit. Goldberg suggested that nonlipid vascular protective effects occur in a similar fashion with TZDs. TZDs may then be reasonable agents for hyperglycemic individuals with reduced HDL cholesterol, although one needs randomized controlled trial evidence with demonstration of vascular protective effects rather than simply lipid changing effects.

Diabetic dyslipidemia

Mechanisms underlying the lipid abnormalities in type 2 diabetes were studied by a number of investigators at the ADA meeting. Tay et al. (abstract 282) administered intravenous lipids with and subsequently without glucose to seven nondiabetic individuals for 2 days, leading to doubling in FFAs. Euglycemic clamp insulin sensitivity decreased, with increases in triglycerides from 114 to 172 mg/dl and (with glucose) to 243 mg/dl, with larger VLDL particle size, decreases in HDL concentration and particle size, increases in blood pressure from 109 to 119 mmHg and 123 mmHg (diastolic), with C-reactive protein increasing 2- and 1.6-fold, respectively, suggesting that elevations in FFAs to levels seen in obese individuals reproduces abnormalities seen in metabolic syndrome. Smiley et al. (abstract 283) administered intravenous lipid plus heparin to 12 normotensive diabetic individuals for 48 h, showing a 13/6 mmHg elevation in blood pressure and 14% reduction in brachial artery flow-mediated dilatation. After a 6-week period of treatment with 8 mg rosiglitazone daily, although FFA levels increased to a similar degree, the lipid plus heparin infusion failed to increase blood pressure or flow-mediated dilatation. These authors (abstract 699) studied 19 normotensive obese diabetic and 13 normotensive obese non-diabetic individuals with the lipid infusion protocol, showing that along with a similar increase in blood pressure, serum insulin and C-peptide levels increased more than threefold, with tripling of C-reactive protein and doubling of tumor necrosis factor-α levels, suggesting that FFAs reduce insulin sensitivity, increase blood pressure, have proinflammatory effects, and cause endothelial dysfunction, while TZDs potentially prevent these adverse effects. De Serna et al. (abstract 476) studied 12 type 2 diabetic individuals with placebo and, following 1 week of 120 mg nateglinide three times daily, 5 mg glyburide twice daily, and 5 mg glipizide extended release (XL) daily, finding relatively abrupt increases in plasma FFAs from 3 through 6 h after a standardized lunch meal, with the greatest increase in the nateglinide group, suggesting a mechanism of postprandial worsening in insulin resistance.

Moon et al. (abstract 263) studied the association of insulin resistance with hepatic steatosis, despite increased hepatic triglyceride secretion, showing evidence that in the liver, insulin promotes lipogenesis with relative decrease in apolipoprotein B secretion in an animal model, potentially contributing to this phenomenon. Dziurko et al. (abstract 264) reported that intestinal apolipoprotein B48 particle production was almost doubled in nondiabetic individuals with hyperinsulinemia, suggesting that gut, as well as hepatic lipid handling, must be considered in assessing the lipid abnormalities of insulin resistance. Baysen et al. (abstract 266) studied 12 type 2 diabetic individuals receiving metformin and sulfonylurea with mean A1C 8.2% after a 20-week course of treatment with pioglitazone versus rosiglitazone, showing similar 1.1–1.3% fall in A1C, with triglyceride decreasing from 218 by 18 mg/dl versus increasing from 219 by 34 mg/dl. A significant reduction in de novo lipogenesis, another potential mechanism of dyslipidemia, was seen only in the pioglitazone group, with neither agent changing VLDL triglyceride production or clearance.

Porchia-Baldereali et al. (abstract 4) studied the CETP TaqIB single nucleotide polymorphism in 3,115 type 2 diabetic individuals with high cardiovascular risk, based on urinary albumin ≥20 mg/l, with a mean age of 65 years, followed for 4 years. The HDL concentration was 1.25, 1.33, and 1.39 mmol/l/l in B1 homozygotes, B1:B2 heterozygotes, and B2 homozygotes, respectively. Adjusting for age, sex, and BMI, sudden death occurred in 7% of B1 homozygotes but in 4.8% of those with one or two B2 alleles, an association not explained by further adjustment for HDL cholesterol. Kretowski et al. (abstracts 669) noted the association of apolipoprotein A-IV with coronary artery disease in individuals with type 2 diabetes and studied the Gln360His polymorphism in 484 type 1 diabetic individuals and 501 nondiabetic control subjects, finding the histidine allele to be associated with 34% progression of coronary artery calcification over a mean 2.6 years in the diabetic but not in the nondiabetic
individuals. Alaupovic et al. (abstract 855) studied the relationship between the ATP III metabolic syndrome criteria and lipoproteins among 1,134 adult and 545 adolescent Oklahoma Cherokee individuals without diabetes, finding a particularly strong association with apolipoprotein C-III levels, suggesting that its effects on particle clearance and arterial wall binding may be relevant to insulin resistance and atherosclerosis progression in this population.

**PROactive Trial**

The concepts developed by Goldberg were extended in a symposium on the potential applicability of PPAR agonists to CVD. Charles Burant (Ann Arbor, MI) discussed potential effects of PPARγ agonists on cardiovascular events, giving his interpretations of the PROactive (Prospective pioglitAzone Clinical Trial in maCroVascular Events) Trial. Clearly, glycemic and lipid control can impact cardiovascular risk. The PPARγ agents have effects on triglycerides, LDL size, and inflammatory mediators, although worsening obesity is a potential adverse effect. In the PROactive Trial, diabetic individuals with a history of macrovascular disease were treated under International Diabetes Federation care guidelines (A1C goal of 6.5%, with LDL lowering, aspirin, and good blood pressure control) with or without pioglitazone. The primary end point was a composite of mortality, nonfatal myocardial infarction, leg revascularization, major leg amputation, and a number of additional adverse outcomes. A forced titration of pioglitazone to 45 mg daily led to >95% of patients reaching the highest dose. A total of 5,238 individuals were enrolled, with only 2 lost to follow-up. There were 177 vs. 186 deaths, without significant difference between the pioglitazone and control groups in the primary composite end point, although Burant noted that event rates only diverged during the latter half of the 34.5-month mean observation period. The principle secondary end point was the first occurrence of all-cause mortality, nonfatal myocardial infarction, and stroke, and this did show a significant 2.1% decrease with pioglitazone. An important observation complicating interpretation of the study was the increase in leg revascularization seen with pioglitazone treatment. Burant noted that loop diuretic use increased with pioglitazone treatment and that the rate of leg revascularization was particularly great in pioglitazone-treated individuals during the 1st year of the study, suggesting that lower-extremity edema might have led clinicians to interpret leg symptoms as reflecting ischemia. A similar line of reasoning leads to the concept that some of the excess in diagnosis of heart failure may also be due to the association of TZDs with edema.

Multivariate analysis of the primary end point showed that older individuals and those who had a stroke had higher risk, while those with statin use, a prior cardiac event, or allocated to pioglitazone had decreased events. There has been some question as to whether other treatments affected outcomes, with individuals not receiving statins or not receiving a β-blocker having a trend to increase in the protective effect of pioglitazone on the primary end point. A secondary analysis of individuals who had had a prior myocardial infarction showed that recurrent myocardial infarction decreased with pioglitazone treatment, although there has been controversy as to whether it was valid to perform this analysis given the negative primary outcome.

Burant noted that much of the controversy over the study has revolved around what was in essence an arbitrary decision on the part of the investigators as to the primary end point. Event rates were somewhat lower than expected, thus a larger study might have been better. Alternatively, the study might have more clearly shown the effect of pioglitazone with longer treatment exposure. More optimal risk factor reduction could have been pursued, particularly given the somewhat lower A1C, lipid levels, and blood pressure in the pioglitazone group. He concluded that, on balance, the high dose of pioglitazone was well tolerated and that the study showed benefits of TZDs in achieving target glycemic control. “It is not certain whether TZDs can prevent CVD,” he stated, “beyond the effect” on metabolic parameters.

**FIELD Study**

Lawrence Leiter (Toronto, Canada) discussed PPARα and cardiovascular events in the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) and other studies. Ligands of PPARα include fatty acids and eicosanoids, as well as the fibrates. PPARα agonists increase apolipoprotein A-I and A-II, lipoprotein lipase, and scavenger receptor and reverse cholesterol transport, lower triglycerides, and exhibit pleiotropic effects, including evidence of reduction in inflammation. The fibrates include clofibrate, now rarely used, gemfibrozil, most widely used in the U.S., fenofibrate, and bezafibrate. In the Helsinki Heart Study of >4,000 individuals without known heart disease treated with gemfibrozil versus placebo, a 34% reduction was found in CVD risk (20), the VAHIT individuals with CVD and isolated low HDL showed a 22% risk reduction with gemfibrozil, and the Beza-fibrate Infarction Program showed a nonsignificant 9% reduction in CVD (21). The Diabetes Atherosclerosis Intervention Study of individuals with diabetes and preexisting coronary artery disease treated with fenofibrate versus placebo showed angiographic benefit, with a nonsignificant 24% decrease in clinical outcomes (22).

Diabetes and high triglyceride or low HDL-to-LDL ratio in the Helsinki Heart Study were associated with particularly great reduction in CVD. In the Beza-fibrate Infarction Program, those with triglyceride levels >200 mg/dl had significant risk reduction, as did individuals with metabolic syndrome. In the VAHIT, comparison of risk reduction among individuals with versus without diabetes, and in nondiabetic individuals with versus without hyperinsulinemia, suggest that most of the benefit observed with gemfibrozil was seen in the insulin-resistant subgroup, members of which appear then to particularly benefit from use of these agents.

In the FIELD Study, 9,795 diabetic individuals not receiving lipid treatment were randomized to fenofibrate versus placebo, with coronary death/nonfatal myocardial infarction, total CVD events, and microvascular events studied (23). Two-thirds were male, 22% had prior known CVD, and only 37% had dyslipidemia as defined when the study was initiated. Statins were frequently added during the course of the study, particularly in the placebo group. A nonsignificant 11% decrease in coronary events and a nonsignificant 11% increase in coronary mortality were found. Enrolled patients without prior CVD had 19% risk reduction, while those with prior CVD did not show benefits. Rhabdomyolysis rates were low in both groups, although safety data for individuals receiving a statin with fenofibrate have not yet been reported. There is evidence of decrease in microvascular disease, with reduced requirement for laser therapy for retinopathy and a lower rate of progression of albuminuria with fenofibrate, an observation also made in the Diabetes Atherosclerosis Intervention Study (24), although fenofi-
brate was also associated with an increase in the serum creatinine level.

The event rate in the placebo group was lower than that in VAHIT and other earlier studies, suggesting that CVD risk may have improved among individuals with diabetes and explaining in part the lesser benefit seen in the FIELD Study. The investigators achieved a mean A1C of 7% and blood pressure of 136/77 mmHg, leading Leiter to comment, “this was a very well-treated cohort.” He compared the FIELD Study with the Collaborative Atorvastatin Diabetes Study in which 10 mg atorvastatin daily lowered CVD risk by 37% in diabetic individuals without CHD (25). A1C was lower in the FIELD Study, but annual event rates and baseline lipids were similar in the two studies. HDL-raising and triglyceride-lowering effects were also similar in the Collaborative Atorvastatin Diabetes Study versus the FIELD Study, but there was considerably greater LDL lowering and much greater event reduction, with Leiter noting that the event reduction in the FIELD Study is approximately what would be predicted from the LDL effect.

Leiter pointed out that the increase in HDL cholesterol initially seen was significantly attenuated over the course of the study. Apolipoprotein A-II increased, but the expected increase in apolipoprotein A-I was not seen. This point was extended in a study reported at the meeting, with Huikka et al. (abstract 860) describing changes in HDL subspecies in 165 type 2 diabetic individuals receiving fenofibrate versus placebo treatment without concomitant statin use as part of the FIELD Study. In the overall study, there was an initial 5% increase in HDL cholesterol with fenofibrate, which lessened over 5 years. In the substudy, the baseline HDL cholesterol level was 46 mg/dl, with similar levels at 5 years in the placebo versus fenofibrate groups; among individuals receiving fenofibrate, HDL2 cholesterol decreased from 18 mg/dl by 21 and 33% at 2 and 5 years, respectively, while HDL3 cholesterol increased from 28 mg/dl by 8.1 and 10.4%, and apolipoprotein A-II increased 22 and 29%. Apolipoprotein A-I did not change over time.

There were small but statistically significant increases in deep vein thrombosis, pulmonary embolus, and pancreatitis in the fenofibrate group, perhaps related to the increased homocysteine and creatinine seen with this agent. After discontinuation of fenofibrate, homocysteine and creatinine levels returned to baseline. Leiter noted that recent homocysteine-lowering studies have not shown benefit, leading him to question whether this is actually a mechanism, although a recent meta-analysis did suggest a modest benefit of folate treatment in reducing CVD (26). There is some experimental evidence that fibrates increase cardiac myocyte fatty acid uptake, a potential explanation of the worse outcome in the FIELD Study individuals with existing CVD. Interestingly, gemfibrozil may be less potent in extrahepatic tissues, and this could protect the heart from adverse PPARα effects, contributing to the greater benefit reported in VAHIT than in the FIELD Study. It is possible, then, that adverse effects of fenofibrate may attenuate the increase in HDL over time and reduce fenofibrate’s benefit. On the other hand, fenofibrate does have greater safety than gemfibrozil when used in combination with statins. The ACCORD (Action to Control Cardiovascular Risk in Diabetes) Trial has ~10,000 patients and will use 20–40 mg simvastatin and fenofibrate versus placebo in a substudy, giving us additional information about this topic. At the present time, Leiter concluded, “the overall neutral effects . . . should maintain statins as the primary lipid lowering agents in patients with diabetes.”

**Lipid-lowering treatment for diabetes**

Many aspects of lipid-lowering treatment in individuals with diabetes were addressed in studies presented at the meeting. Ghanim et al. (abstract 857) administered 200 mg fenofibrate daily for 12 weeks to 28 hyperglycemic individuals, 13 with and 15 without type 2 diabetes. In addition to the expected triglyceride-lowering effect, C-reactive protein fell 27 and 22% in those with and without diabetes, respectively, with less marked reductions in serum amyloid A and in the adhesion molecules, vascular cell adhesion molecule-1 and E-selectin, suggesting that the drug has anti-inflammatory effect. Kearney et al. (abstract 920) reported a meta-analysis of 14 randomized trials of a statin versus controls among 18,868 patients with diabetes. Each 1 mmol/l (39 mg/dl) reduction in LDL cholesterol is associated with a 21% reduction in the likelihood of nonfatal myocardial infarction or coronary death, stroke, or coronary revascularization, with similar relative benefit in the 1,466 type 1 diabetic patients as in the 17,220 type 2 diabetic patients, as well as in those with versus without diagnosed vascular disease or hypertension. Deedwania et al. (abstract 619) analyzed the effect of 10 vs. 80 mg atorvastatin in 5,584 participants in the 4.9-year Treating to New Targets Study; 11.3 vs. 8% of those with vs. without metabolic syndrome experienced a major CVD event, comprised of CHD death, nonfatal non-procedure-related myocardial infarction, resuscitated cardiac arrest, or stroke. Among those with zero to two, three, four, and five metabolic syndrome components, as well as diabetes plus metabolic syndrome, taking 10 vs. 80 mg atorvastatin were 8.3 vs. 7.7, 11.5 vs. 8.1, 13.1 vs. 10.3, 17 vs. 11.8, and 17.8 vs. 14%, respectively, experiencing a major CVD event.

Nichols and Koro (abstract 536) compared 10,247 type 2 diabetic individuals who newly initiated statin therapy between 1997 and 2004, ascertained from the electronic records of Kaiser Permanente Northwest, and matched them to 10,247 diabetic patients not exposed to statins. Respective myopathy and myalgia incidences were 25 and 20 per 1,000 person-years in the statin group, both 37% higher than in individuals not receiving statins, with age, obesity, female sex, and use of fibrates, corticosteroids, and sulfonlureas associated with increased risk. Myositis and rhabdomyolysis occurred with incidences of 0.58 and 0.39 per 1,000 person-years, with numbers insufficient to assess statin effects. No interaction was found between diabetes treatment and the statin effect. In further analysis of the latter issue, Koro and Rabatin (abstract 923) studied a pharmaceutical database including 3,823 cases of myopathy and 22,579 control subjects, with 34 and 28% receiving statins, respectively, and 6 and 5% receiving thiazolidinediones; the latter not changing the likelihood of myopathy. Fibrates increased the risk of myopathy by 22%, with increased risk of myopathy also associated with obesity, CVD, and renal and hepatic dysfunction. In another study were gemfibrozil and fenofibrate effects distinguished.

Isley et al. (abstract 861) administered the combination of fish oil (3.3 g eicosapentaenoic acid and docosahexaenoic acid) and 3 g niacin daily versus placebo in a crossover study in eight type 2 diabetic patients on stable doses of medications, none receiving fibrates or thiazolidinediones and three treated with a statin. Triglycerides decreased 53% to
114 mg/dl, and HDL cholesterol increased 58% to 63 mg/dl, without change in LDL cholesterol, glucose, or insulin levels after a 75-g oral glucose load. Chen et al. (abstract 856) studied GW4064, an agonist of the farnesoid X receptor, for which bile acids are endogenous ligands. The agent improved all parameters in a high-fat diet mouse model of obesity, glucose intolerance, insulin resistance, and hypertriglyceridemia, with reduction in liver and muscle triglyceride content, suggesting the potential for benefits in treatment of diabetes, insulin resistance, and metabolic syndrome.

PPARs and heart failure versus edema

Frederick Masoudi (Aurora, CO) discussed the interrelationships between PPARγ agonists, heart failure, and edema. Diabetes is itself an extremely powerful predictor of heart failure, as shown by the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial Study, in which type 2 diabetes was as important a risk factor, as was a history of coronary heart disease, and was more important than cigarette use or left ventricular hypertrophy. The prevalence of diabetes among individuals with heart failure, then, is high, comprising 20–40% of individuals with heart failure in randomized controlled trials and in community samples. Mechanisms include the associated macrovascular disease, hypertension, and obesity of diabetes, as well as coronary microvascular disease, metabolic dysfunction, and fibrosis. Heart failure incidence may be related to the degree of glycemic control among individuals with diabetes, in community studies and in the UKPDS, where each 1% increase in A1C was associated with a 14% increase in the risk of heart failure in animal models, cardiac function worsens with diabetes. Masoudi reviewed such a study in which aminoguanidine improved cardiac function, although the power of the trial to demonstrate an effect is, however, certainly fluid retention with this agent (28).

In the PROActive Trial, individuals receiving pioglitazone had greater risk of heart failure. There was a 1.6% increase in the risk of heart failure hospitalization, without increase in heart failure mortality, although the power of the trial to detect this was low. The risk of heart failure diagnosis has been reported to be increased in some but not all studies, and Masoudi’s study of TZD-treated older diabetic individuals suggests reduced mortality, although with increased risk of heart failure readmission (27), further suggesting that it is not clear whether all individuals hospitalized with the diagnosis of heart failure truly have this as the etiology of their fluid retention. An audience member commented that, in fact, the health care system encourages the diagnosis of heart failure in individuals with edema to justify procedures and to allow hospitalization. Masoudi suggested that the American Heart Association/ADA recommendations that individuals with grade III and IV heart failure avoid TZDs seems reasonable.

Treatment of heart failure and TZD withdrawal usually improves the edema in TZD-treated individuals. When asked about specific treatment for edema in individuals receiving TZDs, he commented that he had “not seen any compelling data yet that using spironolactone or amiloride will necessarily be effective.” A recent study, however, suggested superiority of spironolactone, and to a lesser extent of hydrochlorothiazide, to furosemide and rosiglitazone withdrawal in individuals developing fluid retention with this agent (28).

Many individuals with diabetes have underlying functional and structural abnormalities, putting them at increased risk of heart failure, particularly in the setting of myocardial infarction; thus, it is appropriate to identify individuals at particularly high risk, perhaps with brain natriuretic peptide measurement. In a study presented at the meeting, Grimmeshaye et al. (abstract 660) noted that NH2-terminal pro-brain natriuretic peptide (NT-proBNP) is a marker of adverse outcome in individuals with heart failure, in those with acute coronary syndrome, and among diabetic individuals with nephropathy. NT-proBNP was measured in 285 type 1 diabetic individuals, 19% with hypertension, 18% with microalbuminuria, and 7% with known CVD, finding it to be associated with female sex, age, nephropathy, peripheral neuropathy, and myocardial infarction and angina. Over 5 years of follow-up, NT-proBNP was associated with mortality, even with exclusion of the 90 individuals with known CVD, hypertension, and diabetic nephropathy. Okada et al. (abstract 682) found a negative correlation between circulating CD34+ cells, endothelial progenitors involved in the maintenance of vascular endothelial dysfunction and neovascularization, and NT-proBNP concentration in 37 type 2 diabetic individuals, mean age 70 years, suggesting a role in the development of cardiac dysfunction in individuals with type 2 diabetes.

Dual PPARα/γ agonists

Bart Staels (Lille, France) discussed the dual PPARα/γ agonists. PPARγ receptor activation plays a role in fat metabolism in adipose tissue, appearing to explain the effects of TZDs on glucose homeostasis by increasing adiponectin and decreasing in-
sulin resistance–promoting cytokines, as well as by decreasing FFA release, while PPARs and -α act in muscle and liver, respectively, mainly to stimulate fatty acid oxidation. All appear to have antiinflammatory effects, appearing in part to involve effects on T-cell and macrophage API and nuclear factor-κB (29). PPAR agonists act to stimulate HDL metabolism and reverse cholesterol transport, as well as increasing hepatic fatty acid oxidation. Staels pointed out that bezafibrate is not a PPAR agonist alone but a triple agonist of all three PPARs. The idea of the dual PPARγ agonists is that the α agonists lowers triglyceride and small dense LDL cholesterol, possibly activating adiponectin receptors, while γ agonists increase adiponectin and decrease FFA release, with the combined agents having greater antiinflammatory potency. The lesser effect of fibrates on HDL in diabetic than in nondiabetic individuals further suggests the need to potentiate the lipid benefit of α agonists; with γ agonists improving hepatic insulin sensitivity, leading to greater PPARs effect. Furthermore, β-cell function may improve with dual agonist therapy, as PPARα and -γ are both expressed in the β-cell, and lipotoxicity models show that rosiglitazone improves basal and stimulated insulin release and that fibrates improve basal insulin release. Both PPARα and γ improve vascular endothelial nitric oxide synthase, and atherosclerosis might be reduced in a complementary fashion by the inhibitory effects of PPARγ agonists on matrix metalloproteinases and of PPARα agonists on tissue factor. Pioglitazone has been shown to decrease carotid intima-media thickness independent of glycemic control, and in animal models of restenosis, PPARα agonists decrease neointima formation, again suggesting potential combined benefit. In the FIELD Study, fenofibrate appeared to be more effective in individuals without prior CVD, while pioglitazone in the PROactive Trial appeared of greater benefit in individuals with more evidence of prior CVD, another potential combined benefit of treating both targets.

“Nevertheless,” Staels commented, “there are a number of safety issues” with the class of combined PPARα/γ agonists, both preclinical and clinical. The major preclinical issue has been carcinogenicity in rodent models, as well as some evidence of increased heart weight. In clinical use, PPARγ agonists cause edema, although not convincingly causing other evidence of heart failure, and PPARα agonists might cause myopathy by altering fatty acid oxidation in muscle and in liver, although there is no evidence that this occurs either in animal models or in humans. In studies of muraglitazar, ragaglitazar, tassaglitazar, and other compounds in development, glucose homeostasis and dyslipidemia benefits have been at least as great as those with TZDs, but safety issues have halted development. Ragaglitazar did cause weight gain and edema in phase 2 clinical trials, but its development was stopped because of rodent toxicity with urothelial cancer. MK 767 led to the development of hemangiosarcoma in rodents. A Takeda compound elevated liver enzymes. Muraglitazar and tesaglitazar appeared to be safe in preclinical testing, although prolonging the QT interval in canine studies. In humans, however, muraglitazar caused weight gain, edema, and appeared to increase development of CVD (30), while tesaglitazar development was stopped because of increased creatinine. Staels was encouraged that no “common denominator” led to discontinuation of development of these drugs. All the agents act differently, with weak α agonist effects and effects on γ, which differ between the agents, although all are PPARγ dominant. Staels suggested that there might still be “pros for the dual PPAR agonists,” as agents with greater benefit on combined dyslipidemia/insulin resistance, as well as greater antiinflammatory effect, and with less risk of drug-drug interaction than would be seen with use of PPARα and -γ agonists separately. He commented that it may be possible to design selective PPAR modulators free of adverse effects such as edema; thus, it may be premature to completely abandon research in development of these agents.

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Point: If It Is Important to Prevent Type 2 Diabetes, It Is Important to Consider All Proven Therapies Within a Comprehensive Approach

Type 2 diabetes is a common metabolic disease that is defined on the basis of glucose levels above specific thresholds. Individuals with type 2 diabetes are at high risk of blindness, renal failure, amputation, cardiovascular disease, premature death, dementia, and a variety of other chronic diseases and life-threatening events. However, unlike other risk factors for future events (e.g., hyperlipidemia or hypertension), type 2 diabetes is often associated with symptoms and discomfort related to elevated glucose levels that range from fatigue, nocturia, polyuria, and nonspecific aches and pains to dehydration and coma. Moreover, once diabetes is diagnosed, affected individuals incur additional cost and inconvenience related to disease labeling, dietary and lifestyle modification, glucose monitoring, eye assessments, and higher health and life insurance premiums.

Several trials have shown that aggressive management of type 2 diabetes can reduce the risk of microvascular disease (1–3), and that multifactorial risk factor interventions can reduce the risk of these and other consequences (4–6). These considerations and epidemiologic evidence that the risk of eye and kidney disease is well below the diagnostic thresholds for diabetes suggest that if glucose levels are prevented from rising past these thresholds, or the rise is delayed, these consequences will also be prevented or delayed. Moreover, evidence that the glucose level is a progressive risk factor for cardiovascular events (i.e., that the risk rises with the glucose level) (7–11) supports the hypothesis that preventing or delaying any rise within the nondiabetic range may reduce the risk of cardiovascular events. Finally, if ongoing clinical trials (12) show that therapies that lower elevated glucose levels in individuals with and without diabetes reduce the risk of cardiovascular events, then a therapy that both prevents diabetes and lowers or normalizes glucose levels may also reduce cardiovascular risk. Such a possibility has already been raised by at least one diabetes prevention trial (13).

Today, these are just hypotheses, and whether they are true may depend on the specific means by which the intervention prevents or slows the rise in glucose levels, in addition to whether it lowers nondiabetic glucose levels or just keeps them from rising any higher. For example, a hypothetical drug that dramatically lowers the renal threshold for glucose and causes glucosuria may have a very different effect on the consequences of diabetes than a drug that improves β-cell function, even though both agents could prevent or slow a rise of glucose levels past the diabetes thresholds.

Clinical trials in individuals with impaired glucose tolerance (IGT) have clearly shown that a program of diet and exercise can substantially reduce the incidence of type 2 diabetes by ~60% (14,15), and that the glucose-lowering drugs metformin and acarbose can reduce the incidence of diabetes by 25–30% (14,16). Most recently, the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) trial showed that the addition of the thiazolidinedione rosiglitazone to healthy lifestyle advice can reduce type 2 diabetes by 60% in individuals with either impaired fasting glucose (IFG) or IGT (17), and that this metabolic benefit was accompanied by modest weight gain preferentially localized to the hip versus the abdomen. The most notable adverse effect was non-fatal congestive heart failure that occurred at a low incidence of 0.5% over 3 years.

The possibility that diabetes incidence can be reduced by agents that are not viewed as glucose-lowering agents has also been prospectively tested. One clinical trial (18) of a weight-reducing drug reported a 37% risk reduction in obese individuals. However, the fact that only 43% of the randomized participants were followed for the full study period and that benefits were most apparent in the IGT subgroup make it difficult to generalize these findings to all obese individuals. Most recently, the DREAM trial reported that ramipril did not significantly reduce diabetes incidence in individuals with IFG or IGT at low risk for cardiovascular disease (19), in contrast to a meta-analysis of previous ACE trials that suggested a modest effect on diabetes prevention in individuals at high risk for cardiovascular events (20). However, the data suggested a trend toward benefit after 3 years, and ramipril did significantly increase the secondary outcome of regression to normoglycemia (19).

Table 1 summarizes the key characteristics and results of the trials of non-pharmacologic and pharmacologic interventions that yielded a significant reduction, delay, or prevention of diabetes. It is important to note that from a clinical perspective, the words “reduction,” “de-lay,” and “prevention” are identical. For the group allocated to the interventions that yielded positive results, diabetes incidence was reduced; for the individuals within that group who did not develop diabetes, it was delayed or prevented during the trial. Moreover, if they do not develop diabetes before they die from other causes, it will have been prevented for their life. Whether either diet and exercise or the pharmacologic interventions transiently or permanently altered the underlying metabolic physiology responsible for the rise in glucose levels over time is a mechanistic or biologic question. It can be answered in part by short- and long-term follow-up of participants who did not develop diabetes during the trial and who are no longer following a diet and exercise regimen or who are no longer taking the drugs (i.e., in whom the effects of the intervention are being “washed out”). Such a question is being answered for both ramipril and rosiglitazone during a post-trial follow-up of DREAM trial par-
Therapies proven effective in diabetes prevention trials

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>n</th>
<th>Population</th>
<th>Age (years)</th>
<th>Duration (years)</th>
<th>Follow up</th>
<th>Intervention (daily dose)</th>
<th>Control subjects (%/year)</th>
<th>Relative risk</th>
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</thead>
<tbody>
<tr>
<td>Finnish DPS (15)</td>
<td>522</td>
<td>IGT, BMI ≥ 25 kg/m²</td>
<td>55</td>
<td>3.2</td>
<td>92</td>
<td>Individual diet/exercise</td>
<td>6</td>
<td>0.42 (0.30–0.70)</td>
</tr>
<tr>
<td>DPP (14)</td>
<td>2,161*</td>
<td>IGT, BMI ≥ 24 kg/m², FPG &gt; 5.3 (93)</td>
<td>51</td>
<td>3</td>
<td>93</td>
<td>Individual diet/exercise</td>
<td>10</td>
<td>0.42 (0.34–0.52)</td>
</tr>
<tr>
<td>Pan et al. (22)</td>
<td>259*</td>
<td>IGT (randomized groups)</td>
<td>45</td>
<td>6</td>
<td>92</td>
<td>Group diet/exercise</td>
<td>16</td>
<td>0.62 (0.44–0.86)</td>
</tr>
<tr>
<td>Kosaka et al. (23)</td>
<td>458</td>
<td>IGT (men), BMI = 24 kg/m²</td>
<td>~55</td>
<td>4</td>
<td>92</td>
<td>Individual diet/exercise</td>
<td>2</td>
<td>0.33 (0.10–1.0)†</td>
</tr>
<tr>
<td>Indian DPP (24)</td>
<td>269*</td>
<td>Individual</td>
<td>46</td>
<td>2.5</td>
<td>95</td>
<td>Individual diet/exercise</td>
<td>22</td>
<td>0.71 (0.63–0.79)</td>
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<tr>
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<td>IGT, BMI ≥ 24 kg/m², FPG &gt; 5.3</td>
<td>51</td>
<td>2.8</td>
<td>93</td>
<td>Metformin (1,700 mg)</td>
<td>10</td>
<td>0.69 (0.57–0.83)</td>
</tr>
<tr>
<td>Indian DPP (24)</td>
<td>269*</td>
<td>IGT</td>
<td>46</td>
<td>2.5</td>
<td>95</td>
<td>Metformin (500 mg)</td>
<td>22</td>
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<tr>
<td>STOP NIDDM (16)</td>
<td>1,419</td>
<td>IGT, FPG &gt; 5.6</td>
<td>54</td>
<td>3.2</td>
<td>96</td>
<td>Acarbose (300 mg)</td>
<td>13</td>
<td>0.75 (0.63–0.90)</td>
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<tr>
<td>XENDOS (18)</td>
<td>3,277</td>
<td>BMI &gt; 30 kg/m²</td>
<td>43</td>
<td>4</td>
<td>43</td>
<td>Orlistat (360 mg)</td>
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<tr>
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<td>IGT, BMI ≥ 24 kg/m², FPG &gt; 5.3</td>
<td>51</td>
<td>0.9</td>
<td>93</td>
<td>Troglitazone (400 mg)</td>
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<td>0.25 (0.14–0.43)†</td>
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<tr>
<td>TRIPOD (26)</td>
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<td>Previous GDM</td>
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<td>67</td>
<td>Troglitazone (400 mg)</td>
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<tr>
<td>DREAM (17)</td>
<td>5,269</td>
<td>IGT or IFG</td>
<td>55</td>
<td>3.0</td>
<td>94</td>
<td>Rosiglitazone (8 mg)</td>
<td>9</td>
<td>0.40 (0.35–0.46)</td>
</tr>
</tbody>
</table>

*Number of participants in the indicated comparisons and not the total randomized; †calculated from information in the article. DPP, Diabetes Prevention Program; DPS, Diabetes Prevention Study; GDM, gestational diabetes mellitus; STOP, Study to Prevent Non-Insulin Dependent Diabetes; TRIPOD, Troglitazone in Prevention of Diabetes; XENDOS, Xenical in the prevention of Diabetes in Obese Subjects.

Participants who are taking single-blind placebo.

Thus, strong evidence from randomized clinical trials shows that diabetes can be prevented by dietary modification, increased physical activity, and a growing list of drugs. Moreover, it is likely that the list of drugs will continue to grow with time and that their benefits will magnify the benefits of diet and exercise. If this is true, the impact of combination therapy would indeed be impressive. For example, if the effects of rosiglitazone and a diet and exercise program similar to that offered by the Diabetes Prevention Program (both with a hazard ratio of ~0.4) are completely independent, the combination could theoretically yield a hazard ratio as low as 0.16 or a relative risk reduction of 84%. This would reduce the 3-year risk of diabetes from 26 to 4% in an individual similar to a DREAM participant; the addition of metformin would reduce it even further.

These considerations suggest that we are quickly acquiring the tools to mount a comprehensive approach to diabetes prevention, which will include both non-pharmacologic and pharmacologic approaches. Indeed, as learned from other epidemics, even this will be insufficient to stem the diabetes epidemic without a broader perspective. The response to the diabetes epidemic needs to include societal changes to urban planning, food, education, and social and public health policies so they more effectively promote metabolically healthy behaviors. Public health initiatives that facilitate self assessment of the risk of diabetes with simple tools (21) and routine glucose testing of high-risk patients by health care providers need to be tested and promoted. For high-risk individuals, healthy lifestyle approaches should be recommended first as background therapy. After subsequent evaluation of the risks and benefits for a particular individual, the addition of pharmacologic therapy should be considered when nonpharmacologic approaches are insufficient or inappropriate, and both the response to therapy and adverse effects should be monitored and reevaluated periodically. It is only if we use all of the tools at our disposal that we will be able to reverse the growing threat that diabetes poses to both the length and quality of our lives.

HERTZEL C. GERSTEIN, MD, MSC, FRCP

From the Division of Endocrinology and Metabolism and the Population Health Research Institute, McMaster University and Hamilton Health Sciences, Hamilton, Ontario, Canada.

Address correspondence and reprint requests to Dr. H. C. Gerstein, Department of Medicine, Room 3V38, 1200 Main St. West, Hamilton, Ontario L8N 3S5, Canada. E-mail: gerstein@mcmaster.ca.

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Point-Counterpoint


Counterpoint: Evidence-Based Prevention of Type 2 Diabetes: The Power of Lifestyle Management

The need to prevent type 2 diabetes was recognized as early as the 1920s (1), but surprisingly little was done to apply preventive measures against this disease in subsequent decades (2,3). One of the main problems was the lack of evidence based on well-conducted studies. There were several clinical trials, but they were usually grossly underpowered, had flaws in design and conduct, and most used antidiabetes drugs as the intervention (3). Luckily, firm positive results from several randomized controlled trials (4–10) using lifestyle intervention have become available during recent years. Also, several properly designed and conducted trials using antidiabetes drugs in individuals at high risk, i.e., with intermediate hyperglycemia, have reported favorable results (8,10–14). The bottom line is that these recent trials have unequivocally demonstrated that it is possible to reduce the rate of progression to type 2 diabetes in high-risk individuals with intermediate hyperglycemia.

The Swedish Malmö feasibility study (5) used increased physical exercise and weight control as major intervention strategies to prevent or delay type 2 diabetes in men with impaired glucose tolerance (IGT). Men who received intervention had less than half the risk of developing diabetes in 6 years compared with those who decided not to participate in the diet-exercise program.

In the Chinese Da Qing Study (6), people with IGT were randomized by clinic into one of the four groups: exercise only, diet only, diet plus exercise, and a control group. The cumulative incidence of type 2 diabetes during 6 years was significantly lower in the three intervention groups compared with the control group (41% in the exercise group, 44% in the diet group, 46% in the diet plus exercise group, and 68% in the control group) and remained significant even after adjusting for differences in baseline BMI and fasting glucose.

The Finnish Diabetes Prevention Study (DPS) (7) found that a reduction in body weight achieved through an intensive diet and exercise program was associated with a 58% reduction in the risk of developing type 2 diabetes ($P < 0.001$). Middle-aged men ($n = 172$) and women ($n = 350$) who were overweight and had IGT were individually randomized to an intervention group or to a control group and received conventional advice. The goals of the lifestyle interventions were to achieve a $\geq 5\%$ reduction in body weight, reduce all fat intake to $< 30\%$ of energy consumption, particularly reducing saturated fat intake to $< 10\%$ of energy consumption, increase fiber intake to at least 15 g/1,000 kcal, and undertake a program of moderate physical activity for $\geq 30$ min/day. After 1 year, individuals in the intervention group had achieved a significantly greater mean reduction in body weight compared with the control group ($P < 0.001$). They also demonstrated favorable changes in fasting and postchallenge plasma glucose levels. The reduction in the risk of progression to diabetes was directly related to the magnitude of the changes in lifestyle; none of the participants who had achieved at least four of the five intervention goals in the 1st year developed type 2 diabetes during the trial.

The U.S. Diabetes Prevention Program (DPP) (8) also found that lifestyle modification reduced the incidence of type 2 diabetes by 58% in overweight American adults with IGT. A total of 3,234 adults were randomized to standard lifestyle recommendations plus placebo or 850 mg metformin twice daily or to an intensive lifestyle modification program. The goal of the program was to achieve and maintain $\geq 7\%$ reduction in body weight through a low-calorie, low-fat diet plus physical activity of moderate intensity for at least 150 min/week. Participants in the lifestyle intervention group had a significantly greater mean reduction in body weight ($\sim 5.6$ kg, $P < 0.001$) compared with those in the placebo ($\sim 0.1$ kg) and metformin groups ($\sim 2.1$ kg). The cumulative incidence of diabetes during the follow-up period was lower in the lifestyle intervention and metformin groups than in the placebo group, with incidence rates of 4.8, 7.8, and 11.0 cases per 100 person-years, respectively. This reduction in incidence can be translated to one case of diabetes prevented for every 7 individuals with IGT treated for 3 years in the lifestyle intervention group, compared with 14 in the metformin group. Lifestyle intervention produced almost identical results in all ethnic groups included in the DPP.

A Japanese lifestyle intervention study (9) among 458 men with IGT resulted in a 67% relative risk (RR) reduction compared with control men during a 4-year trial. Recently, in the Indian Diabetes Prevention Program (10), 531 individuals with IGT were randomized into four groups assigned to: 1) metformin, 2) lifestyle modification, 3) both lifestyle modification and metformin, or 4) a control group. The cumulative incidence of type 2 diabetes during the median follow-up period of 30 months was significantly lower in the lifestyle modification group (39%), the metformin group (41%), and the lifestyle modification plus metformin group (40%) compared with the control group (55%). Thus, also in this trial, the absolute risk difference was $\sim 15\%$.

To summarize, these trials have repeatedly confirmed that lifestyle intervention works in all ethnic groups and various social and cultural settings worldwide. Nevertheless, several individuals in the intervention arm of these trials became diabetic. Thus, it seems that lifestyle intervention did not completely remove the risk of diabetes. The DPS, however, has demonstrated that those individuals who changed their lifestyle to the desirable level were protected against diabetes and that those assigned to the intervention group who became diabetic were not able manage to change their lifestyle sufficiently.

LESSONS FROM THE EXTENDED FOLLOW-UP OF THE FINNISH DPS — After a median of 4 years of the intensive intervention period, the active intervention in the DPS has ceased because it had been un-
equivocally demonstrated that lifestyle intervention prevents type 2 diabetes (7). The DPS participants who had remained free of diabetes when the study closed were further followed for a median of another 3 years, making the overall follow-up time 7 years on average. The extended follow-up of the DPS assessed the extent to which the originally achieved lifestyle changes and the reduced risk of diabetes persisted after active lifestyle counseling had been discontinued (15). Diabetes incidence, body weight, physical activity, and dietary intakes of fat, saturated fat, and fiber were measured. During the overall follow-up period, the incidence of type 2 diabetes was 4.3 and 7.4 per 100 person-years in the intervention and control groups, respectively (log-rank test \( P = 0.0001 \)), indicating a 43% (RR) reduction. The 58% RR during the original active trial period was higher, but this was due to the statistical facts. The cumulative incidences became higher in both groups, which reduced the ratio (RR), whereas the absolute risk difference between the original randomization groups remained about the same or even increased a little. The risk reduction was related to the success in achieving the intervention goals of weight loss, reduced intake of total and saturated fat, increased intake of dietary fiber, and increased physical activity during the original randomized trial period. Importantly, beneficial lifestyle changes initially achieved by the intervention group participants were maintained even after the discontinuation of the intervention; the incidence rates during the post-intervention follow-up period were 4.6 in the original intervention group and 7.2 per 100 person-years in the control group \((P = 0.0401)\), indicating a further 36% RR reduction.

Thus, the DPS follow-up for the first time has demonstrated that lifestyle intervention in individuals at high risk for type 2 diabetes not only reduces diabetes risk in the short term when the actual intervention is carried out, but also that effects on lifestyle changes and reduced diabetes risk are long term. For public health services planning, the message is clear that an intensive lifestyle intervention lasting for a limited time can yield marked long-term reduction in the risk of type 2 diabetes in individuals with IGT without a further investment.

ANTIDIABETES DRUGS LOWER BLOOD GLUCOSE, AS LONG AS THEY ARE TAKEN — Since the early invention of insulin and oral antidiabetes agents, it has been clear that it is possible to lower elevated blood glucose by pharmacotherapy. All clinical trials have shown that blood glucose levels may be reduced to some extent if an antidiabetes drug is taken. Several long-term studies have, however, found that despite active antidiabetes drug therapy, glycemic levels gradually increase in diabetic patients and even exceed the pretreatment values in <10 years (16).

As expected, trials of antidiabetes pharmacotherapy among individuals with elevated plasma glucose have confirmed that plasma glucose levels can be reduced, and similarly, several studies among individuals with IGT have reconfirmed that these drugs also lower plasma glucose in nondiabetic individuals. In diabetes prevention trials, such glucose lowering by drugs has been called “the prevention of diabetes.” Whether antidiabetes treatment can really be labeled as the prevention of type 2 diabetes requires a thorough discussion.

The pharmacologic effect of antidiabetes drugs on plasma glucose will gradually disappear after the drug use is discontinued, as shown by placebo-controlled trials with a cross-over design and studies using a “wash-out” period. Similarly, in recent diabetes prevention trials in individuals with IGT, the effect of metformin (8,10), acarbose (11), and troglitazone (12,13) began to disappear after relatively short wash-out periods. The Troglitazone in Prevention of Diabetes study in premenopausal women with previous gestational diabetes, however, reported (12) that troglitazone might have resulted in an improvement in insulin secretion in some women that remained even after the discontinuation of the drug. It is possible that this was due to the selection of the target population since in the DPP in older individuals with IGT such a long-lasting effect of troglitazone was not seen (13).

Recently, the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) trial showed that the incidence of type 2 diabetes was 60% lower in individuals with IGT/impaired fasting glucose (IFG) when treated with rosiglitazone compared with placebo (14). This risk reduction was, as one may expect given the 1.6 mmol/l fall in 2-h post-challenge plasma glucose, 56% (95% CI 54–65%) (17). Thus, there was no additional effect on diabetes incidence over and above the glucose-lowering effect of rosiglitazone. Until today, the results from the wash-out period after the randomized treatment in the DREAM trial have not been published, but it would be surprising if glucose levels would not increase after stopping rosiglitazone.

DREAMS AND THE REALITY IN THE HOPE TO PREVENT THE ONSET OF DIABETES BY ACE INHIBITORS — It has been suggested that action of ACE to increase angiotensin II and promote the breakdown of bradykinin may promote the development of diabetes, and that by inhibition of ACE and/or blockade of angiotensin II, the risk of diabetes may be reduced. The post-hoc analysis of the Heart Outcomes Prevention Evaluation study was the first placebo-controlled trial to suggest that the ACE inhibitor ramipril may prevent diabetes (18). Subsequently, additional post-hoc analyses of large controlled trials have also reported that ACE inhibitor use is associated with a lower incidence of diabetes in comparison with placebo or various active comparators (19). A growing number of post-hoc analyses from trials that used angiotensin II receptor blockers also suggest that they may have a similar effect (20). These trials were however not designed to test diabetes prevention as a primary hypothesis, and a proper diagnostic method (an oral glucose tolerance test) was not used, and most cases of diabetes were self-reported. Thus, it has remained unclear whether these drugs will really reduce the risk of diabetes.

The recent data from the DREAM trial in individuals with intermediate hyperglycemia have tested this hypothesis with the ACE inhibitor ramipril (21). The results were negative, i.e., ramipril did not reduce the incidence of diabetes compared with placebo, while this large trial clearly had the power to find a 20–25% effect that has been observed in previous post-hoc analyses (19). Next, we need to see whether angiotensin II receptor blockers will influence the risk of diabetes in placebo-controlled trials that are currently ongoing.

THE MYTH OF REVERTING TO “NORMOGLYCEMIA” — It is obvious that antidiabetes drugs will bring plasma glucose levels of some indi-
individuals with IGT or IFG to a range that is considered normal. There is no trial that has attempted to maximally lower plasma glucose, and thus, claims about “reversion” are not appropriate. Regarding lifestyle trials where the aim has been to stop the progression from IGT to diabetes, the regression is not supported by the design. Nevertheless, in trials on serum LDL cholesterol and blood pressure, the statement “the lower the better” has been proven to be true. At present, for several biological parameters, the aim is to find the most adequate level, which is often close to that observed after birth.

SAFETY OF PREVENTIVE MEASURES — For any treatment, the safety profile is very important, and similarly, measures to prevent diseases must be safe. To bring less harm compared with benefits is necessary for treatment. For preventive measures, an even stricter rule is needed; harm should be kept at minimum. Regarding pharmacologic and lifestyle interventions, there is a distinct difference in the potential regarding safety. Lifestyle interventions are safe, and they will typically promote healthy behaviors (diet, weight control, physical activity, etc.) that have multiple health benefits beyond diabetes prevention. Thus, the risk-to-benefit ratio of lifestyle intervention may be more favorable than what a single outcome assessment such as plasma glucose may indicate. While some drugs are known or believed to have multiple (pleiotropic) effects, modern drug development attempts to design drugs that have a specific target and mode of action. Pharmacologic interventions, on the other hand, often result in undesired effects that may increase with increasing dose of the drug. These may reduce the risk-to-benefit ratio of a drug and reduce the compliance with such an approach for long-term prevention of type 2 diabetes. For instance, significant weight gain associated with rosiglitazone in the DREAM trial (14) clearly works against the lifestyle advice to lose weight given to high-risk subjects and will place the individual in a very difficult situation.

ECONOMICS OF PREVENTION — Cost-effectiveness or cost-benefit estimates of various interventions to prevent chronic diseases play an important role when deciding about their applicability for large-scale implementation. Important but rather limited information about cost-effectiveness of preventive measures can be derived from data collected during prevention trials. The conclusion of the DPP investigators was that preventive interventions were cost-effective and that lifestyle intervention was better than metformin (22). Metformin is one of the cheapest antidiabetes drugs and has relatively few side effects that are mostly mild. To use more recently developed drugs such as glitazones for prevention of diabetes would increase costs dramatically, while overall benefits might not increase in the similar degree, as seen in the DREAM trial.

The new follow-up data from the DPS will further strengthen the case of cost-effectiveness of lifestyle intervention for type 2 diabetes. After the intensive lifestyle intervention that was provided to the intensive intervention group for 4 years on average, additional benefits in terms of lower risk of type 2 diabetes were still obtained during at least 3 years without any effort from health personnel (19). This will improve the long-term cost-effectiveness estimates markedly. With pharmacologic intervention, such long-term effects after stopping the treatment are unlikely, and if treatment is continued for the long term, it will require efforts from health care providers in addition to the cost of the drug itself.

COMMUNITY-WIDE APPROACH TO PREVENT TYPE 2 DIABETES — While it is obvious that a population-based strategy to fight the pandemic of type 2 diabetes is urgently needed, it is also evident that an individualized approach to guide people at high risk is warranted. A relatively simple lifestyle intervention seems to work well. However, further research is needed to reveal the optimal and most cost-efficient strategy, intensity, and duration of such an intervention. The results from the extended follow-up of the DPS nevertheless have demonstrated that the effect of lifestyle intervention on diabetes risk does not disappear after stopping active lifestyle counseling. This message is very important for planning and implementing community-based diabetes prevention programs. Antidiabetes drugs are nevertheless needed in such programs for the next stage, i.e., for effective pharmacotherapy to lower elevated blood glucose as early as possible to prevent deleterious effects of hyperglycemia.

JAakko Tuomilehto, MD, MPOLSC, PHD
From the Department of Public Health, University of Helsinki, Helsinki, Finland.
Address correspondence and reprint requests to Jaakko Tuomilehto, Department of Public Health, University of Helsinki, Mannerheimintie 172, 00300 Helsinki, Finland. E-mail: jaakko.tuomilehto@ktl.fi.
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**Editorials**

**Point: Pulmonary Inhalation of Insulin: Another “Brick in the Wall”**

The latest innovation for advancing diabetes care is not a new pharmacologic class; it represents a new “twist” on one of the oldest pharmacologic agents known for treatment of diabetes. After >80 years of clinical use and after many years of research for alternative means of delivery (including dermal, nasal, and oral approaches), insulin delivered by pulmonary inhalation is finally a clinical reality. The availability of inhaled insulin could not have come at a better time. At a time when the prevalence of diabetes is increasing at alarming rates worldwide and when the majority of individuals with diabetes have not achieved the recommended glycemic goal, new insights into the disease itself are being revealed at a rapid pace and are allowing for the development of novel approaches to better manage the disease. As such, inhaled insulin now joins the glucagon-like peptide 1 (GLP-1) agonists, dipeptidyl peptidase-IV inhibitors, and synthetic analogs of amylin as the latest tools available to the clinician. However, it is somewhat surprising that despite the promise that inhaled insulin could contribute to a paradigm shift in the clinical management of diabetes, considerable concern is openly expressed regarding its routine use.

There is no question about the need for insulin therapy in an individual with type 1 diabetes. The use of insulin in type 2 diabetes and, in particular, earlier in the course of management is supported by the natural history of the disease, which is characterized by progressive β-cell dysfunction. However, as a medical community, we need to do a much better job in advancing therapy in order to achieve glycemic control. Data from the National Health and Nutrition Examination Survey (NHANES) III and NHANES 1999–2000 suggested, if anything, a decrease in the percentage of individuals achieving glycemic targets (1). At the same time, the percentage of individuals treated with insulin, either as monotherapy or in combination with oral agents, remained essentially unchanged. Since these initial findings, additional data have suggested a slight improvement in glycemic control, but the majority of individuals with diabetes are still not at goal (2). Although new guidelines suggest continual titration of therapy over a period of months (which includes initiation of insulin) in order to achieve glycemic targets (3,4), the reality is that in many circumstances, providers fail to intensify management despite inadequate glycemic control on the current regimen, an observation referred to as “clinical inertia” (5). Therefore, providers who care for patients with type 2 diabetes appear to accept less than optimal control on combination oral therapy because of their concerns of using insulin or because of the concerns of the patients. This resistance to advance to insulin therapy is particularly disturbing given that insulin remains as the sole clinically available agent that allows the clinician to continuously titrate until the patient is at glycemic goal. An additional limitation of insulin therapy is that in order to optimize glucose control, the regimen may require multiple insulin injections that, in turn, may increase the complexity and effort required to comply with the regimen. Therefore, barriers to insulin use and intensification exist from both patients and physicians (6–8). Thus, it would appear that inhaled insulin, by overcoming some of the barriers to insulin use, would be well received and judged as a valuable addition to our treatment options based on the data suggesting need, efficacy, patient acceptability, and safety.

Based on the feasibility of delivering insulin via pulmonary inhalation, there are a number of devices and insulin formulations currently in development by pharmaceutical companies for pulmonary delivery and include the Pfizer/Nektar Exubera, Lilly/Alkermes AIR, MannKind Technosphere AERx iDMS, and Novo Nordisk/Aradigm AERx Pulmonary Insulin Delivery System. The major differences in these systems currently in development include the insulin formulation used, e.g., dry powder versus liquid, and the specific mechanics of the devices. Despite the apparent differences of the insulin formulations and devices, a consistent observation is that inhaled insulin has a faster onset of action than subcutaneous regular insulin and an onset of action that is comparable to fast-acting analogs such as lispro insulin.

Inhaled insulin has demonstrated its efficacy in numerous clinical trials. Studies have been conducted in individuals with type 1 diabetes using the Pfizer/Nektar Exubera Pulmonary Insulin Delivery System and have compared preprandial inhaled insulin, with basal injection at night, with both conventional insulin and intensive insulin dosing (9,10). These studies, for the most part, demonstrate comparable glycemic control between subcutaneous insulin regimens and regimens incorporating premeal use of inhaled insulin. Similar findings were reported for the Lilly/Alkermes AIR system for individuals with type 1 diabetes. These findings included comparisons with regimens using fast-acting insulins, such as lispro (11). Regimens using preprandial inhaled insulin, along with one injection of basal insulin, were comparable with conventional insulin injection regimens (mixed-regular/NPH insulin) in subjects with type 2 diabetes (12). Patients with either type 1 or type 2 diabetes receiving inhaled insulin reported enhanced overall satisfaction, quality of life, and acceptance of intensive insulin therapy (9,12,13). However, the most important use of inhaled insulin may be in the treatment of individuals with type 2 diabetes who fail combination oral therapy. A phase 3 study (14) of 309 patients with type 2 diabetes suboptimally controlled on oral therapies revealed improved glycemic control (as assessed by A1C) in the patients taking inhaled insulin alone and in combination by 1.2 and 1.9%, respectively, compared with those receiving oral agents alone.

The argument most frequently used against the widespread use of inhaled insulin would be long-term safety. However, long-term safety would need to be definitively established for any new agent. Yet, the frequency and nature of adverse events such as hypoglycemia reported with inhaled insulins appear, in general, to be comparable with subcutaneous insulin, with the exception of cough (although it decreases in incidence and prevalence with continued use). Pulmonary function tests, including forced expiratory volume in 1 s (FEV$_1$), forced vital capacity (FVC), total lung capacity (TLC), and carbon monoxide diffusing capacity (DLCO) have been conducted for all inhaled insulin studies. Some of the earlier
studies (9,10) reported differences in the more variable DLCO relative to subcutaneous insulin. However, longer-term studies (15) have been conducted, and 2-year data are available. Treatment group differences in changes from baseline in FEV1 and DLCO were small, occurred early, remained stable, and were nonprogressive for up to 2 years of follow-up (15). Patients treated with inhaled insulin have been shown to develop increased serum insulin antibody levels (9,10,12,14). However, the increase in antibodies observed did not result in any apparent clinical change and were not related to changes in pulmonary function (16). In addition, extensive preclinical and clinical studies, which have included 2-year controlled studies utilizing high-resolution computerized tomography of the thorax, have not revealed evidence of inflammatory, fibrotic, or proliferative responses in the lung. Based on the efficacy of inhaled insulin, the safety data reported to date, the medical need, and the sponsor’s commitment to conduct a comprehensive postmarketing risk management plan, the Pfizer/Nektar Exubera insulin inhaler was given U.S. Food and Drug Administration approval in January 2006 and is currently the only insulin inhaler available for routine clinical use.

Because of the effects noted on pulmonary function, it is currently recommended that all patients have spirometry (FEV1) assessed before initiating inhaled insulin, after the first 6 months of therapy, and yearly thereafter. It is important to understand that the studies that evaluated the safety and efficacy of inhaled insulin were not done in subjects for which the baseline FEV1 was <70%. One major question would be whether adjustments in the dose of inhaled insulin should be considered in the face of respiratory infections. A retrospective analysis of pooled data from 14 controlled phase 2 and 3 clinical trials, ranging in duration from 3 to 24 months, revealed no apparent changes in glycemic control or hypoglycemic rates for inhaled insulin during intercurrent respiratory infections. As such, it was felt to be safe and efficacious even during these intercurrent respiratory illnesses (17). However, smoking has been shown to greatly alter the pharmacokinetics of inhaled insulin, and inhaled insulin should not be used in patients with diabetes who chose to continue smoking (18).

Although the new inhaler provides a means to deliver prandial insulin and appears to be comparable on glycemic control when compared with injections, two observations appear noteworthy. First, patients delivered insulin via pulmonary inhalation appear to have less of a tendency for weight gain as reported in a presented abstract (19) on retrospective data from a number of completed studies. Clearly, head-to-head comparisons will need to be made with analog insulins for both postprandial control and weight before marketing claims can be made. Second, studies have shown that use of insulin with the inhaler results in greater reductions in fasting blood glucose (15). The mechanisms behind the weight and fasting glucose effects are not yet known.

The availability of the insulin inhaler should not be viewed as the sole answer to the problem of compliance and inadequate glycemic control commonly seen in clinical practice today. However, inhaled insulin has been demonstrated to be an effective therapy compared with subcutaneous insulin regimens and appears superior when compared against failed oral therapies. Based on the efficacy and side-effect profile, the insulin inhaler should be considered as another viable therapeutic option available to the clinician and should be used as part of a comprehensive program with other new and established agents in an attempt to improve and maintain glycemic control. In this context, the new insulin inhaler can be considered as another “brick in the wall.”

**WILLIAM T. CEFALU, MD**

From the Division of Nutrition and Chronic Diseases, Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, Louisiana.

Address correspondence and reprint requests to William T. Cefalu, MD, Professor and Chief, Division of Nutrition and Chronic Diseases, Pennington Biomedical Research Center, Louisiana State University System, 6400 Perkins Rd., Baton Rouge, LA 70808. E-mail: cefaluwt@pbrc.edu

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Counterpoint: No Time to Inhale: Arguments Against Inhaled Insulin in 2007

Much of the storied history of insulin has revolved around attempts to make its administration easier for patients who have to inject it to survive. The search for alternative routes of administration began almost immediately after its discovery—inulin was administered by inhalation, with modest effectiveness, and then within several years of its first administration by subcutaneous injection (1). The now almost unimaginable use of 20-gauge needles, sharpened by hand, and glass syringes that had to be sterilized regularly made the development of less painful and more convenient injections highly desirable. Moreover, before the development of intermediate- and long-acting formulations of insulin in the 1930s, four to five daily injections of the available rapid-acting formulation were required if patients wanted to avoid hyperglycemia and accompanying polyuria and polydipsia.

The introduction of “protamine insulin” in 1936 (2), followed by protamine zinc insulin, NPH, and the lente series of insulins, made it possible to maintain generally asymptomatic levels of glucose control, based on the longer-acting profile of the formulations, with only two injections per day. Although more convenient for patients with type 1 diabetes, the intermediate-acting insulins, and long-acting insulins that followed, had the unintended consequence of distracting attention from the more physiologic administration of insulin by multiple injections (3). When the glycohemoglobin assay became widely available in the early 1980s (4), it was clear that the chronic glycemic control achieved with these nonphysiologic, albeit convenient, regimens was far from normal. More importantly, the elevated levels of chronic glycemia were strongly associated with all of the long-term complications of diabetes that resulted in severe morbidity and premature mortality (5).

It took almost 60 years after the introduction of intermediate-acting insulins to establish the long-term benefits of intensive therapy. As defined in the Diabetes Control and Complications Trial (DCCT), intensive therapy included at least three injections per day or continuous subcutaneous insulin administration with an external pump (6). The need to frequently administer rapid- or very-rapid–acting insulin in order to achieve near-normal glucose control and delay or prevent the long-term complications once again placed a major burden on patients with type 1 diabetes. However, this time the burden was not owing to limited insulin formulations; rather, the demands of therapy arose from strong evidence that individuals with type 1 diabetes could live a healthier and longer life if they injected more frequently. The development of a whole range of insulin formulations to provide basal and bolus delivery, along with increasingly sharp small gauge needles, disposable syringes, and insulin delivery devices (e.g., insulin pens, pumps), has made injection therapy more tolerable; however, the need to frequently inject insulin remains a burdensome feature of the modern therapy of type 1 diabetes.

Now, the latest innovation in insulin delivery, inhaled insulin, promises to free type 1 diabetic patients from frequent injections, although the provision of basal insulin will still require injections. The development of inhaled insulin is based on the technology used to deliver pulmonary medicine for respiratory diseases. The limited, ~10%, absorption of the insulin powder from the respiratory tract has been solved by delivering doses that are 10-fold larger than would be given by the subcutaneous route. Concerns regarding potential pulmonary toxicity (insulin is a potent growth factor, and there are insulin receptors in the pneumocyte, raising the specter of potential changes in the alveolar or bronchiolar structure that could interfere with gas exchange) have been addressed through studies in animals and long-term (generally 2-year) studies in patients with diabetes. Only modest changes in DLCO have been observed, and the increased anti-insulin antibody titers generated with inhaled insulin have been found not to adversely affect the availability or biologic activity of insulin (7,8).

Given the enthusiasm of investigators and manufacturers of inhaled insulin, and the uniform approval of the patients who use it, why would anyone object to its use? My primary objection to inhaled insulin is not that it is unsafe or that it will cost more than injected insulin. I do not find fault with the obscenely large inhaler that must be carried everywhere. Patients who have inhaled insulin have coped with these barriers and continue to inhale. My primary objection is that the level of glycemic control achieved in the clinical trials using inhaled insulin has been substandard. The recent excellent meta-analysis by Ceglia et al. (7), which reviewed seven controlled clinical trials in >1,500 type 1 diabetic patients, noted that all of the efficacy trials were noninferiority studies. Thus, the investigators only needed to demonstrate that the inhaled insulin was no worse than an active comparator, usually preprandial injections of rapid- or very-rapid–acting insulin. This level of proof is apparently satisfactory to the U.S. Food and Drug Administration (FDA), as evidenced by its approval of inhaled insulin in January 2006; however, given the critical importance of long-term near-normal glycemia, the level of control achieved by the comparator and inhaled insulin must be scrutinized. As noted in the meta-analysis, the A1C achieved with preprandial inhaled insulin in type 1 diabetic patients was slightly higher than with subcutaneous insulin regimens. More worrisome was that none of the long-term inhaled insulin regimens achieved a mean A1C as low as that in the DCCT, even though the baseline A1C value was substantially lower in the inhaled insulin studies than in the DCCT. The failure of the comparator therapy in the inhaled insulin studies to reach the A1C levels achieved in the DCCT is peculiar, considering that they had access to the very-rapid–acting and newer very-long–acting insulin analogs that were not available during the DCCT.

The data presented to the FDA during the approval process showed that during two 24-week studies in >200 subjects with type 1 diabetes using inhaled insulin, the mean A1C at study end was 7.5% in one study and 7.7% in the other. A1C...
type 2 diabetic patients who refuse to use inhaled insulin may be an alternative for diabetic patients is a “distraction” (13). In a sense, inhaled insulin for type 2 diabetic patients may require more basal insulin, compared with subcutaneous regimens utilizing twice-per-day regimens with intermediate-acting insulins resulted in poor diabetes control and long-term complications. Inhaled insulin may turn out to be a wonderful addition to our therapeutic arsenal, combining patient convenience and comfort with acceptable glycemic control. However, until inhaled insulin is shown to achieve the chronic glycemic levels that effectively prevent or delay complications, patients would be well advised not to inhale.

DAVID M. NATHAN, MD

From the Diabetes Center and Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts.

Address correspondence and reprint requests to David M. Nathan, MD, MGH Diabetes Center, 50 Staniford St., Suite 340, Boston, MA 02114 E-mail: dnathan@partners.org.

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References
**OBSERVATIONS**

**Is Metformin Safe in Patients With Mild Renal Insufficiency?**

Among the first million patients who received metformin in the U.S., 47 patients developed metformin-associated lactic acidosis (MALA), with 43 having predisposing factors for lactic acidosis (including moderate to severe renal failure and congestive heart failure) (1). Although there was initial concern, studies have suggested that MALA is secondary to underlying conditions and represents a coincidental finding (2,3). While the current consensus is that the risk of lactic acidosis is negligible when metformin is used as labeled (4), we present a patient who developed MALA in the absence of currently recognized contraindications to metformin.

A 55-year-old man with hypertension, type 2 diabetes, and mild renal insufficiency (measured creatinine clearance 91 ml/min) presented with sudden onset of fatigue, vomiting, and altered mental status after performing strenuous yard work without sufficient hydration. His medications included nifedipine, captopril, hydrochlorothiazide, glyburide, and metformin.

The patient rapidly developed respiratory distress and hypotenison necessitating intubation and vasoactive agents. Laboratory studies revealed a serum creatinine level of 9.4 mg/dl, pH 6.98, CO2 6 mmol/l, and lactic acid 27 mmol/l. Evaluation using a computed tomography scan and magnetic resonance angiography of the abdomen/pelvis, various cultures and cardiac echocardiogram could not reveal an etiology for lactic acidosis. Serum metformin level (ARUP Laboratories, Salt Lake City, UT) was 8 mg/l (therapeutic range 1–2). Continuous venovenous hemofiltration was initiated immediately. Conservative management was followed by rapid amelioration of his general status. He was extubated within 24 h, continuous venovenous hemofiltration was stopped after 36 h, and he was discharged 6 days after presentation without deficits.

This case is unique in that MALA developed in the absence of currently recognized risk factors or predisposing conditions. Although this patient had mild impairment of kidney function, contraindication criteria for the use of metformin were not met (5). The patient was taking 2 g metformin per day, which is within the recommended therapeutic range.

In our opinion, a threshold serum creatinine level above normal range should not be considered safe for metformin use because renal function can rapidly deteriorate in patients with even mild underlying kidney disease, resulting in accumulation of metformin and development of MALA. We suggest that consideration be given to avoiding metformin in patients with any degree of renal dysfunction.

**Malignant Melanoma Misdiagnosed as a Diabetic Foot Ulcer**

A male patient aged 48 years with type 2 diabetes presented with a painless nonhealing ulcer of 18 months duration under his right first metatarsal head. The ulcer was not a typical appearing neuropathic foot ulcer and had mushrooming granulation tissue and areas of intact epidermis in a lenticular fashion over the wound bed (Fig. 1). The patient also complained of a “knot” in his right inguinal area. An incisional biopsy was taken from the foot lesion, which revealed a poorly differentiated melanoma covered by an intact epidermis and granulation tissue. The incisional biopsy was 0.8-cm thick, and melanoma extended to the deep margin. At presentation, the size and poor differentiation of the tumor made it impossible to assess the subtype of the original melanoma. The S-100 and HMB-45 stains (positive in melanoma cases) were strongly positive. A computed tomography of the chest, abdomen, and inguinal areas revealed metastasis to the inguinal lymph nodes and liver. The patient died 6 months later.

Although rare, melanomas can present as neuropathic foot ulcers in individuals with diabetes (1,2). Melanomas are located on the plantar surface in ~7% of cases (3) with the exception of Japanese patients, in whom the plantar surface is more common.

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**References**


**Figure 1—Malignant melanoma tumor that was misdiagnosed as a neuropathic foot ulcer.**
the most common location (4). Acral lentiginous melanoma is the most common melanoma type that presents on the plantar aspect of the foot (3). This type of melanoma is commonly amelanotic, frequently ulcerates (5), and does not exhibit the classic signs of malignant melanoma associated with the mnemonic aid “ABCD” (asymmetry, border, color, diameter). In a review (6) of 53 lower extremity melanomas, 11 of 18 (61%) misdiagnosed cases were on the plantar foot. All misdiagnosed lesions were histopathologically acral lentigious melanomas. Initial misdiagnoses included nonhealing ulcer, wart, tinea pedis, and onychomycosis. Another retrospective review (7) of palmoplantar melanoma found that misdiagnosis led to a median delay of treatment for 12 months and was associated with increased tumor thickness (5.0 vs. 1.5 mm) and a lower 5-year survival rate (15.4 vs. 68.9%).

We are not supposing that plantar melanoma occurs more frequently in individuals with diabetes. However, we believe there is a greater chance of misdiagnosis given this population’s predilection toward plantar ulceration. An individual with peripheral sensory neuropathy is more likely to unknowingly ampute on a plantar foot lesion, and this increased pressure and trauma can cause a lesion to initially resemble a diabetic foot ulcer. This case and short review emphasizes the importance of performing biopsies on chronic and atypical wounds early in the treatment algorithm of diabetic foot ulcers.

Lee C. Rogers, DPM
David G. Armstrong, DPM, PhD
Andrew J.M. Boulton, MD, FRCPATH
Anthony J. Freemont, MD, FRCP
Rayaz A. Malik, MB, CHB, MRCP, PhD

From the 1Center for Lower Extremity Ambulatory Research (CLEAR), Rosalind Franklin University of Medicine and Science, Chicago, Illinois; the 2Division of Cardiovascular and Endocrine Science, University of Manchester, Manchester, U.K.; and the 3Department of Regenerative Medicine, University of Manchester, Manchester, U.K.

Address correspondence to Lee C. Rogers, DPM, Scholl’s Center for Lower Extremity Ambulatory Research (CLEAR), Rosalind Franklin University of Medicine, 3333 Green Bay Rd., North Chicago, IL 60064. E-mail: lee.rogers@rosalindfranklin.edu.

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COMMENTS AND RESPONSES

An Open, Randomized, Parallel-Group Study to Compare the Efficacy and Safety Profile of Inhaled Human Insulin (Exubera) With Glibenclamide as Adjunctive Therapy in Patients With Type 2 Diabetes Poorly Controlled on Metformin

Response to Barnett et al.

In response to the interesting article by Barnett et al. (1), we would like to offer the following comments. Diabetes control has been shown to improve with diet and exercise regimens (2,3). The degree of study participants' compliance with diet and exercise regimens may have contributed the change in A1C reported in the study (1). Also, the independent effect of BMI on both diabetes control and response to therapy has been studied extensively (4). The effect of modification of baseline BMI on diabetes control among various strata of BMI in both study groups needs clarification.

The open-blinded design of the study (1), especially since it involves diabetes education and self-monitoring, can significantly impact internal validity due to both performance bias of the subject with respect to compliance with lifestyle modifications as well as detection bias of the health care providers in ascertaining adverse outcomes (5). In addition, the non-inferiority design offers no protection against a predetermined idea of equivalence by the investigator, who could allocate similar scores to responses and events of all study subjects (6).

Balavenkatesh Kanna, MD, MPH1,2
Heidi Abreu-Pacheco, MD1

From the 1Department of Internal Medicine, Lincoln Medical & Mental Health Center, Bronx, New York; and the 2Weill Medical College of Cornell University, New York, New York.

Address correspondence to Balavenkatesh Kanna, MD, MPH, Department of Medicine, Suite 8-22, 8th Floor, 234 E 149th St., Bronx, NY 10451. E-mail: balavenkatesh.kanna@nychhc.org.

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anemia, age, gender, body mass index (BMI), triglycerides, and lipoprotein(a).

Negative effects of smoking include cardiovascular disease, chronic obstructive pulmonary disease, and lung cancer.

Diabetes Care 27:2067–2073, 2004

An Open, Randomized, Parallel-Group Study to Compare the Efficacy and Safety Profile of Inhaled Human Insulin (Exubera) With Glibenclamide as Adjunctive Therapy in Patients With Type 2 Diabetes Poorly Controlled on Metformin

Response to Kanna and Abreu-Pacheco

We thank Kanna and Abreu-Pacheco (1) for their comments on our study (2). As Kanna and Abreu-Pacheco point out, overweight and obesity are strongly linked to the development of type 2 diabetes and can complicate its management. While most patients with type 2 diabetes are overweight (3), this study (2) included individuals with a range of BMI values typical of those seen in clinical practice; mean BMI in the inhaled insulin and glibenclamide groups was 31.8 (range 19–51) and 31.1 (22–47), respectively. When analyzed by baseline BMI values, the mean change from baseline A1C in the moderately high A1C arm (≥8.0 to ≤9.5%) was −1.6, −1.3, and −1.5% in patients with baseline BMI values of <30, 30–35, and ≥35 kg/m², respectively, compared with −2.9% for all subjects. The results show no meaningful differences between the BMI categories, and the authors therefore believe it to be unlikely that the baseline BMI values could have confounded the A1C results.

For the duration of the study, patients were required to follow an American Diabetes Association diet (with 30% fat and calories sufficient to maintain ideal body weight) and to perform 30 min of moderate exercise at least 3 days per week. There was no specific measure of compliance with diet and exercise regimens during the study, but patients were reminded of their importance at each clinic visit.

Finally, we would like to point out that our study was open label and not blinded. As highlighted in the article, a double-blind study, while desirable, was not possible for two principal reasons: 1) it was not possible to manufacture a suitable placebo for inhaled insulin, and 2) it is generally inappropriate to blind treatment when individualized flexible dose titration is needed for effective management with exogenous insulin.

Anthony H. Barnett, BSc, MD, FRCP
Manfred Dreyer, MD
Peter Lange, MD
Marjana Serdarevic-Pehar,

On behalf of the Exubera Phase III Study Group

From the 1University of Birmingham and Heart of England National Health Service Foundation Trust (Teaching), Birmingham, U.K.; the 2Department of Diabetes and Metabolism, Bethanien Krankenhaus, Hamburg, Germany; the 3Department of Respiratory Medicine, Hvidovre University Hospital, Hvidovre, Denmark; and 4Pfizer, Ltd., Sandwich, U.K.

Address correspondence to A.H. Barnett, Undergraduate Centre, Birmingham Heartlands Hospital, Bordesley Green East, Birmingham, B9 5SS, U.K.
E-mail: anthony.barnett@heartofengland.nhs.uk.
M.S.-P. is an employee of Pfizer.
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Hyperglycemia and Diabetes in Patients With Schizophrenia or Schizoaffective Disorders

Response to Cohen et al.

We commend Cohen et al. (1) on their report on hyperglycemia and diabetes in patients with schizophrenia and schizoaffective disorders. To our knowledge, this is the first large study of oral glucose tolerance tests in this population.

Cohen et al. found that the prevalence rate of diabetes was significantly higher in patients with schizophrenia and schizoaffective disorders than in the general population. They did not detect a differential effect of antipsychotic monotherapy in diabeticogenic effects, and they consequently proposed a modification of the consensus statement on antipsychotic drugs, obesity, and diabetes, i.e., measurement of fasting glucose in all patients with schizophrenia irrespective of the prescribed antipsychotic drug. We argue that the differences in the metabolic effects of different antipsychotic agents are too clear in the literature to justify any notion that the antipsychotic agents are comparable in their metabolic effects.

Comparative studies of antipsychotic agents are limited in their scope by the difficulty in conducting randomized controlled trials of antipsychotic agents. For many patients, specific antipsychotic agents are indicated ahead of the others based on the information available at that time. For example, clozapine is difficult to study in comparative investigations because it is not recommended by most as a first-line treatment. A recent study (2) addressed this issue to some extent by conducting a randomized controlled trial of risperidone and olanzapine in dogs. The dogs who received olanzapine developed hepatic insulin resistance, whereas those who received risperidone did not. Fur-
thermore, the usual compensatory increase in insulin secretion in response to insulin resistance was lacking in the olanzapine-fed dogs. Apart from the evidence of differential effects of the two agents, the results suggest that olanzapine may induce insulin resistance even in the absence of psychopathology. The lack of compensatory increase in insulin secretion suggests that olanzapine may also impair insulin secretion.

A recent correlational analysis (3) of receptor affinities of individual antipsychotic agents and their diabetogenic effects suggests that muscarinic M3 receptor affinity is the best predictor of risk for development of type 2 diabetes. The study was limited by its use of data from different laboratories, collected under different conditions. Nevertheless, the results are not surprising given the clinical knowledge that two of the antipsychotic agents with the most anticholinergic activity, clozapine and olanzapine, seem to present the greatest risk for development of type 2 diabetes. Among the first generation agents, there are reports (4) of diabetes in patients taking chlorpromazine, an agent with considerable anticholinergic activity. To our knowledge, however, there are no reports of diabetes in those taking haloperidol, an agent without significant anticholinergic activity. Muscarinic receptor affinity may also be the reason why a comparative study (5) of clozapine and chlorpromazine did not find a significant difference between treatments and their effects on weight or glucose metabolism. The study was cited by Cohen et al. (1) in support of their contention that all antipsychotic agents present risks of diabetes.

Taken together, these studies suggest that antipsychotic agents differ from one another in their effects on glucose metabolism. Until this issue is completely resolved, it would be prudent to monitor measurement of fasting glucose in all patients with schizophrenia, irrespective of the prescribed antipsychotic drug, with special attention provided to those taking olanzapine, clozapine, and chlorpromazine.

RIPU D. JINDAL, MD,1,2 MATCHERI S. KESHAVAN, MD1,2

From the 1University of Pittsburgh, Pittsburgh, Pennsylvania; and 2Wayne State University School of Medicine, Detroit, Michigan.

Address correspondence to Ripu D. Jindal, MD, University of Pittsburgh, 3811 O’Hara St., Pittsburgh, PA 15213. E-mail: jindalr@upmc.edu.

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Hyperglycemia and Diabetes in Patients With Schizophrenia or Schizoaffective Disorders

Response to Jindal and Keshavan

We thank Jindal and Keshavan (1) for their contribution explaining the results of our study (2), which stated that in a cross-sectional design (n = 200), no differences in the prevalence of diabetes or hyperglycemia between typical- or atypical-treated patients were found. We would like to make two comments on this statement. First, although the muscarinic M3 receptor affinity fits well with the diabetogenic properties of antipsychotic drugs, so does H1 histaminergic (but not muscarinic M3) receptor affinity with short-term weight gain, a factor that is often, but not always, present in antipsychotic-related diabetes (3,4). Second, it has been suggested (5) that risk factors of diabetes exert less predictive power in schizophrenia than in the general population. This hypothesis was tested (6) by examining the effect of the two major risk factors for diabetes: age and weight. In 200 patients with schizophrenia, typical (but not atypical) antipsychotic drugs modified the effect of these risk factors, confirming a less straightforward relationship between diabetes risk factors in schizophrenia than in the general population.

The statement by Jindal and Keshavan (1), that no cases of diabetes have been reported with haloperidol, may be interpreted as stressing the same point. Taken literally, it is simply untrue, as the following cases (7) have been reported: 10 of new-onset diabetes, 2 of worsening of existing diabetes, and 1 with an unknown preexisting status (on haloperidol monotherapy) with 4, 2, and 1 cases on haloperidol-risperidone combination therapy, respectively. More broadly speaking, Jindal and Keshavan (1) justly criticize the typical-atypical classification of antipsychotics as a scientifically unproductive dichotomy. This was shown (8) in cell culture, for instance, where haloperidol’s inhibiting effect on cell proliferation was comparable with the atypical clozapine but not to the typical chlorpromazine and fluphenazine. In this very complex matter, the ability to take any stance on explanatory pathways is currently precluded by the fact that research into the diabetogenic properties of antipsychotic medication and its pathways is just beginning.

DAN COHEN, MD, PHD1,2

From the 1De Dijk, GGZ-NHN, Heerhugowaard, the Netherlands; and the 2Department of Clinical Epidemiology, Rijks Universiteit Groningen, Groningen, the Netherlands.

Address correspondence to Dan Cohen, De Dijk, GGZ-NHN, Hectarlaan 19, 1702 CT Heerhugowaard, Netherlands. E-mail: d.cohen@ggz-nhn.nl.

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Modeling Chronic Glycemic Exposure Variables as Correlates and Predictors of Microvascular Complications of Diabetes

Response to Dyck et al.

We read with interest the article by Dyck et al. (1), in which the authors described a chronic glycemic exposure variable (GEi) in the Rochester Study. They examined GEi and its individual components (A1C, duration, and age at onset) in terms of prediction/correlation with complications and concluded that GEi is generally predicted better than its individual components (see Table 3 of ref. 1).

Dyck et al. compared their results with our previously published analyses (2) using a different chronic glycemic exposure variable, A1C months, noting that (as also reported by the Diabetes Control and Complications Trial [3]) this combination variable did not predict better than its components (A1C and duration). Our analytic approach, however, was different; we compared the fit of models, including the components to a model, with the composite alone. The differences in fit were small but favored the separate components. It would thus be interesting to compare the total R² of alternate models, one with GEi, and another with its components, in the current study. We suspect that, as in our case, differences would be small.

Another interesting issue is the use of “age at onset” and “duration” (1) together effectively defining age itself. Could any enhanced prediction be related to age itself? Inclusion of the partial R² for age in Table 3 (see ref. 1) would be useful.

Dyck et al. further suggested that differences between these studies may be explained by the “choice of patients” and differences in outcome assessment. As the Epidemiology of Diabetes Complications study (4) is comprised of community-treated type 1 diabetic individuals from a childhood-onset cohort shown to be epidemiologically representative of type 1 diabetes, selection bias was unlikely. However, the inclusion of type 2 diabetic subjects in the Rochester Study may have influenced results. Nevertheless, we agree that a continuous neuropathy outcome measure may be preferable and that this difference also may have contributed to the differences reported. Consequently, a comparison of A1C months and GEi would be more informative if performed for the outcome common to both studies (Diabetes Control and Complications Trial protocol neuropathy).

Finally, one motivation behind developing the A1C month measure was to address whether a glycemic threshold exists above which complications develop. Were the authors able to examine this issue using GEi? While unable to determine a clear threshold, we found that ~1,000 A1C months were experienced before the advent of advanced complications. This translates to 42 years of A1C 2% above normal or 18 years at 5% above normal, which reflects another motivation for our chronic glycemic exposure variable—a clinically useful concept of risk.

TREVOR J. ORCHARD, MD
TINA COSTACOU, PHD
RACHEL G. MILLER, MS
CATHERINE T. PRINCE, BA
GEORGIA PAMBIANCO, MS, MPH

References

Modeling Chronic Glycemic Exposure Variables as Correlates and Predictors of Microvascular Complications of Diabetes

Response to Orchard et al.

We are pleased to respond to the letter by Orchard et al. (1), especially since they first raised the following question: Do composite measures of chronic glycemia correlate or predict complications better than individual components? Orchard et al. reported evidence against the hypothesis, while we (2) reported evidence for the hypothesis. Having considered their suggestions, we offer an explanation for why their conclusions differed from ours.

Orchard et al. (3) compared the fit
from two models, one consisting of only
the composite and the other consisting of
a regression model that included both
components. The regression model is a
linear combination of the two compo-
nents in which the weights are chosen to
obtain an optimal fit; thus, the regression
model itself is a composite, though one in
which the fit to the data should be better
than A1 months (which is exactly what
they found).

Since comparing two composites was
not the goal of our study (2), we ap-
proached the analyses differently. We de-
volved one regression model including
all variables that were significant in the
developed one regression model including
approached the analyses differently. We de-
volved one regression model including
all variables that were significant in the
developed one regression model including

We also agree that the patient popu-
lation under study and the choice of out-
comes to be analyzed can influence the
results and that a continuous neuropathy
measure is desirable. Although use of a
common outcome measure would assist in
comparing our results with those of Or-
chard et al. (3), such a comparison was
not the focus of our study (2). Finally,
determining the threshold of chronic gly-
cemia, which induces complications, is
a worthy goal, but before we do this we
want to include studies of normal subjects
and glucose-impaired individuals cur-
cently being studied.

L. Joseph Melton, III, MD
Peter C. O’Brien, PhD

From the 1Department of Neurology, Mayo Clinic
College of Medicine, Rochester, Minnesota; the 2Di-
vision of Endocrinology, Mayo Clinic College of
Medicine, Rochester, Minnesota; the 3Department of
Ophthalmology, Mayo Clinic College of Medi-
cine, Rochester, Minnesota; the 4Division of Neuro-
pathy, Mayo Clinic College of Medicine, Rochester,
Minnesota; the 5Division of Biostatistics, Mayo
Clinic College of Medicine, Rochester, Minnesota.

Address correspondence to Peter J. Dyck, MD,
Mayo Clinic College of Medicine, Department of
Neurology, 200 First St., SW, Rochester, MN
55905. E-mail: dyck.peter@mayo.edu.

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A Critical Appraisal of the Continuous Glucose-Error Grid Analysis

Response to Wentholt et al.

In a recent publication, Wentholt et al. (1)
noticed that their aim was to critically
explore the continuous glucose-error grid
analysis (CG-EGA) (2) and to com-
pare it with traditional techniques using
data previously reported from two sen-
sors. As developers of the CG-EGA, we
hoped that our method might stimulate a
discussion on the important problem of
the accuracy of continuous monitoring
sensors (CGS); therefore, we read this cri-
tique with interest.

The methods used by Wentholt et al. (1)
unfortunately failed to take into ac-
count the basic structure of CGS data,
which represent time series (i.e., sequen-
tial readings that are ordered in time) (3).
This structure leads to two fundamental
requirements in their analysis. First, con-
secutive sensor readings taken from the
same subject within a relatively short time
are highly interdependent. Therefore,
standard statistical analyses such as t tests,
while appropriate for independent data
points, will produce inaccurate results if
applied to CGS data. Second, the order of
the CGS data points is essential for clinical
decision making. For example, the se-
quences 90 → 82 → 72 mg/dl and 72 →
82 → 90 mg/dl are clinically very differ-
ent. Standard accuracy measures, such as
the mean absolute deviation (MAD) used
by Wentholt et al. (1), do not account for
the data’s temporal order; if reference-
sensor data pairs are reshuffled, the MAD
remains the same.

As a result, the primary statistical
analysis used by Wentholt et al. is flawed,
both to demonstrate significant differ-
ences between the sensors and to imply
that CG-EGA is insensitive. The CGS data
from 13 subjects were pooled to compare
2 MADs (15.0 ± 12.2 vs. 13.6 ± 10.2%).
The result was reported as significant
(P = 0.013), but for these highly overlap-
ing MADs to differ statistically required
a large number (>1,000) of degrees of
freedom, which was calculated by pool-
ing the total number of CGS data points
(735 and 1,156) across all subjects. Such
an approach led to inaccurate conclusions
because there were only 13 independent
subjects, and the data points within each
subject were highly dependent. If the cor-
rect number of degrees of freedom is
used, the MADs of the two sensors are not
different (P > 0.5), which confirms the
CG-EGA results showing no differences.

Other conclusions by Wentholt et al.
also deserve comment. First, they stated
that CG-EGA is time consuming. Indeed,
alyses of temporal data are intrinsically
more sophisticated than standard time-
deependent statistics, but such analyses
are essential for this type of data. CG-EGA
software is available. Second, Wentholt et
al. stated that “poor accuracy rate is barely
noticeable in the final CG-EGA outcome,”
implying that this result of the CG-EGA
is incorrect. However, this result is not in-
correct because better combined (rate and
point) accuracy during hypoglycemia is
observed with the sensor, showing poorer
rate accuracy in this critical region. It is
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clinically apparent that when blood glucose is <3.9 mmol/l point accuracy should be given more emphasis than rate accuracy. A strength of CG-EGA is its ability to vary the input of either rate or point accuracy to overall clinical accuracy depending on blood glucose range. Third, the results of CG-EGA vary with time intervals. This is also an intuitive strength of CG-EGA, which is designed to account for increased noise associated with frequent sampling. We advocated (2) adopting a uniform sampling protocol with reference and/or sensor pairs taken every 10–15 min to standardize comparisons of rate accuracy, which is a sampling scheme based on physiological considerations of possible glucose change rates. Fourth, Wentholt et al. (1) questioned the appropriateness of the formulae to shift point EGA based on interstitial time lag. However, the authors reported an average time lag of ~7 min in one of their sensors, which is identical to that assumed for CG-EGA, thus confirming that ~7 min is a reasonable average for blood-to-interstitial diffusion delays. CG-EGA software allows setting this parameter to any value <7 min.

We are pleased that both the discussion regarding CG-EGA and the analysis of time series data have begun, and we look forward to continuing this important dialogue. However, we also recommend careful consideration of basic statistical assumptions when analyzing sensor-generated glucose data; their inherent temporal structure should be taken into account.

WILLIAM L. CLARKE, MD1
LINDA GONDER-FREDERICK, PHD2
DANIEL COX, PHD2
BORIS KOVATCHEV, PHD2

From the 1Department of Pediatrics, University of Virginia Health System, Charlottesville, Virginia; and the 2Department of Psychiatry and Neurobehavioral Sciences, University of Virginia Health System, Charlottesville, Virginia.

Address correspondence to Boris Kovatchev, Department of Psychiatry and Neurobehavioral Sciences, University of Virginia Health System, Box 800137, Charlottesville, VA 22908. E-mail: bori@virginia.edu.

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A Critical Appraisal of the Continuous Glucose–Error Grid Analysis

Response to Clarke et al.

We thank Clarke et al. (1) for their thought-provoking response to our article (2). With their comments (1), they not only took on the important issue of how to optimally assess the accuracy of continuous glucose monitors (CGMs); they moved the discussion one step further.

In our study (2), we did indeed take the statistical liberty of deriving degrees of freedom from all pooled data points—in contrast to the proposal by Clarke et al. (1) who compared the accuracy of two sensors using one average mean absolute deviation (MAD) value per patient. The latter approach may be too rigid because not all readings are interdependent. For example, postprandial glucose sensor readings at lunch and at night depend little on each other, if at all. It is common practice to derive degrees of freedom from pooled data in the sensor field. In a previous study, Clarke et al. (3) compared the accuracy of two CGMs in 16 type 1 diabetic patients by using the continuous glucose–error grid analysis (CG-EGA).

The difference in pooled readings in the hypoglycemic area that ended up in zones A and B was reported to be highly significant between both sensors (88 vs. 62.8%, respectively) (P < 0.0005). This level of significance implies that degrees of freedom were derived from all data pairs in the hypoglycemic range (250 mg/dl) rather than from the actual amount of participants (n = 16). Even with a strict statistical policy, the better MAD for the microdialysis sensor in the hypoglycemic area in our study (2) (12.0% for the 7-min corrected microdialysis sensor vs. 25.2% for the needle-type sensor, calculated per patient [df = 12], P = 0.036 by Wilcoxson’s signed-rank test) and the larger sensitivity for hypoglycemia associated with this sensor (75.0 [75 data pairs] vs. 55.9% [56 data pairs], P = 0.018 by Pearson’s χ², with 16 of 16 and 12 of 15 hypoglycemic episodes detected by the microdialysis and needle-type sensor, respectively, P = 0.06 by Pearson’s χ²) contrasted with the CG-EGA that noted no difference (51.5 vs. 60.0% accurate readings and benign errors in the hypoglycemic range [df = 42], P = 0.841 by Pearson’s χ² for the microdialysis and the needle-type sensor, respectively). Therefore, even with a mild statistical approach (i.e., deriving degrees of freedom from 43 data pairs rather than 13), CG-EGA could not confirm the different accuracy of the sensors in the hypoglycemic range.

As to the order of CGS data points, the sensor’s ability to follow the rate and direction of glucose changes is nicely reflected by the MAD: A sequence of glucose values that has been incorrectly reported by a given sensor (e.g., 90 → 82 → 72 mg/dl instead of 72 → 82 → 90 mg/dl) will result in a worsened MAD.

In reaction to the comment by Clark et al. (1) in regards to time consumption, we were happy to learn that the software for CG-EGA has become available. Nevertheless, the laborious collection of frequent blood samples on fixed intervals (in addition to the construction of a rate, a point accuracy plot, and, finally, a combining matrix) will remain inevitable drawbacks of CG-EGA.

With the attempt to standardize the length of the time intervals, Clark et al. clearly tried to improve the CG-EGA methodology. Nevertheless, a time interval that can vary by 5 min (10–15 min) still leaves the door open for interobserver variability.

As to our finding in a previous study (4) of a 7-min delay that was inherent to the microdialysis instrument itself and not seen in the needle-type sensor, Clarke et al. (1) alluded to a (much-disputed) constant 7-min physiological delay resulting from the relationship between interstitial and blood glucose. This physiological delay has been reported to be anywhere between 0 and 30 min, so the 7-min assumption made for the CG-EGA is questionable. Fortunately, Clarke et al. have now implemented into the software the possibility of setting the delay <7 min.

Currently, the optimal way to assess a CGM seems to be the combination of MAD calculated per glucose range, com-
bined curve fitting with assessment of horizontal and vertical shift, sensitivity, and positive predictive value for detecting hypoglycemia.

IRIS M. WENTHOLT, MD
JOOST B. HOEKSTRA, MD, PHD
J. HANS DeVRIE, MD, PHD

From the Department of Internal Medicine, Academic Medical Center, Amsterdam, the Netherlands.
Address correspondence to Iris M. Wentholt, MD, Department of Internal Medicine, Academic Medical Center, P.O. Box 22660, Amsterdam 1105 AZ, Netherlands. E-mail: i.m.wentholt@amc.uva.nl.
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References

Breast-Feeding and Risk for Childhood Obesity

Response to Mayer-Davis et al.

The study by Mayer-Davis et al. (1) reflects the fact that maternal nutrition plays an important role in the pathogenesis of childhood obesity. Breast milk contains linoleic acid (of the n-6 polyunsaturated fatty acids [PUFA] series) and α-linolenic acid (of the n-3 PUFA series) as well as longer chain derivatives, such as arachidonic acid (of the n-6 PUFA series) and docosahexaenoic acid (of the n-3 PUFA series). Maternal intake determines content of breast milk, which ultimately affects the infant’s future health.

Childhood obesity is probably an immune inflammatory response to a faulty diet of the mother (before and during gestation and lactation) consisting of high n-6 PUFAs, low n-3 PUFAs, and deranged n-6-to-n-3 ratio (2). In those who are breast-fed, breast milk provides longer-chain n-3 PUFAs, which prevent ectopic accumulation of fatty acids in muscle and liver (3,4). Formula feeding does not provide this benefit. Cow’s milk content depends on whether it is pasture fed (more n-3 PUFAs) or given commercial feeds (more n-6 PUFAs). Breast-fed infants have a muscle membrane fatty acid composition similar to insulin-sensitive adults, and formula-fed infants have a muscle membrane fatty acid composition similar to insulin-resistant adults (5). Correcting n-6 and n-3 PUFAs in the diet is currently needed for changing global health for one and all.

MANISHA TALIM, MBBS, DD

From Shrusrusha Hospital, Mumbai, India.
Address correspondence to Dr. Manisha Talim, Shrusrusha Hospital, 698-B Ranade Rd., Dadar, Mumbai 400028, India. E-mail: drmanishatalim@yahoo.com.
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References

Breast-Feeding and Risk for Childhood Obesity

Response to Mayer-Davis et al.

We read with great interest the recent study by Mayer-Davis et al. (1) on the impact of breast-feeding on childhood obesity risk in the presence of maternal diabetes or obesity. The authors drew conclusions that seem to directly oppose previous observations from our group (2,3). However, we would like to deliver three arguments suggesting that the presented data can also be interpreted in a completely different manner and in no way exclude, but rather support, a potentially negative dose–depending effect of early neonatal breast-feeding on overweight risk in offspring of diabetic/overweight mothers, as observed by us.

First, the majority of fully adjusted estimates for the effect of maternal diabetes have 95% CIs that include decreased as well as increased odds ratios over a wide range (e.g., odds ratio 0.79 [0.29–2.16] for breast milk only vs. formula only). By statistical definition, one therefore cannot exclude the possibility that the true effect of breast-feeding on overweight risk in the presence of maternal diabetes/obesity is not beneficial but deleterious, at least in a considerable number of cases.

Second, breast-feeding during the 1st month by diabetic mothers increased overweight risk compared with formula feeding. This, in fact, confirms rather than rejects our observations. Moreover, this is unlikely to be accounted for by reverse causation, since no dose response–like relation between duration of breast-feeding and risk of overweight was observed in offspring of diabetic mothers. These data may even support our hypothesis of a crucial and probably even deleterious impact of breast-feeding by diabetic mothers during the early neonatal period.

Finally, the authors stated that our observations might reflect “appropriate” growth rather than untoward effects. This, however, does not correspond with increased prevalence of overweight in the highest tertile of early neonatal intake of diabetic breast milk, using the symmetry index (2) additionally validated against BMI (4). Most importantly, this interpretation completely ignores deleterious ef-
Letters

Breast-Feeding and Risk for Childhood Obesity

Response to Plagemann et al.

We appreciate the interest and comments of Plagemann et al. (1) regarding our study (2) on maternal status as a potential modifier of association of breast-feeding on childhood obesity. As noted, for the contrast of breast milk only versus formula only, the 95% CI excluded the null value, thus necessarily including values >1.0. From a statistical perspective, however, the best estimate for this contract is an odds ratio (OR) of 0.79, not a value >1.0. Furthermore, the test for dose response suggested a statistically significant trend in the direction of protection by breast-feeding for both groups. Finally, there is no indication of a differential effect of breast-feeding according to maternal status. Specifically, for both exclusivity and duration of breast-feeding, the interaction term from fully adjusted models was P = 0.50 and P = 0.66, respectively. Thus, our interpretation of the data is that there is no evidence of a deleterious effect of breast-feeding according to maternal obesity or diabetes status.

With regard to the second point raised by Plagemann et al. (1), the OR of 1.11 for overweight among children of diabetic mothers who were breast-fed <1 month compared with those who were formula fed was in the same direction (i.e., potentially deleterious) as observed in the previous work by Plagemann et al. (3). We note that for this specific contrast, the 95% CI was quite wide (0.22–5.60), making interpretation difficult. Interestingly, the OR for the same contrast for nondiabetic mothers with BMI >25 kg/m² was also >1.0 (OR 1.46 [95% CI 1.01–2.13]). We noted in our original article (2) that to interpret this finding, one must consider potential circumstances related to the decision to stop breast-feeding at such a young age, as well as the infant-feeding behaviors in response to those circumstances. Here, while we agree with Plagemann et al. (1) that there is a need for further work in this regard, we note that the context of breast-feeding duration during the neonatal period (i.e., choosing to stop or to continue breast-feeding) is extremely important to consider, rather than simply focusing on this time period in isolation.

Finally, our work was, in fact, very specifically motivated by that of Plagemann et al., and thus we certainly agree that this topic is of considerable importance. Our comments in our study (2) regarding potential explanations for differences in our findings were speculative; thus, we have no further comments in this regard.

Elizabeth J. Mayer-Davis, PhD
Sheryl L. Rifas-Shiman, MPH
Li Zhou, BS
Frank B. Hu, MD, PhD
Graham A. Colditz, MD, PhD
Matthew W. Gillman, MD, SM

References

From the 1Center for Research in Nutrition and Health Disparities, Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, Columbia, South Carolina; the 2Department of Ambulatory Care and Prevention, Harvard Medical School and Harvard Pilgrim Health Care, Boston, Massachusetts; the 3Channing Laboratory, Brigham and Women’s Hospital, Boston, Massachusetts; and the 4Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts.

Address correspondence to Elizabeth J. Mayer-Davis, PhD, Center for Research in Nutrition and Health Disparities, University of South Carolina, Arnold School of Public Health, 2718 Middleburg Dr., Columbia, SC 29208. E-mail: ejmayer@gwm.sc.edu.

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Breast-Feeding and Risk for Childhood Obesity

Andreas Plagemann, MD
Thomas Harder, MD, MSCE
Elke Rodekamp, MD
Joachim W. Dudenhausen, MD

From the Experimental Obstetrics Research Group, Clinic of Obstetrics, Charité – University Medicine Berlin, Campus Virchow-Klinikum, Berlin, Germany.

Address correspondence to Prof. Andreas Plagemann, MD, Head of Experimental Obstetrics, Charité – University Medicine Berlin, Campus Virchow-Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany. E-mail: andreas.plagemann@charite.de.

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References

From the 1Center for Research in Nutrition and Health Disparities, Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, Columbia, South Carolina; the 2Department of Ambulatory Care and Prevention, Harvard Medical School and Harvard Pilgrim Health Care, Boston, Massachusetts; the 3Channing Laboratory, Brigham and Women’s Hospital, Boston, Massachusetts; and the 4Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts.

Address correspondence to Elizabeth J. Mayer-Davis, PhD, Center for Research in Nutrition and Health Disparities, University of South Carolina, Arnold School of Public Health, 2718 Middleburg Dr., Columbia, SC 29208. E-mail: ejmayer@gwm.sc.edu.

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Errata


A typographical error appears in Fig. 1 of the above-listed article. In the lower left box, “any explained hypoglycemia” should read “any unexplained hypoglycemia.”

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In the above-listed article, a typographical error occurred in the title. The correct citation appears above, and the online version reflects the change.


In the above-listed article, a typographical error occurred in the title. The correct citation appears above, and the online version reflects the change.