The Journal of Pediatrics

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Anemia—“IRONIC,” isn’t it!

Iron deficiency anemia continues to be a serious risk to low-income children, although its overall prevalence has fallen in the past two decades. There are still, however, some unanswered questions about the demographics of this risk.

In the current issue of *The Journal*, Cusick et al from the CDC present the results of a fascinating series of analyses of data from the CDC’s Pediatric Nutrition Surveillance System. The size of the cohorts studied in these analyses is impressive, numbering hundreds of thousands of mostly low-income children.

The data were divided into cohorts, representing children who were studied for anemia at about 12, 18, 24, and 36 months, and who had a subsequent examination a year later. The fascinating observation from this study was that in each cohort, most of the children identified as anemic at follow up were not anemic at the time of original measurement; most of the anemia was “incident.” This reinforces the importance of continued anemia surveillance in low-income at risk children, and continued attention to dietary prevention discussions at the time of health maintenance visits.

—Thomas R. Welch, MD

Is duration of sleep a cardiovascular risk factor?

Sleep abnormalities have been shown to be related to obesity, insulin resistance, hypertension and increased risk of cardiovascular disease in adults. In this issue of *The Journal*, Flint et al evaluated 40 obese children and found a relatively high prevalence of obstructive sleep apnea. They also found that children with a shorter duration of sleep had higher fasting insulin levels and greater insulin resistance. In addition, obstructive sleep apnea was associated with higher triglyceride levels and insulin resistance. In a multiple regression analysis, duration of sleep appeared to be the most important independent variable. These findings suggest that promoting a longer duration of sleep could be useful in lowering risk of cardiovascular disease in children and adolescents. These relationships are further reviewed and put in broader context in the editorial by Amin.

—Stephen R. Daniels, MD, PhD

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Treatment regimens for acute lymphoblastic leukemia in the 1970s and 1980s are associated with adult short stature today

The cross-sectional study reported by Chow et al of adult height of 2,434 survivors of childhood acute lymphoblastic leukemia (ALL) compared with their siblings provides several sound conclusions about treatments in the 1970s and 1980s, as well as worthy hypotheses for testing of current treatments. The investigators report decreased adult height and increased risk of short stature in cancer survivors, with graded adverse effects for diagnosis prior to puberty, increasing doses of cranial radiotherapy, and radiotherapy of any dosage to the spine. The study provides evidence that there was no “catch-up” growth post treatment.

Doses of radiation in protocols for treatment of ALL since this cohort was treated have been reduced or eliminated, but have been replaced by more aggressive chemotherapy regimens. Because even the “milder” chemotherapy circa 1970-1986, without radiation therapy, was associated with decreased adult height in the current report of Chow et al, there is reasonable concern for adverse long-term growth effect of protocols circa 2000s.

—Sarah S. Long, MD

Fitness or fatness—which is more important for cardiovascular risk?

There is no question that the epidemic of obesity is resulting in increased risk for cardiovascular disease across the population of children and adolescents. However, there is still much to learn about the mechanisms of this increased risk. One important question relates to the relative role of fitness and fatness. Are there some children who are at lower risk, despite being overweight, because of increased fitness?

In this issue of The Journal, two articles report on studies that begin to evaluate elements of these relationships. Allen et al studied overweight middle school children. They found that both percent body fat and V02 Max were associated with the level of fasting insulin. However, particularly in boys, the relationship of cardiorespiratory fitness to fasting insulin was stronger than the relationship of percent body fat and fasting insulin.

Rizzo et al examined the association of physical activity and cardiorespiratory fitness with a clustering of metabolic risk factors. They found that cardiorespiratory fitness was more strongly correlated with metabolic risk than total physical activity. However, body fat seems to play an important role in the association of cardiorespiratory fitness and metabolic risk.

Both studies emphasize the role of fitness, but also support the role of fatness. This would seem to put physical activity in an important role. However, it should be emphasized that fitness is only partially determined by physical activity. Fitness also has an important genetic component. Further research is needed to evaluate developmental and other aspects of these interrelationships.

—Stephen R. Daniels, MD, PhD

Do we need chest radiographs in infants with uncomplicated bronchiolitis?

The question of whether we need routine chest radiographs in infants with uncomplicated bronchiolitis is addressed in an article by Schuh et al from The Hospital for Sick Children, Toronto. They performed a prospective cohort study of more than 200 infants in the pediatric emergency department. The results showed that infants with typical bronchiolitis do not need imaging; invariably, it just confirms the diagnosis. In fact, the use of routine chest radiographs appeared to increase the chances of prescribing antibiotics in patients who were otherwise uncomplicated. The risk of air space disease such as pneumonia was particularly low in infants with mild to moderate respiratory distress and oxygen saturations in room air above 92%. These results will help guide the use of radiographic studies in clinical care.

—Robert W. Wilmott, MD
**Postnatal corticosteroids for bronchopulmonary dysplasia**

The occurrence and slow resolution of bronchopulmonary dysplasia (BPD) is one of the most frustrating management problems in neonatology. As with most intractable diseases, corticosteroid treatments are tried. In the case of BPD, initial results were positive but long-term follow-up suggested that postnatal corticosteroids increased brain injury and inhibited brain growth. These adverse effects resulted in recommendations by the American Academy of Pediatrics and the Canadian Pediatric Association that postnatal corticosteroids not be used to try to prevent or treat BPD. This recommendation did decrease the excessive use of these potent agents, but studies of their use continue because corticosteroid treatments do improve short-term respiratory outcomes. A recent report by Doyle et al (Pediatrics 2005;115, 655-661) argues that corticosteroids benefit infants at high risk of severe BPD.

In this issue of *The Journal*, Rademaker et al reports that hydrocortisone treatments for BPD did not adversely affect the brain MRI or neurodevelopmental outcomes at school age. Nixon et al further report that postnatal dexamethasone treatments may improve respiratory outcomes at school age. An editorial by Watterberg puts this contentious area of treatment into perspective and points out the multiple questions that remain.

—Alan H. Jobe, MD, PhD

**An effective strategy for neonatal hyperbilirubinemia**

Hyperbilirubinemia remains a major cause of prolonged hospital stays and readmissions for newborns. Careful initial screening of all infants and targeted follow-up for higher-risk infants will identify most infants at risk of bilirubin toxicity in a timely fashion. The exceptions are occasional infants with G6PD and other genetic abnormalities that can cause acute hemolysis in newborns. However, initial detection and follow-up systems are seldom ideal and infants continue to develop bilirubin toxicity.

Kaplan et al report an efficient management approach that combined pre-discharge screening with culturally-based community awareness to detect and effectively treat hyperbilirubinemia. However, 42% of the infants that required re-hospitalization were not identified as being at risk by pre-discharge screening. The study points out the need for effective follow-up for successful detection of some infants with hyperbilirubinemia.

—Alan H. Jobe, MD, PhD

**B-cell depletion in pediatrics**

B-cell depletion is being used in a wide range of disorders. El-Hallak et al at Harvard Medical School have reviewed their experience with treatment of refractory autoimmune disorders in this issue of *The Journal*. Although only 10 patients are reported, this is the largest pediatric experience reported for children with autoimmune diseases.

The article is discussed in some detail by Claas Hinze and Alexei Grom from Cincinnati Children’s Hospital Medical Center, Division of Rheumatology.

—Robert W. Wilmott, MD

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**Graph**

![Graph showing prednisone dose over time](image-url)

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Robert M. Jacobson, MD, Rochester, Minnesota

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Postnatal Steroids for Bronchopulmonary Dysplasia: Where Are We Now?
Kristi L. Watterberg, MD, Albuquerque, New Mexico

Is EGF the Holy Grail for NEC?
Michael Caplan, MD, Evanston, Illinois

The Systemic Effects of Short Sleep Period
Joseph Crisalli, MD, and Raouf S. Amin, MD, Cincinnati, Ohio

Childhood Cancer Cures: The Ongoing Consequences of Successful Treatments
Sharon E. Oberfield, MD, New York, New York

B Cell Depletion: On the Rise
Claas H. Hinze, MD, and Alexei A. Grom, MD, Cincinnati, Ohio

MEDICAL PROGRESS

Treatment with Rituximab in Benign and Malignant Hematologic Disorders in Children
Lisa B. Giulino, MD, James B. Bussel, MD, Ellis J. Neufeld, MD, PhD, and the Pediatric and Platelet Immunology Committees of the TMH Clinical Trial Network, New York, New York, and Boston, Massachusetts

ORIGINAL ARTICLES

Follow-up Study of a Randomized Controlled Trial of Postnatal Dexamethasone Therapy in Very-Low-Birth Weight Infants: Effects on Pulmonary Outcomes at Age 8 to 11 Years

Neonatal Hydrocortisone Treatment: Neurodevelopmental Outcome and MRI at School Age in Preterm-born Children
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Association between Inadequate Sleep and Insulin Resistance in Obese Children
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Eric J. Chow, MD, MPH, Debra L. Friedman, MD, MS, Yutaka Yasui, PhD, John A. Whitton, MS, Marilyn Stovall, PhD, Leslie L. Robison, PhD, and Charles A. Sklar, MD, Seattle, Washington, Edmonton, Alberta, Canada, Houston, Texas, Memphis, Tennessee, and New York, New York

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50 Years Ago in The Journal of Pediatrics—Neonatal Hepatitis in Siblings
Veena L. Venkat, MD, Cincinnati, Ohio

Fitness is a Stronger Predictor of Fasting Insulin Levels than Fatness in Overweight Male Middle-School Children
David B. Allen, MD, Blaise A. Nemeth, MD, R. Randall Clark, MS, Susan E. Peterson, MS, Jens Eickhoff, PhD, and Aaron L. Carrel, MD, Madison, Wisconsin

Relationship of Physical Activity, Fitness, and Fatness with Clustered Metabolic Risk in Children and Adolescents: The European Youth Heart Study
Nico S. Rizzo, MSc, Jonatan R. Ruiz, BSc, Anita Hurtig-Wennlöf, PhD, Francisco B. Ortega, BSc, and Michael Sjöström, MD, PhD, Huddinge and Örebro, Sweden, and Granada, Spain

Six-Minute Walk Test in Children and Adolescents
Ralf Geiger, MD, Alexander Strasak, MD, Benedikt Treml, MD, Klaus Gasser, MD, Axel Kleinsasser, PhD, Victoria Fischer, MD, Harald Geiger, MD, Alexander Loeckinger, PhD, and Joerg I. Stein, PhD, Innsbruck and Dornbirn, Austria

Sonia Partap, MD, Palo Alto, California

Impaired Endothelial Function in Healthy African-American Adolescents Compared with Caucasians
Mary M. Duck, BS, and Robert P. Hoffman, MD, Columbus, Ohio

Cardiac Manifestations in Oxidative Phosphorylation Disorders of Childhood
J. Yaplito-Lee, MD, R. Weintraub, MBBS, K. Jamsen, BSc(Math), C. W. Chow, MBBS, D. R. Thorburn, PhD, and A. Boneh, MD, PhD, Melbourne, Australia

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Evaluation of Discharge Management in the Prediction of Hyperbilirubinemia: The Jerusalem Experience

Michael Kaplan, MB, ChB, Ruben Bromiker, MD, Michael S. Schimmel, MD, Nurit Algur, MSc, and Cathy Hammerman, MD, Jerusalem and Be’er Sheva, Israel

50 Years Ago in The Journal of Pediatrics—Pediatric Use of Dioctyl Sodium Sulfosuccinate

Dean R. Focht, MD, Honolulu, Hawaii

Pre-ductal and Post-ductal O₂ Saturation in Healthy Term Neonates after Birth

Gonzalo Mariani, MD, Pablo Brener Dik, MD, Analía Ezquer, MD, Adolfo Aguirre, MD, Mirta Lucia Esteban, MD, Cecilia Perez, MD, Silvia Fernandez Jonusas, MD, and Carlos Fusiñana, MD, Buenos Aires, Argentina

Continuing Anemia Prevention Strategies Are Needed Throughout Early Childhood in Low-income Preschool Children

Sarah E. Cusick, PhD, Zuguo Mei, MD, MPH, and Mary E. Cogswell, DrPH, RN, Atlanta, Georgia

50 Years Ago in The Journal of Pediatrics—Premature Infant Mortality

K.S. Joseph, MD, PhD, and Michael S. Kramer, MD, Halifax, Nova Scotia, and Montreal, Quebec, Canada

Evaluation of the Utility of Radiography in Acute Bronchiolitis

Suzanne Schuh, MD, Amina Lalani, MD, Upton Allen, MBBS, David Manson, MD, Paul Babyn, MD, Derek Stephens, MSc, Shannan MacPhee, MD, Matthew Mokanski, RN, Svetlana Khaikin, RN, and Paul Dick, MD, Toronto, Ontario, Canada

GRAND ROUNDS

Ethical and Legal Implications of Genetic Testing in Androgen Insensitivity Syndrome

Jonathan S. Berg, MD, Shannon L. French, MD, Laurence B. McCullough, PhD, Soledad Kleppe, MD, Vernon R. Sutton, MD, Sheila K. Gunn, MD, and Lefkothea P. Karaviti, MD, PhD, Houston, Texas

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Hearing Loss in Biotinidase Deficiency: Genotype-Phenotype Correlation

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Pyruvate Kinase (PK) Deficiency in Newborns: The Pitfalls of Diagnosis

Serge Pissard, MD, PhD, Mariane de Montalembert, MD, Dora Bachir, MD, Isabelle Max-Audit, MD, PhD, Michel Goossens, MD, Henri Wajcman, MD, PhD, and Brigitte Bader-Meunier, MD, Créteil and Paris, France

Hospitalizations with Primary versus Secondary Discharge Diagnoses of Asthma: Implications for Pediatric Asthma Surveillance

David G. Bundy, MD, MPH, Chapel Hill, North Carolina

INSIGHTS

Esophageal Stenosis Due to Vascular Ring

Kazushi Yasuda, MD, Norihisa Koyama, MD, Hiroomi Murayama, MD, and Takashi Watanabe, MD, Aichi, Japan
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**Definition of Metabolic Syndrome**
Elizabeth Goodman, MD, Stephen R. Daniels, MD, PhD, and Lawrence M. Dolan, MD, Boston, Massachusetts, Denver, Colorado, and Cincinnati, Ohio

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Carolyn Chi, MD, Darrell Wilson, MD, and Thomas Robinson, MD, MPH, Stanford, California

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**Finding a Direction for Pediatric Assent**
Suzanne Manning, RN, JD, MA, Toronto, Ontario, Canada

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May 2007

The Programme for Global Paediatric Research Symposium: “Global Childhood Diseases Which Can Impair Development” and Workshop: “Outcome Studies.” May 8-9, 2007, Toronto. Sponsored by The Programme for Global Paediatric Research. PGPR’s fifth symposium will be held May 8, 2007 in conjunction with the annual meeting of the Pediatric Academic Societies. To register for the PAS Meeting, at which this symposium will be held, please go to www.pas-meeting.org. To register for the follow-up workshop on May 9, 2007, please contact Alvin Zipursky, Chair and Scientific Director; phone: 416-813-8762; E-mail: Alvin.zipursky@sickkids.ca; Website: www.globalpaediatricresearch.org.

18th Annual Spring Conference on Pediatrics. May 16-19, 2007, Marriott Frenchman’s Reef Beach Resort, St. Thomas, United States Virgin Islands. Sponsored by Symposia Medicus. For more information, contact Symposia Medicus; phone: 925-969-1789, 800-327-3161; E-mail: info@symposiamedicus.org; Website: www.symposiamedicus.org.

July 2007

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Pediatrics and Evidence-Based Medicine Revisited

ROBERT M. JACOBSON, MD

In 2006, Belamarich et al found with the AAP guidelines is not a condition limited to primary care; it is just the largest and most obvious target. Pediatric subspecialties also suffer from a deficit of evidence-based guidelines and a plethora of expert opinions. Sackett, by the way, not only calls for the mandatory retirement of experts but for a general policy of refusing to believe them altogether.

Now, the deficit of evidence is not necessarily for a lack of trying. All too often, the literature searches directed toward answering questions raised in the clinical pediatric subspecialties fail to find clinical studies and especially high-quality studies. In pediatrics, we are often dealing with a deficit of data, an absence of study, and an evidence-less base from which to operate.

Features of pediatrics limit the evidence base in ways that those practicing adult medicine can only imagine. First, many of the diseases that concern us, those with significant morbidity and mortality rates, are relatively rare. In contrast to our colleagues in adult medicine, we cannot easily access large patient numbers to have adequate statistical power for study.

Second, even when we can achieve the numbers, either by the nature of the disease or through networking or collaboration, we face significant ethical and regulatory concerns. Institutional review boards applying the Common Rule 45 CFR 46 hold pediatric studies to a higher standard. For studies that exceed minimal risk, issues of direct benefit and relevance to the individual subject become germane. Critical information such as normal values for certain diagnostic tests (particularly in radiographic imaging) are lacking because of restrictions on enrolling normal children in studies that exceed minimal risk. Even for those studies that do not exceed minimal risk, we not only need parental permission for enrollment but, depending on the age of the child or teenager, their assent as well. Our ethical concerns complicate our decisions regarding control or comparative maneuvers as well. Where standard care exists, with or without an evidence base to support it, we are loath to use a placebo control in minors. Although adults may consent to a randomization that may leave them untreated and at risk for worsening of their condition, children pose concerns because they cannot consent.

Third, running clinical trials are more difficult in the pediatric age group. One not only has to recruit parent and child rather than a single individual, one must retain the child through the study procedures, exposure to discomfort and boredom, and the parents often suffer additional costs of participating in the study that adult volunteers do not have—including school attendance, their own work schedules, and care for the siblings.

Fourth, much of pediatric subspecialty care suffers from a
therapeutic orphaning syndrome. Again, the volumes of these diseases are small. Pharmaceutical companies focus on adult disease where the numbers are greater. Clinical trials and studies are expensive. The multi-prong approach the Food and Drug Administration has taken to accelerate pediatric drug development is, of course, an important effort, as is the National Institutes of Health requirement for pediatric study where appropriate.

One of the more important passages in Frohna and Park’s 2002 call-to-arms addressed the problem that teaching and practicing EBM in pediatrics will rapidly reveal this deficit of evidence. The authors wrote that, in such situations, we must not abandon evidence-based pediatrics. Specifically, they wrote that in our efforts to teach EBM, we must not abandon teaching the skills of critical reasoning and the art of medical decision-making, which includes making decisions that deal with uncertainty and incorporate clinical experience and family preference as well. Critical thinking is not something that intelligent individuals have as a gift or an art that one learns by osmosis or through experience. One must study and practice logical and critical thought to obtain and retain that skill. We cannot assign that topic to the reading of a paper, the attendance of a conference, or the assignment to a rotation. In fact, one must practice it every day with each patient encounter regardless of whether the evidence-base is strong, weak, or absent. Callahan stressed the importance of supplementing EBM with rules regarding medical decision-making that one learns from mentors and experience: History and physical examination are the cornerstones of diagnosis. Be cautious in assuming “it’s probably nothing.” Trust your intuition. Follow-up when questions remain. Never stop becoming pediatricians. Read the textbook (or something). Be cautious about releasing responsibility for a patient to a consultant. Never underestimate parents’ desire to provide the best for their children. The physician should appreciate the parents’ level of concern. Good physicians can make bad mistakes.

Furthermore, when our literature searches of a clinical question fail to find studies specific to our patients, Phillips lays out an approach that allows pediatricians to adapt and adopt evidence. First, we should ask if the biologic differences between our patients and those studied really matter. Second, we should ask whether the demographic differences between our patients and those studied will really matter. Third, we should consider the risk for adverse events of those apparently beneficial and feasible therapies. We need to consider the baseline risks in our patients compared with those studied and weight that accordingly. Fourth, we should consider all of the outcomes measured and consider whether these outcomes, including the negative ones, are comprehensively assessed and applicable to our patients. By doing so, Phillips argues that we may be able to extend our reach into the pool of evidence and find applicable data.

Finally, part of our difficulty with the deficit of data is our perception that the data are lacking. Searching for studies is methodologically difficult and all too often incomplete. The Cochrane Collaboration has dedicated particular effort to the pediatric database to improve pediatric access to the data that do exist. The Cochrane Collaboration now includes a Child Health Field (http://www.cochranchildhealth.ualberta.ca/) that features a trials registry of more than 20,000 clinical trials in children and youth, as well as a new Cochrane review journal dedicated to those who care for children, entitled, Evidence based Child Health: A Cochrane Review Journal.

EBM should appeal to trainees and practitioners of pediatrics especially. We must protect our patients, with their higher degree of vulnerability, from the dangers of available technology. Even if a useless treatment was absolutely harmless, given the disparities in funding pediatric care, we must avoid any waste in time, cost, and effort. Also, given the relative rarity of pediatric subspecialty disease, our subspecialties are for the most part limited to academic centers and thus by necessity (and fortunately) remain scholarly fields for all of the practitioners. We are therefore more naturally attracted to seeking a scientific basis for our practice. Still, one does not learn EBM through the research experience but through clinical training and experience. We clearly need to improve the effectiveness of our teaching EBM and the use of EBM in practice. We should extend that academic focus of our specialty and subspecialties to include empirical evidence of diagnostic and therapeutic field-effectiveness whenever possible. That the evidence will often be limited or nonexistent is no reason to abandon the technique. In fact, those revelations should drive our quest for investigation and clarifying the role that critical thinking must have in medical decision-making when dealing with uncertainty.

REFERENCES
Almost 25 years ago, the first peer-reviewed publication reporting a randomized, masked trial of postnatal dexamethasone to treat bronchopulmonary dysplasia (BPD) appeared in The Lancet. Mammel et al treated 6 infants who had established BPD with 3 days of dexamethasone (0.5mg/kg/day) or placebo in a crossover design. The trial was stopped when sequential analysis showed significant respiratory benefit from dexamethasone, and the drug was then tapered for 1 to 4 months. Although the authors cautioned that “the therapy cannot be recommended without further study of patient selection, dosage schedules, short- and long-term side effects, and the mechanism of its action,” this and other small studies of short-term benefit led to early enthusiasm and widespread adoption of dexamethasone therapy to treat BPD.

In a stunning application of the “more is better” theory and in contrast to accepted principles of new drug testing (start with a low dose and escalate after assessing safety), this early enthusiasm resulted in treatment with high doses of dexamethasone for as long as 6 weeks and treatment ever earlier in life. Within 5 years, infants were being enrolled in studies of dexamethasone therapy starting on the first postnatal day. At its most widespread use, in the late 1990s, >25% of all very low birth weight infants were exposed to postnatal steroid therapy, and contamination of placebo groups with open-label dexamethasone treatment became a major confounder for larger randomized trials.

Along the way, numerous cautions were sounded both by the authors of these studies and in associated commentaries, but the beneficial effect of dexamethasone on respiratory function overrode the reports of numerous short-term adverse effects such as hyperglycemia, short-term growth failure, and hypertrophic cardiomyopathy. The first convincing reports of adverse effects of high-dose dexamethasone therapy on subsequent growth and development appeared 15 years after publication of the Mammel study. Although animal studies as early as the 1960s had consistently shown adverse effects on brain development from administration of high-dose glucocorticoids, the neonatology community was shocked. These new reports of long-term adverse outcomes led to a joint statement by the American Academy of Pediatrics and the Canadian Paediatric Society condemning further “routine use” of postnatal dexamethasone to treat or prevent BPD. Although the statement recommended additional clinical trials of dexamethasone and other glucocorticoids, the negative atmosphere made continuing enrollment in dexamethasone studies essentially impossible, ironically cutting short a randomized trial whose primary end point was neurodevelopmental outcome after a lower dose and shorter course of dexamethasone.

Further trials of dexamethasone now appear unlikely, even in view of new information suggesting benefits in specific circumstances. First, a meta-analysis indicated that the benefits of dexamethasone in decreasing death or cerebral palsy may outweigh its risks in infants at highest risk. Second, in this issue of The Journal, Nixon et al report improved respiratory outcomes at 8 years of age in infants treated with dexamethasone, compared with those treated with a placebo. Logistic regression analysis suggested that this improvement resulted at least in part from fewer days of mechanical ventilation. Because dexamethasone facilitates extubation in infants who are chronically dependent on a ventilator, the benefits of a brief course of therapy in such infants could outweigh the risks.

However, dexamethasone is not the only glucocorticoid available, and the rationale for choosing it in the first place is unclear, as is the choice of an initial dose as high as that used for acute meningitis or spinal cord compression. Synthetic glucocorticoids without mineralocorticoid activity may produce deleterious effects on neurodevelopment not only by exposing the immature organism to excess glucocorticoid, but also by suppressing endogenous cortisol production, thus producing a “chemical adrenalectomy.” At physiologic concentrations, cortisol binds to mineralocorticoid and glucocorticoid receptors in the brain and appears to be an important regulator of neuronal function. Experimental adrenalectomy results in degeneration of neurons, specifically in the hippocampus. Thus, adrenal insufficiency and glucocorticoid excess can both be associated with adverse neurologic consequences.

If the aforementioned principle pertains, treatment of premature infants with lower doses of hydrocortisone should result in significantly improved neurologic outcome, compared with dexamethasone or other synthetic glucocorticoid. In this issue of The Journal, Rademaker et al report neurodevelopmental and
magnetic resonance imaging outcomes at school age (7-8 years old) in a large cohort of premature infants, comparing 62 infants treated with hydrocortisone for BPD (5 mg/kg/day for 1 week, tapered for a minimum of 15 days) with 164 infants who were not treated with postnatal glucocorticoids.12

In comparison with the infants who did not receive postnatal hydrocortisone, who were larger, more mature, and less acutely ill, the infants receiving hydrocortisone had no apparent functional disadvantage or structural impairment with magnetic resonance imaging. This information is consistent with earlier reports from these authors, which showed improved outcomes for a cohort of infants treated with hydrocortisone compared with a separate cohort treated with dexamethasone.12 These findings are also consistent with information from a multicenter randomized trial, in which infants treated with early low-dose hydrocortisone (1 mg/kg/day) showed no evidence of neurodevelopmental compromise at 18 to 22 months adjusted age compared with infants who were treated with placebo.13

These studies provide an encouraging foundation for pursuing future studies of hydrocortisone therapy for BPD. As we proceed with such studies, however, let us learn from the dexamethasone experience and apply a more scientific approach. For example, as was the case for dexamethasone, the authors’ reason for choosing a particular dose of hydrocortisone was not clear. Although 5 mg/kg/day of hydrocortisone is a much lower glucocorticoid dose than 0.5 mg/kg/day of dexamethasone, it may still be higher than needed to achieve the desired effect. Data on pharmacokinetics of hydrocortisone in extremely premature infants are even more limited than information on pharmacokinetics of dexamethasone in this population.14

In the life cycle of a new therapy, early enthusiasm on the basis of small positive studies should lead to large, multicenter trials in which adverse effects are revealed and enthusiasm tempered. As the full range of a therapy’s benefits and risks become apparent through careful evaluation of these randomized, controlled studies, informed decisions can be made about the usefulness of the therapy itself. In the case of dexamethasone, the early small studies and the clinical frustration of treating infants with BPD led instead to widespread clinical use before, rather than after, large randomized trials. Thus began what now appears to be a long detour on the road of postnatal glucocorticoid therapy, with the use of an excessive dose of what may be the wrong medication at the wrong time.

So where are we now? Just as Mammel cautioned about dexamethasone in 1983, “the therapy cannot be recommended without further study of patient selection, dosage schedules, short and long-term side effects, and the mechanism of its action.” Clearly, the effects of glucocorticoid therapy will be a consequence of the drug, the dose, the timing, and the length of therapy. Much work remains to be done to evaluate those factors. Who to treat? When? With how much and for how long? By testing lower doses of a more appropriate medication and starting with careful evaluation of long-term outcomes from the first patients enrolled, we may be heading in a better direction.

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Is EGF the Holy Grail for NEC?

It has been 55 years since Schmidt and Quaiser reported the pathological and clinical findings from 85 patients with a new disease that they named necrotizing enterocolitis (NEC). Much has been learned in the ensuing years, although the morbidity and mortality from this complex disease has not significantly improved, and there are no effective strategies for prevention or treatment, except for delaying preterm delivery and providing breast-milk feeding. Nonetheless, new information from basic science and clinical studies provides hope for identifying early predictive markers, clarifying the pathophysiologic cascade, and providing an effective preventive approach for this dreaded intestinal catastrophe.

Epidermal growth factor (EGF) is one of many growth factors that stimulates receptors present on intestinal epithelium and promotes gut maturation and health. Studies have demonstrated the importance of EGF in preserving gut barrier function, increasing intestinal enzyme activity, and improving nutrient transport. In addition, EGF receptor (EGFR) knockout mice develop epithelial cell abnormalities and hemorrhagic necrosis of the intestine similar to neonatal NEC, suggesting that this molecule may play a role in the human condition. In support of this hypothesis, Halpern, Dvorak, and Clark and their colleagues have shown that EGF supplementation reduces the incidence of NEC in a neonatal rat model of NEC, in part by reducing apoptosis, barrier failure, and hepatic dysfunction, which have all been suggested as components of the pathophysiologic cascade.

Human studies have suggested that urinary and salivary EGF (sEGF) levels correlate with gestational age and inversely with NEC. In this issue of The Journal, Warner et al report on a robust series of 327 sEGF levels from premature and term infants and demonstrate that (1) initial levels directly correlate with gestational age and increase with postnatal age; (2) lower sEGF during the first week was associated with an increased incidence of NEC; and (3) greater increases in sEGF values in subsequent weeks 2 and 3 was associated with increase NEC. Additional data of interest suggested that Caucasian patients, nil per os status, and postnatal antibiotics were associated with lower sEGF levels. These findings suggest that EGF may play a role in neonatal NEC, and leads one to consider EGF as a predictive marker of disease, as a component of the pathophysiologic cascade, and/or as a preventive approach.

Neonatal NEC presents with acute clinical symptoms and signs, and in severe cases, it progresses rapidly to irreversible bowel necrosis and/or death. Early predictive markers might allow clinicians to alter feeding patterns and other clinical variables that might influence the incidence, onset, and severity of disease. Unfortunately, to date, the search for a reliable and consistent predictor of NEC has been unsuccessful. Investigators have considered blood cytokine values, blood and stool platelet activating factor (PAF) levels, blood cytosolic β-glucosidase, c-reactive protein, intestinal and liver fatty acid binding protein, t-crypt antigen on red blood cells, volume of gastric residuals, and several other markers, but none have proven reliable, easy to measure, and clinically useful. In this context, it would be exciting if sEGF could be an early predictive marker for NEC. Unfortunately, because of the wide ranges of values and somewhat unpredictable changes from week to week observed in the Warner study, this measurement may be no more predictive of NEC than the most reliable indicator—gestational age.

The pathophysiology of NEC has not been clearly elucidated, although substantial evidence suggests that in the preterm infant (who has an immature mucosal barrier) an unbalanced predilection toward the proinflammatory response results from stress factors that include feeding, bacterial colonization, and intestinal ischemia/reperfusion. Low levels of endogenous EGF as measured by sEGF during the first week of life by Warner et al are consistent with this hypothesis. EGF matures the mucosal barrier, decreases intestinal epithelial apoptosis, and down-regulates the proinflammatory response via signal transduction through EGF receptor and activation of PI3 kinase, JAK-STAT, and the Ras-MEK pathways. Of note, there are single nucleotide polymorphisms (SNPs) identified in the human EGF gene, and a higher risk for some clinical conditions such as malignant melanoma and gastric cancer have been associated with the EGF A61G genotype. Ethnic diversity has been associated with particular SNP patterns, and it seems likely that these differences can account for variations of disease incidence in different ethnic populations. Because epidemiologic data suggest an increase in NEC incidence in African-Americans, identification of SNP patterns in the EGF or EGFR gene (or multiple other genes) may help identify the importance of a variety of molecules in the pathophysiology of NEC.

Regardless of the successful identification of an early predictive marker or a complete understanding of the

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**Abbreviations:**

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<tr>
<th>EGF</th>
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<tr>
<td>EGFR</td>
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<td>NEC</td>
<td>Necrotizing enterocolitis</td>
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<td>sEGF</td>
<td>Salivary EGF</td>
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<td>SNP</td>
<td>Single nucleotide polymorphisms</td>
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pathophysiology of neonatal NEC, an effective preventive therapy is the ultimate "Holy Grail." There have been multiple human trials reported to reduce the incidence of NEC, including human milk, IgA supplementation, prophylactic antibiotics, dexamethasone, polyunsaturated fatty acids, and, most recently, probiotic supplementation. Because of the inability to conclusively reproduce some results or because of some unexpected deleterious effects, human milk feeding is the only currently accepted modality for NEC prevention. Nonetheless, probiotic supplementation has garnered significant interest, and large phase III trials will soon be underway to confirm this potentially beneficial approach of gut colonization with commensal bacteria. Of interest, human milk contains bioactive EGF with concentrations similar to those found in the saliva reported in the Warner study. Human milk is replete with multiple antimicrobial and antiinflammatory factors that might contribute to a reduction in NEC incidence, but it is intriguing to consider EGF as one of the key components. Further, it has been shown that postnatal steroid therapy increases EGF levels in preterm infants and that probiotics can improve EGF binding to EGFR following pathogenic infection. These observations, coupled with the compelling animal studies and human data published by Warner et al and others, suggest that EGF supplementation in feedings may be an effective strategy for the prevention of NEC in preterm infants. In conclusion, there is a growing body of evidence that suggests abnormal EGF regulation in the preterm infant may contribute to the development of neonatal NEC. Warner et al should be congratulated for their extensive contributions to our understanding of the clinical relevance of EGF as well as the biochemical responses that regulate intestinal apoptosis, proliferation, and healing. Further investigation is needed to confirm the importance of EGF in the pathophysiological cascade and to identify a potential role in the prevention of NEC.

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The Systemic Effects of Short Sleep Period

The prevalence of obesity in children continues to increase at an alarming rate. In 2004, 17% of children in the United States were obese, an increase of more than 20% in 6 years. Parallel to this trend in obesity, the prevalence of impaired glucose tolerance and type 2 diabetes mellitus in children has increased dramatically. One study found that more than 20% of obese children referred to a weight management program had impaired glucose tolerance, and 4% of the obese adolescents had type 2 diabetes mellitus. There is substantial evidence linking obesity to an increased risk of insulin resistance. Insulin resistance is predictive of the development of type 2 diabetes mellitus and is central to the development of the metabolic syndrome. Thus, prevention of obesity and insulin resistance in children is of great public health importance.

A key to effective prevention strategies is identifying risk factors for disease. Along these lines, attention has been directed toward gaining more insight into the possible role of sleep disorders in the development of obesity, insulin resistance, and other risk factors for cardiovascular disease in children.

In the current issue of The Journal, Flint et al report on an investigation into the relationships among nocturnal sleep duration, sleep-disordered breathing (SDB), and markers of insulin resistance in a cohort of 40 obese children evaluated for sleep-related complaints. Children receiving less than 6 hours of sleep on overnight polysomnography demonstrated increased insulin resistance as measured by the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) compared with those receiving more than 6 hours of sleep. There was no difference in mean body mass index between the 2 groups. Similarly, children with SDB had significantly higher HOMA-IR values than those without SDB, indicating a greater degree of insulin resistance. In multiple linear regression analysis, increasing age and decreasing percentage of rapid eye movement sleep were associated with a higher index of HOMA-IR. The severity of SDB, as measured by the apnea-hypopnea index, was not predictive of HOMA-IR. Notably, increased—not decreased—sleep duration was associated with a greater degree of insulin resistance in the regression model.

A strong association has been demonstrated between short sleep period and obesity in children. In a large cross-sectional study of 6- to 7-year-old Japanese children, Sekine et al found a dose-response relationship between short sleep period and obesity. After adjusting for physical activity and television watching, children receiving less than 8 hours of sleep per night were almost 3 times as likely to be obese than children receiving 10 or more hours of sleep per night. Multiple studies of pediatric cohorts have replicated these findings.

The relationships among sleep period, glucose metabolism, and insulin resistance have been studied primarily in adults. In an experimental study of healthy adult subjects, Spiegel et al found that restriction to 4 hours of sleep for 6 consecutive nights was associated with impaired glucose tolerance. Normalization of the sleep period resulted in resolution of glucose intolerance. Gonzalez-Ortiz et al reported decreased insulin sensitivity after 24 hours of sleep deprivation in healthy subjects. Numerous observational studies in adults have also demonstrated an increased prevalence of type 2 diabetes mellitus in subjects with habitually short sleep periods. However, in 2 of these studies, the association was no longer significant after adjusting for potential confounders, including obesity. The association between obesity and both short sleep period and insulin resistance raises the question of whether the observed relationship between short sleep period and insulin resistance is at least partially mediated through the development of obesity.

The possibility that sleep loss may play a role in the development of obesity and insulin resistance has biologic plausibility. Studies have demonstrated that short sleep period is associated with decreased serum leptin and increased ghrelin levels, which promote increased appetite. Furthermore, sleep loss has been associated with increased levels of the counterregulatory hormone cortisol and with activation of the sympathetic nervous system, creating a milieu favoring insulin resistance. In addition, the up-regulation of proinflammatory mediators, such as tumor necrosis factor, interleukin-6, and C-reactive protein (CRP), in association with sleep loss represents an alternative pathway linking sleep disorders to insulin resistance. Increasing levels of CRP have been demonstrated to predict the development of type 2 diabetes mellitus. Therefore, it is plausible that habitual sleep loss may lead to a chronic inflammatory state predisposing to the development of insulin resistance.

The study of Flint et al is an important first step in investigating the relationship between short sleep period and insulin resistance in children. This study has several limitations, however.

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CRP C-reactive protein
HOMA-IR Homeostasis Model Assessment of Insulin Resistance
SDB Sleep-disordered breathing
The cross-sectional design prevents assessment of the direction of causation in the relationships among short sleep period, SDB, and insulin resistance. Moreover, in multiple linear regression analysis, increased—not decreased—sleep duration predicted a greater degree of insulin resistance. Therefore, the finding of increased insulin resistance in children with short sleep period may be due to confounding factors. Alternatively, the relationship between sleep period and insulin resistance may be nonlinear in nature. Several studies in adults have demonstrated increased insulin resistance with both long and short sleep periods relative to 7 or 8 hours of sleep per night.12,14-15

Prospective studies are needed to characterize the complex interactions among sleep disorders, obesity, and insulin resistance. The study of such a complex model requires sophisticated statistical approaches to determine the relative contributions of SBD and sleep loss to the risk of developing insulin resistance. In future investigations of the relationships among sleep period, SDB, and insulin resistance, incorporating measures of statistical mediation22 will prove essential in determining direct causation versus effects mediated through intermediate processes such as obesity.

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REFERENCES

Childhood Cancer Cures: The Ongoing Consequences of Successful Treatments

In 1979, with colleagues in hematology/oncology and neurology at Memorial Sloan-Kettering Cancer Center, New York, we began a Late Effects Endocrine Clinic. Patients were only a few months post-treatment, and most of the patients were survivors of childhood brain tumors. Our treatment options were limited, little was known about long-term disease survival, risks of endocrine therapy, or the development of secondary malignancies.

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We published our preliminary findings on a small cohort of patients and noted marked impairment of statureal growth and the development of primary hypothyroidism. Over the past more than 25 years, multiple studies have documented the development of endocrinopathies that impact normal growth and development. Indeed, some of these analyses have resulted in the modification of treatment protocols.

Today, there are close to 270,000 survivors of childhood cancer, and nearly 1 in 640 persons between the ages of 20 and 39 years has had some form of childhood malignancy. Long-term consequences—now close to 30 years after diagnosis and treatment—include “early death, secondary neoplasm, organ dysfunction . . . reduced growth and development, decreased fertility, impaired intellectual function, difficulties obtaining employment and insurance, and overall reduced quality of life.” The good news is that most survivors do not experience serious untoward effects as a direct consequence of their cancer or its treatment. However, ongoing vigilance is crucial, as was recently shown by Oeffinger et al, who evaluated chronic health conditions in adult survivors of childhood cancer by using data from the Childhood Cancer Survivor Study (CCSS). The CCSS is a retrospective cohort of 5-year survivors of childhood cancers in whom a diagnosis was made before the age of 21 years between 1970 and 1986, with 26 sites contributing data from the United States and Canada. This study also compares results with those of siblings (>20,000 initially enrolled, with approximately 5000 siblings amassed). Cancer diagnoses include leukemia, central nervous system (CNS) tumors (excluding craniopharyngiomas), Hodgkin’s disease, non-Hodgkin lymphoma, Wilms tumor, neuroblastoma, and sarcoma and bone tumors. This study determined the prevalence of chronic conditions in survivors and siblings with a grading scale for untoward outcomes (grade 1, mild, through grade 4, life-threatening or disabling) and assessed whether a person had ≥2 health conditions. Chronic conditions included replacement of joints, congestive heart failure, second malignancy, severe cognitive dysfunction, coronary artery disease, cerebrovascular accident, renal failure or dialysis, loss of hearing or eyesight, and ovarian failure in female patients. Sixty-two percent of survivors, compared with 37% of siblings, reported having at least 1 chronic health condition, but only 28% reported a grade 3 or 4 condition. Twenty-five percent of survivors (compared with 5% of siblings) reported having ≥3 conditions. The cancer survivors were 8 times more likely than their siblings to have a severe or life-threatening chronic health condition; persons at highest risk were survivors of bone tumors, CNS tumors, or Hodgkin’s disease. Furthermore, severe life-threatening conditions were increased when cancer treatment included chest, abdominal, or pelvic radiation therapy (RT). Sex differences also were noted; female survivors were at greater risk than male survivors for growth hormone (GH) deficiency and obesity.

Previous papers from the CCSS have also assessed the risk in survivors of childhood brain cancers for reduced final height, alterations in body mass index (BMI), use of GH treatment, and related outcomes of GH treatment and risk of secondary malignancy. It appears that although GH therapy does not increase the risk of recurrence of the primary disease, secondary malignancies are 2.15 times more likely (5%-95% CI, 1.3-3.5) to develop in survivors treated with GH, with meningiomas being the most common tumor reported in the group treated with GH.

Perhaps the most worrisome late effect in survivors of childhood cancer and acute lymphoblastic leukemia (ALL), specifically, is the onset of obesity and associated co-morbidities. In another, recent CCSS study by Oeffinger, 1765 adult survivors were compared with 2565 siblings. The risk of obesity was greatest in female survivors in whom ALL was diagnosed before the age of 4 years who were treated with cranial RT doses  20 Gy (RR 3.81), whereas no increased risk was seen when treatment involved only chemotherapy or lower doses of cranial irradiation. Most recently, a study from Australia compared the prevalence of overweight/obesity, abdominal adiposity, hyperinsulinemia, impaired glucose tolerance, and diabetes mellitus in 248 survivors of childhood cancers. Although in this study the prevalence of overweight/obesity was not increased, the incidence of abdominal adiposity in both prepubertal and pubertal children was nearly doubled. Body RT, untreated hypogonadism, and abdominal adiposity were found to be independent risk factors for the development of hyperinsulinemia, impaired glucose tolerance, and diabetes mellitus. A total of 116 patients had ALL, at a mean age of 3.8 years, followed to a mean age of 20.4 years; 21 patients underwent bone marrow transplants, 13 patients underwent total body RT, and 92 patients underwent pituitary RT. Of the 39 subjects in whom hyperinsulinemia and impaired glucose tolerance were diagnosed, 15 had ALL, 10 of whom had undergone bone marrow transplants, 8 of whom had undergone total body RT, and 6 of whom underwent pituitary RT at doses >30 Gy. Sixteen adult patients with ALL who had undergone bone marrow transplants, compared with the 82 who did not, had a >25-fold risk for the development of hyperinsulinemia, impaired glucose tolerance, and diabetes mellitus. Because of the worldwide epidemic of obesity, children with ALL would seem to benefit from ongoing nutritional counseling and prudent dietary intake.

In this issue of The Journal, the paper by Chow et al adds to the current body of knowledge on the long-term consequences of cancer treatment for ALL. It also addresses the need for future outcome analysis in ALL, specifically for the use of various chemotherapeutic agents, without CNS radiation. Earlier reports on growth in survivors of ALL have shown loss of height after RT doses >24 Gy, but consistent patterns of growth are not well defined for treatment with chemotherapy alone. In a cross-sectional study by Chow et al, adult height was determined in 2434 survivors of ALL who participated in the CCSS and was compared with that of 3009 siblings, of whom 818 were actual siblings of survivors of ALL. The mean age of the survivors was 27 years, and the mean age of the siblings was 31 years.
Exposure and outcome assessment reviewed cumulative chemotherapy doses for anthracyclines, epipodophyllotoxins, and methotrexate (given intravenously, intramuscularly, or intrathecally). Cumulative scores were created to define the overall intensity of treatment. CNS irradiation doses were assessed, and 95% of the survivors received between 15 and 29 Gy to the spine. Height was determined either with self-report or proxy-reported points. Although self-reporting of heights may overestimate true height by at most 2 cm, these findings still revealed a significant height deficiency in the cancer cohort. Data also were analyzed as to whether the patient was prepubertal or pubertal at the time of initial treatment. The authors state that year of diagnosis or date of birth did not affect statistical models, so these were not included for additional analysis. This study is unique because, to date, it reports on the largest group of survivors of ALL who were evaluated for true adult height, and it allows for evaluation of final height in a number of patients with ALL who were exposed to chemotherapy alone.

This paper reports that patients with ALL, irrespective of treatment exposure (chemotherapy alone or in combination with cranial or craniospinal RT), have decreased adult heights and are at a 12.5-fold risk of decreased final height (height > 2 SD below that of siblings). Furthermore, even with chemotherapy alone, there was a >3-fold increased risk of decreased stature. Patients who were prepubertal at the time of diagnosis and received ≥20 Gy, compared with <20 Gy, or who had any spinal RT or were female had the greatest risk (12.5% versus 5.5%).

The greatest impact on height occurred in male survivors who had received craniospinal RT ≥20 Gy plus chemotherapy; their mean adult height was 168.5 ± 10 cm or 5’6”, compared with 180.1 cm or 5’11” in male siblings. For female survivors, the lowest mean final height also was in this group and was reported as 155.7 cm or 5’1½”, compared with 165.5 cm or 5’5½” for female siblings. None of the patients received GH. The mean final heights were still within 2 SDs for sex and height for >18 years of age, although the data demonstrated a loss of ~0.69 SD for male survivors and ~0.74 SD for female survivors compared with sibling control groups.

These findings are important, particularly because the most recent studies suggest that the overall risk of a secondary neoplasm developing in survivors of childhood cancer is 2.15 higher for those who have undergone GH treatment than for those who did not, although the risk appears to be decreasing with time of follow-up.10 Patients who had leukemia and received GH had a 2.3 greater relative risk of a second malignancy developing than patients who did not receive GH. These second malignancies included osteosarcoma of bone and lower extremity, astrocycoma, and glioma.10 Because many of these children also are known to have early onset or rapid pubertal progress, treatment of these children with growth augmenting therapies, such as GH or agents designed to halt pubertal progress (gonadotropin releasing hormone agonists) still needs to determined on a case-by-case basis.

Chow et al provide yet another reminder that the treatment of childhood cancers is no longer simply within the realm of a few pediatric endocrinologists and oncologists. Optimal outcome for these young adults will result from continued review of long-term multicenter outcome studies and aggressive vigilance. Early supportive care and intervention should allow, ultimately, for appropriate modification of treatment regimens, anticipation of co-morbidities, and further reduction of severe, untoward effects.

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REFERENCES

B cell depletion using rituximab, initially developed as a treatment modality for B cell neoplasms and US Food Drug Administration-approved for that purpose in 1997, is a rising therapy for various immune-mediated diseases. In this issue of The Journal, El-Hallak et al retrospectively describe their experience with B cell depletion therapy in the treatment of pediatric autoimmune diseases in a single pediatric institution. Their 10 patients had a variety of conditions. Some of these conditions, such as Evans syndrome or systemic lupus erythematosus (SLE), are well known to respond to rituximab. Several other patients had less well-categorized autoimmune conditions for which there has been no previous experience with B cell depletion. Although the literature on the use of rituximab in immune-mediated diseases is expanding, the data on pediatric patients are still very limited; therefore, this report is important. Multiple clinical trials are currently underway studying a wide variety of diseases (Table). In addition, case reports suggest that B cell depletion therapy is effective in many other immune-mediated conditions, such as myasthenia gravis, chronic inflammatory demyelinating polyneuropathy, renal transplantation, or ABO-mismatched transplantation, to name just a few.

Rituximab is currently Food and Drug Administration-approved only for 1 autoimmune condition, moderately to severely active rheumatoid arthritis in adults. Unfortunately, controlled clinical trials for pediatric patients are much harder to perform because of the small number of eligible patients. Therefore, the interpretation for the safety and efficacy of B cell depletion therapy in pediatric autoimmune disease largely relies on the analysis of case series and extrapolation from adult studies.

Rituximab is a chimeric anti-CD20 monoclonal antibody, comprising a human IgG1κ constant region and a murine variable region. CD20 is a signature B cell differentiation antigen that is expressed on the surface of pre-, naïve, mature, and memory B cells, but not on plasma, early pro-B, or stem cells. Depletion of B cells is thought to be mediated by the induction of antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, and apoptosis. B cells are not only precursors of antibody-producing plasma cells, but also highly effective antigen-presenting, cytokine-producing, and regulatory cells. The effects of rituximab probably rely on interference with all these functions. Rituximab not only depletes B cells, but can also alter B cell homeostasis and improve B cell abnormalities. Although plasma cells are not depleted, a reduction in pathogenic autoantibodies is seen. One example is a decrease in rheumatoid factor titers in rheumatoid arthritis. This is presumably caused by interference with activation and differentiation of B cells toward the effector plasma cell. Total immunoglobulin levels usually remain within reference range. The effect obtained by rituximab is transient in most cases. El-Hallak et al reported that B cell repopulation occurred at a mean of 8 months, although 2 patients continued to have prolonged B cell depletion 5 years and 17 months after initial treatment. This observation is consistent with earlier studies that showed B cell repopulation at a mean of 8 months after rituximab therapy. Ng et al report that repeat treatment with rituximab is effective; their series included 7 adult patients with refractory SLE who responded to as many as 3 separate cycles of rituximab.

Is rituximab safe? Overall, rituximab appears to be a well-tolerated treatment with a modest toxicity profile, although data are very limited for pediatric patients. More than 540,000 patients worldwide have received rituximab, most for the treatment of B cell malignancies. Accordingly, most of the safety information stems from the treatment of malignancies rather than autoimmune disease; this makes the safety assessment somewhat difficult, because the concomitant aggressive chemotherapeutic regimens often have significant toxicities in their own right. El-Hallak et al also reported an acceptable toxicity profile for a prolonged follow-up period in their study. One death was reported, but was probably caused by the severity of the underlying disease process. Serious infections occurred, but at the same rate as before rituximab therapy. Interpretation of their data is limited by the small number of patients. According to the literature, the most common adverse effects observed with rituximab are infusion-related and relatively easy to manage, similar to those seen with other chimeric monoclonal antibodies. Infusion-related adverse effects include transient fever, chills, nausea, headache, and less common allergic reactions, such as bronchospasm, urticaria, pruritus, rash, flushing, and angioedema. More severe reactions, such as hypotension, or frank anaphylaxis, occur rarely. Infusion-related adverse effects are more common with the initial infusion. Edwards et al reported that in adult patients with autoimmune diseases, delayed respiratory reactions occur as long as 10 days after rituximab infusion, with symptoms suggestive of bronchitis, pneumo-
Table. Immune-mediated conditions studied for their response to B cell depletion therapy

<table>
<thead>
<tr>
<th>Rheumatic diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Systemic lupus erythematosus</td>
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<tr>
<td>- Rheumatoid arthritis</td>
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<tr>
<td>- Sjögren syndrome</td>
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<tr>
<td>- Inflammatory myopathy (dermatomyositis, polymyositis, juvenile dermatomyositis)</td>
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<tr>
<td>- ANCA-associated vasculitis</td>
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<td>- Systemic sclerosis</td>
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<tr>
<th>Hematologic diseases</th>
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</thead>
<tbody>
<tr>
<td>- Cold agglutinin disease</td>
</tr>
<tr>
<td>- Aplastic anemia</td>
</tr>
<tr>
<td>- Acquired pure red cell aplasia</td>
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<tr>
<td>- Idiopathic thrombocytopenic purpura</td>
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<tr>
<td>- Thrombotic thrombocytopenic purpura</td>
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<tr>
<td>- Evans syndrome</td>
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<tr>
<td>- Acquired hemophilia</td>
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<td>- Hemophilia with high titer inhibitor</td>
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<table>
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<tr>
<th>Neurologic diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Chronic focal encephalitis (Rasmussen’s encephalitis)</td>
</tr>
<tr>
<td>- Multiple sclerosis</td>
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<tr>
<td>- Opsoclonus myoclonus syndrome</td>
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<td>- Polyneuropathy with immunoglobulin M autoantibodies</td>
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<td>- Stiff person syndrome</td>
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<tr>
<th>Renal diseases</th>
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<tbody>
<tr>
<td>- Lupus nephritis</td>
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<tr>
<td>- Membranoproliferative glomerulonephritis</td>
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<tr>
<th>Gastrointestinal diseases</th>
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<tr>
<td>- Ulcerative colitis</td>
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<td>- Primary biliary cirrhosis</td>
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<tr>
<th>Endocrine diseases</th>
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<tr>
<td>- Graves disease</td>
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<td>- New onset type I diabetes mellitus</td>
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<table>
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<tr>
<th>Other diseases</th>
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<tbody>
<tr>
<td>- Pemphigus</td>
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<tr>
<td>- Bullous pemphigoid</td>
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<tr>
<td>- Chronic urticaria</td>
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<tr>
<td>- Chronic graft versus host disease</td>
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nitis, or serositis; those reactions are hard to differentiate from infections. Severe adverse effects include hematologic toxicity, such as thrombocytopenia and neutropenia. Many of those cases may have been caused by the concomitant use of myelosuppressive agents rather than rituximab itself. However, cases of severe neutropenia and thrombocytopenia have also been reported in childhood autoimmune hemolytic anemia when rituximab was used as a single agent. The pathogenesis of this complication is unclear. Clearly, there is a lower incidence and severity of adverse events in the treatment of autoimmune diseases when compared with malignancies, presumably because of the lack of a tumor lysis and cytokine release syndrome. According to Bennett et al, one exception may be the occurrence of serum sickness, which occurred at a rate of 12% in pediatric subjects who were treated for chronic idiopathic thrombocytopenic purpura. Infectious complications, particularly opportunistic infections, have been described, but it is unclear how significant this risk is, because patients often receive concomitant immunosuppressive medications. Long-lasting effects of rituximab on the developing immune system are not known. In 1 case report of the use of rituximab during pregnancy, complete B cell depletion was noted in the newborn. B cell repopulation occurred by the age of 4 months, leading to a normal immune status at the age of 20 months. To what extent this observation can be extrapolated to pediatric patients is not clear.

How should rituximab therapy be monitored? In addition to disease-specific clinical and laboratory markers, are there rituximab-specific monitoring parameters that can help to fine tune the therapy? An obvious monitoring parameter is the number of circulating B cells with flow cytometry. A low number of circulating B cells is indicative of successful B cell depletion. However, re-population of B cells is not necessarily associated with clinical relapse. Leandro et al report that approximately 50% of their patients with rheumatoid arthritis experience a relapse of disease with the return of B cells, and the other 50% at a variable time thereafter, often after prolonged periods, similar to this study’s findings. What differentiates these patients is unclear. Patients who relapse early tend to repopulate with memory B cells, rather than with naïve B cells. This might help in identifying patients who are at high risk for relapse after B cell depletion.

The assessment of changes in B cell abnormalities and recurrence of specific B cell subtypes requires specific studies and assessment of B cell panels. Primary B cell abnormalities are also common. In SLE, an expansion of memory B cells and CD27+ plasmablasts is often seen and may correct with rituximab therapy. Other specific markers useful in rituximab therapy may include the B cell activating factor of the tumor necrosis factor family (BAFF), also known as B lymphocyte stimulator. BAFF plays a major role in B cell activation and survival. It is produced mostly by monocytes/macrophages and acts on B cells through different receptors. BAFF levels have been shown to be elevated in many autoimmune diseases, most prominently in SLE. BAFF levels are not increased in all patients with SLE, but only in approximately 50% of these patients. BAFF levels increase after rituximab therapy. Presumably, this increase occurs by different mechanisms: An early increase simply by a decrease in corresponding receptors, therefore increasing free BAFF levels, and by a delayed upregulation of BAFF through transcriptional mechanisms. After rituximab therapy, BAFF levels initially rise sharply, but then decrease with B cell repopulation. On one hand, it remains to be seen whether monitoring of BAFF is helpful in determining which patients are at risk for disease relapse. However, the co-administration of rituximab and anti-BAFF therapy is, at least theoretically, a very tempting approach for obtaining long-term depression of B cell function, and, thus, disease remission. A BAFF antagonist, belimumab, has already been used in phase II clinical trials.

In summary, B cell depletion therapy is a very promising treatment modality for a wide spectrum of immune mediated diseases, but larger controlled trials are needed. Much
remains to be learned about the appropriate monitoring of therapy.

*Added while in print:* A recent report by the FDA indicates that patients treated with rituximab may be at an increased risk for progressive multifocal leukoencephalopathy (http://www.fda.gov/cder/drug/infopage/rituximab/default.htm).

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REFERENCES

Monoclonal antibodies have had a major impact on the diagnosis and treatment of many disorders. First discovered in the 1980s, the original monoclonal antibodies were composed entirely of mouse protein, which resulted in the development of human anti-mouse antibodies, preventing repeated use. Advances in antibody engineering resulted in chimeric antibodies with both human and mouse components and, more recently, humanized antibodies composed almost entirely of human protein (>90%). The reduction in the mouse components of monoclonal antibodies has allowed for repetition of these treatments by decreasing and in some cases eliminating the development of human anti-mouse antibodies. Tens of thousands of monoclonal antibodies have been produced, but to date fewer than 20 have been licensed for use in patients in the United States.

Rituximab is a chimeric monoclonal antibody specific for CD20, an antigen expressed on B cells. In 1997, it was the third monoclonal antibody approved by the Food and Drug Administration, after OKT3 and abciximab. The efficacy of rituximab in treating non-Hodgkin’s B-cell lymphoma (NHL) and its relative lack of toxicity have lead to its adoption in most standard treatment protocols for B-cell lymphomas and its use to treat a wide spectrum of B-cell disorders, including autoimmune diseases and other malignancies. More than 540,000 patients worldwide have been treated with rituximab, primarily for B-cell lymphomas. Only 2 monoclonal antibodies have been more widely used, both of which are also chimeric: abciximab, an anti-integrin used in more than 1 million cardiac patients, and infliximab, an anti–tumor necrosis factor (TNF)-α agent used in approximately 770,000 patients with Crohn’s disease and rheumatoid arthritis.

Although rituximab has been widely used in adults for 10 years, studies in the pediatric population are limited and include primarily case reports and small retrospective and prospective cohort studies. Specifically, there have been no randomized controlled studies on the safety or efficacy of rituximab in children. The package insert states that rituximab has not been studied in children; however, rituximab has been explored in children for a number of hematologic conditions, including autoimmune hemolytic anemia (AIHA), chronic immune thrombocytopenic purpura (ITP), antibodies to factors VIII and IX, posttransplantation lymphoproliferative disease (PTLD), pre–B-cell acute lymphoblastic leukemia (ALL), and B-cell NHL. This article reviews the current literature on these uses of rituximab in children, including articles and abstracts through July 2006.

**MECHANISM OF EFFECT**

Rituximab is a chimeric monoclonal antibody composed of murine variable regions fused with human IgG1 heavy chains and kappa light chains. Rituximab is specific for the CD20 antigen, a 35-kd transmembrane protein expressed on all normal and malignant mature B-lymphocytes. CD20 is not expressed on stem cells, pro-B-cells, most pre-B-cells, most plasma cells, or any other normal human tissue. It does not circulate in plasma, is not shed from or internalized by the B cell, and is not down-regulated on antibody binding. Its physiological role is poorly understood. There is no known natural ligand, and CD20 knockout mice show no significant defects in B-cell function. Based on structural homologies, CD20 is hypothesized to be a calcium channel subunit involved in B-cell activation, differentiation, and/or cell cycle progression.

Rituximab binds to CD20, causing rapid depletion of circulating CD20+ B cells. Circulating B cells are profoundly depleted, and the number of B cells in lymph nodes is
Treatment with Rituximab in Benign and Malignant Hematologic Disorders in Children

SAFETY PROFILE

The standard dose of rituximab is 375 mg/m²/dose administered intravenously on a weekly schedule for 4 weeks. This dose was not chosen based on any studies of pharmacokinetics or the response to the drug, however, and other dosing schedules have been used successfully. Rituximab has been generally well tolerated in adult and pediatric patients. Most reported side effects are mild or moderate and infusion-related.

The first infusion often causes a syndrome of chills, fever, headache, and occasionally dyspnea, nausea, pruritis, angioedema, or hypotension. These symptoms are mediated by the release of inflammatory cytokines such as TNF-α, interleukin (IL)-8, and interferon (IFN)-γ both directly secondary to B-cell destruction, as well as indirectly by macrophage activation. Among lymphoma patients, reactions are more common and more severe in patients with a high tumor burden. The symptoms usually resolve completely with slowing or temporary interruption of the infusion. Premedications such as antihistamines, antipyretics, and corticosteroids are commonly used and can be readministered to mitigate the reactions. Subsequent infusions are much less likely to cause such reactions.

Among the approximately 540,000 patients worldwide who have been treated with rituximab, tumor lysis syndrome and severe infusion reactions have been reported only rarely, with a small number of fatalities. The most severe cases were in patients with high tumor burden, multiple rounds of previously administered chemotherapy, advanced age, and comorbid conditions, such as pulmonary or cardiovascular disease. Anaphylactoid reactions have been reported, which may be exaggerated “first infusion” cytokine reactions. Severe mucocutaneous syndromes, including Stevens-Johnson syndrome, paraneoplastic pemphigus, and toxic epidermal necrolysis, also occur occasionally. In October 2004, the manufacturer issued a warning stating that carriers of hepatitis B are at risk for its reactivation with development of fulminant hepatitis. A recent FDA alert reported death in two patients being treated with rituximab from progressive multifocal leukoencephalopathy secondary to JC virus reactivation. The patients were both being treated for SLE.

Serum sickness is relatively infrequent, despite the fact that rituximab is chimeric rather than humanized. Two recent reports, however, suggest that serum sickness may develop in 5% to 10% of children receiving rituximab to treat ITP.

An important concern with the use of rituximab has been the risk of developing new infections, which generally do not occur despite the immunologic changes. Treatment with rituximab results in immediate, marked B-cell depletion, with levels remaining low for 2 to 6 months and returning to pretreatment levels only after 6 to 12 months. Physicians should not administer immunizations at any age until the B-cell population has returned. Despite substantial prolonged B-cell depletion, an increased incidence of infection has not been demonstrated. Furthermore, studies (primarily in adults) have generally found very little to no change in immunoglobulin levels. This is likely due to the persistence of both plasma cells and B cells in lymph nodes. Despite the general lack of change of immunoglobulin levels, prophylactic treatment with intravenous immunoglobulin (IVIG) has been used after rituximab therapy in younger children, who are thought to be at higher risk of transient hypogammaglobulinemia. Studies of rituximab in combination with chemotherapy for lymphoma report a higher rate of neutropenia than that seen with chemotherapy alone, but again without a significant increase in infections. A small number of case reports have described serious viral infections in patients receiving rituximab in conjunction with pretransplantation and posttransplantation chemotherapy.

METHODS

A literature search of articles and abstracts published on the use of rituximab in pediatric hematologic and oncologic disorders was performed. Cohorts, case reports, and abstracts published by July 2006 were included. Reports on the use of rituximab for the following diseases were reviewed: AIHA, chronic ITP, antibodies to factor VIII/IX, PTLD, pre-B-cell ALL, and B-cell NHL. No articles were identified describing the use of rituximab in pediatric patients with thrombotic thrombocytopenic purpura. Although there have been a number of reports on the use of rituximab in other autoimmune disorders, such as SLE, they are outside the scope of this review.

Case reports were presented separately from cohort studies given their bias in favor of positive results. Case reports were arbitrarily defined as 3 or fewer patients. Publications were analyzed for the following variables: age, sex, diagnosis, treatment before rituximab therapy, rituximab dose, response to treatment and duration of response, and adverse events. Results are summarized by treatment indication in the Table.
<table>
<thead>
<tr>
<th>Diagnosis–study type</th>
<th>n</th>
<th>% male/female</th>
<th>Age range (median)</th>
<th>Other diagnoses</th>
<th>Previous treatment</th>
<th>Doses of rituximab, range (median)</th>
<th>Response rate</th>
<th>Follow-up range (median)</th>
<th>Toxicity/side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIHA cohort studies</td>
<td>25</td>
<td>38/62</td>
<td>4 mo-15 yr (35 mo)</td>
<td>SLE, rheumatoid arthritis, ITP</td>
<td>Steroids, IVIG, cyclosporine, cyclophosphamide, splenectomy, azathioprine, tacrolimus</td>
<td>1-6 (4)</td>
<td>92%</td>
<td>7-28 mo (16 mo)</td>
<td><em>Escherichia coli</em> pyelonephritis, bronchitis, varicella pneumonia, varicella zoster (2), <em>Pneumocystis carinii</em> pneumonia</td>
</tr>
<tr>
<td>AIHA case reports</td>
<td>14</td>
<td>54/46</td>
<td>2 mo-18 yr (7 yr)</td>
<td>Beta-thalassemia, severe combined immunodeficiency, SLE, ITP, diabetes mellitus, Evans syndrome</td>
<td>Steroids, IVIG, cyclophosphamide, azathioprine,</td>
<td>2-8 (4)</td>
<td>93%</td>
<td>1-30 mo (14 mo)</td>
<td>Fatal RSV pneumonia, fungal infection</td>
</tr>
<tr>
<td>ITP cohort studies</td>
<td>82</td>
<td>48/52</td>
<td>2 yr-19 yr (11 yr)</td>
<td>Evans syndrome (n = 6)</td>
<td>Splenectomy, IVIG, anti-D, danazol, cyclophosphamide, azathioprine, vincristine</td>
<td>1-4 (4)</td>
<td>43%</td>
<td>3-30 mo (9 mo)</td>
<td>Neutropenia (3), serum sickness (3), hypotension with third dose, primary varicella</td>
</tr>
<tr>
<td>ITP case reports</td>
<td>10</td>
<td>33/67</td>
<td>3 mo-16 yr (8 yr)</td>
<td>SLE</td>
<td>Anti-D, steroids, IVlg, splenectomy</td>
<td>3-4 (4)</td>
<td>70%</td>
<td>80 days-22 mo (12 mo)</td>
<td>None</td>
</tr>
<tr>
<td>Antibody to Factor VIII/IX case reports</td>
<td>8</td>
<td>100/0</td>
<td>7 yr-17 yr (14 yr)</td>
<td>None</td>
<td>Immune tolerance</td>
<td>4 every 10 weeks (4)</td>
<td>63%</td>
<td>11-15 mo (13 mo)</td>
<td>None</td>
</tr>
<tr>
<td>PTLD cohort studies</td>
<td>71</td>
<td>not reported</td>
<td>11 mo-16 yr (8 yr)</td>
<td>Transplantation: bone marrow (12), umbilical cord blood (5), liver (11), small bowel (3), heart (15), kidney (11), lung (9), other (5)</td>
<td>Reduction of immunosuppression, ganciclovir, CMV Ig</td>
<td>1-9 (4)</td>
<td>70%</td>
<td>12 days-41 mo (28 mo)</td>
<td>Fatal staphlococcal sepsis, anaphylaxis</td>
</tr>
<tr>
<td>PTLD case reports</td>
<td>14</td>
<td>69/31</td>
<td>9 mo-19 yr (5 yr)</td>
<td>Transplantation: bone marrow (6), umbilical cord blood (1), heart (2), liver (3), kidney (1), small bowel (1)</td>
<td>Reduction of immunosuppression, maternal lymphocytes, acyclovir</td>
<td>1-4 (4)</td>
<td>93%</td>
<td>6 mo-3 yr (10 mo)</td>
<td>None</td>
</tr>
<tr>
<td>Lymphoma case reports</td>
<td>6</td>
<td>67/33</td>
<td>4 yr-14 yr (9 yr)</td>
<td>None</td>
<td>Conventional chemotherapy</td>
<td>4-8 (4)</td>
<td>83%</td>
<td>4-5 yr</td>
<td>None</td>
</tr>
<tr>
<td>Leukemia case reports</td>
<td>6</td>
<td>67/33</td>
<td>2 yr-12 yr (6 yr)</td>
<td>None</td>
<td>Conventional chemotherapy</td>
<td>1-5 (4)</td>
<td>100%</td>
<td>6 mo-4 yr (12 mo)</td>
<td>None</td>
</tr>
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</table>
Autoimmune Hemolytic Anemia

AIHA, an autoimmune disorder characterized by anti-red cell antibodies, is usually a self-limited disorder treated with short courses of steroids. Resistant disease has been difficult to manage, however. Immunosuppressants and splenectomy are not consistently effective and may carry a risk of infection. Children often require prolonged use of high-dose steroids, which is associated with serious adverse effects.

Cohort studies have described the use of rituximab for AIHA in a total of 25 children, all of whom had failed conventional treatments. The standard dose of rituximab was given weekly for 1 to 6 weeks (median, 4 doses). Remarkably, 23 of the 25 children (92%) exhibited a complete response ongoing from 7 to 28 months (median, 16 months) at the time of the last follow-up. Only 3 of these 23 responders had relapsed, including 1 patient who experienced 2 relapses. All relapses were successfully retreated with rituximab.

In the 14 single case reports published on the use of rituximab for AIHA in children, 93% of children demonstrated a complete response. The median response duration was 14 months, and all patients had ongoing responses at the time of publication.

These results differ in comparison with adult studies. Adults with AIHA often have cold agglutinin disease with IgM anti-red cell autoantibodies, unlike children, who most commonly have warm-antibody AIHA. Although some adult studies report a high response rate, the largest study of 27 adults receiving 37 courses of rituximab treatment for cold agglutinin disease reported a response in 54% (20 out of 37 courses), with a complete response in only 1 patient.

Given the high degree of efficacy of rituximab in pediatric patients and the difficulty of treating AIHA, it seems appropriate to consider the early use of rituximab in these patients, especially in steroid-intolerant children. Despite the absence of data on serial immunoglobulin levels, prophylactic monthly IVIG has been used in small children.

Chronic Immune Thrombocytopenic Purpura

Chronic ITP, characterized by autoantibodies to platelets, is treated with observation, corticosteroids, immunosuppressants, anti-D, and/or IVIG. Splenectomy and immunosuppressants are reserved for those with persistent disease.

Wang et al (n = 24), Taube et al (n = 22), and Bennett et al (n = 36) reported series of children with chronic ITP refractory to multiple previous treatments treated with rituximab. Children in the Wang and Bennett studies were given the standard dose of rituximab weekly for 4 weeks; those in the Taube study received only a single dose. A response, defined as platelet count > 50,000/µL, was seen in 71% in the Wang study, most of which were a complete response (CR), and in 31% in the Bennett study. Taube reported CR (platelets > 100,000/µL) in 32% and partial response (platelets > 30,000/µL) in 27%. The lower response rate in the Bennett study was probably a result of a more severely affected patient population. All but 1 CR in the Wang study occurred within 4 weeks of the first rituximab infusion. Case reports published on a total of 10 children with ITP receiving 4 doses of rituximab showed a response rate of 70%.

Neither the Wang study nor the Bennett study could identify clear predictors of response to rituximab. Of note, these 2 studies reported serum sickness in 5 children (Bennett, 2; Wang, 3), a rate apparently higher than in any other rituximab-treated patient group.

Bennett et al noted in that IgG levels remained unchanged in their children (age range, 2.6 to 18.3; median, >10 years), and IgM levels fell significantly but not to levels outside the normal range. Pharmacokinetic analyses on 14 children demonstrated that clearance of rituximab was more rapid after the first infusion than after the fourth infusion.

Data in adults with ITP treated with rituximab are similar to those reported in children, except that children with ITP who respond to rituximab appear to do so more quickly than adults. Conversely, 15 of 17 adults in 1 study who attained a CR maintained that response for >1 year, whereas in the Wang study children appeared to relapse sooner.

In conclusion, rituximab therapy has the potential to avoid splenectomy in children with chronic ITP, but apparently in no more than 50% of cases. Specific indications for rituximab therapy and when best to initiate treatment remain uncertain. The development of serum sickness can be readily managed with prednisone, but this requires discontinuation of treatment.

Antibodies to Factor VIII/IX (Inhibitors)

Approximately 20% to 30% of patients with severe hemophilia A and 5% of those with severe hemophilia B develop inhibitory antibodies. Hemophiliacs with inhibitors commonly receive bypassing agents for acute bleeding and immune tolerance in an attempt to eliminate the inhibitor.

No cohort studies have yet been published on the use of rituximab for inhibitors. Four case reports have described 8 children with congenital hemophilia treated with rituximab for antibodies against factors VIII and IX. Five of the 8 (63%) had an initial response. Two experienced relapses at 8 and 11 months, only 1 of which responded to a second course of rituximab.

Two studies have reported 14 adults (13 with acquired hemophilia) treated with rituximab. A response was seen in 11 of the 13 patients with acquired hemophilia and in the 1 patient with congenital hemophilia. The 2 patients who did not initially achieve remission both had inhibitor levels of >100 Bethesda units; both achieved a complete response with a combination of rituximab and cyclophosphamide.

A projected single-arm study of the efficacy of rituximab in 43 children and adults with inhibitors in congenital hemophilia was started August 15, 2006.
Posttransplantation Lymphoproliferative Disease

PTLD is a B-cell proliferative disorder induced by infection with Epstein-Barr virus in the setting of chronic immunosuppression. It is particularly prevalent in the post-transplantation setting. Standard treatment is withdrawal of immunosuppression, acyclovir, and IFN-α.

A total of 71 children with PTLD have been treated with rituximab, including preliminary results of a phase II trial.65 Twelve patients underwent bone marrow transplantation, 5 underwent umbilical cord blood transplantation, and 54 underwent solid organ transplantation. The patients who had received solid organ grafts had refractory PTLD, defined as no response to reduced immunosuppression, progressive or relapsed disease, and/or concomitant allograft rejection. The 12 children who had undergone bone marrow transplantation received rituximab as first-line treatment.

The 71 children received the standard dose of rituximab weekly for 1 to 9 weeks. Fifty (70%) had a complete response followed for a mean of 28 months with few adverse events. Case reports on 14 children treated with rituximab describe similar results. The authors of one study suggested that rituximab should be first-line treatment for refractory PTLD after solid organ transplantation.

Two large studies in adults reported lower response rates of 46% and 65%. A recent study piloted the use of rituximab in combination with cyclophosphamide and prednisone; complete responses were seen in 5 of 6 adults. Overall, it may be appropriate to use rituximab as a first-line therapy in PTLD in children in view of the high response rate.

Lymphoma

Rituximab was licensed in the United States based on its efficacy in adults with B-cell NHL. Treatment of children with Burkitt’s lymphoma has been far more restricted. This is due in large part to the much smaller number of cases of B-cell NHL in children compared with adults.

No cohort studies have been published on the use of rituximab for pediatric lymphoma. Five case reports have described the use of rituximab in 6 children with B-cell NHL. All 6 had relapsed disease. Rituximab was infused at the standard dose weekly for 4 to 8 weeks, and, in 1 case, repeated every 6 months. Five children had a response to rituximab, which was sustained in 4. Two deaths were reported, 1 from relapse of disease and 1 from graft-versus-host disease related to stem cell transplantation 5 years after rituximab treatment.

Current Children’s Oncology Group protocols incorporate rituximab in first-line treatment protocols for B-cell NHL (Burkitt’s lymphoma).

Leukemia

Because most pre-B cells do not express CD20, the great majority of pre-B-cell ALL in children is CD20-negative. Four case reports have described 6 children with relapsed, CD20+ pre-B-cell ALL treated with rituximab. All 6 had been treated previously with conventional chemotherapy and subsequently relapsed. Rituximab added to chemotherapy produced remission in all 6 patients. One patient died of a CD20-negative relapse 6 months after treatment; the other 5 patients are in remission at the time of this writing.

CONCLUSION

Rituximab has emerged as an important new drug for treating autoimmune B-cell–mediated disease and B-cell malignancies in both adult and pediatric populations. Although there are relatively few published reports in children describing rituximab treatment of hematologic diseases and the sample size of such studies is typically small, the reports appear to demonstrate that rituximab is a safe and effective treatment especially for AIHA, chronic ITP, and PTLD. Although rituximab is generally used when conventional treatments have failed, the findings suggest a role for rituximab as first-line therapy in PTLD and AIHA. In these diseases, and perhaps in chronic ITP as well, rituximab appears to be more effective in children than in adults. The toxicities in children appear to be similar to those in adults, with the exception of serum sickness in children with ITP.

Future studies should include randomized controlled trials to examine long-term outcomes, as well as pharmacokinetic and pharmacodynamic studies to help determine optimal dosing. Other questions that remain to be answered include:

1. Are there reliable predictors of response?
2. Is there a benefit to monthly IVIG infusion after rituximab, beyond infection prophylaxis, by virtue of suppression of rebound autoantibody production?
3. In cases where rituximab is effective, is there a role for maintenance infusions?
4. When is it appropriate to initiate rituximab treatment?

Given the more limited follow-up available in studies of nonmalignant disease, caution regarding long-term potential side effects of rituximab is warranted. The effect of rituximab in transient B-cell depletion generally is not accompanied by infectious complications, but little data exist on the long-term effects on immune response. Questions regarding reactivation of existing infections and acquisition of new infections remain to be addressed. Nonetheless, early results of treatment of hematologic B-cell–mediated diseases with rituximab remain impressive and worthy of further investigation.

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Treatment with Rituximab in Benign and Malignant Hematologic Disorders in Children
Follow-up Study of a Randomized Controlled Trial of Postnatal Dexamethasone Therapy in Very Low Birth Weight Infants: Effects on Pulmonary Outcomes at Age 8 to 11 Years

PATRICIA A. NIXON, PHD, LISA K. WASHBURN, MD, MICHAEL S. SCHECHTER, MD, MPH, AND T. MICHAEL O’SHEA, MD, MPH

Objective To determine whether postnatal dexamethasone (DEX) exposure affects pulmonary outcomes at school age in children born with very low birth weight (VLBW).

Study design Follow-up study of 68 VLBW children who participated in a randomized controlled trial of postnatal DEX. Pulmonary function was assessed by spirometry. Current asthma status was obtained from a parent.

Results Sixty-eight percent of the placebo group had below-normal forced expiratory volume in 1 second (FEV₁), compared with 40% of the DEX group (χ² = 4.84; P = .03), with trends for lower forced vital capacity (FVC) and FEV₁ values in the placebo group. Fifty percent of the placebo group and 34% of DEX group had below normal FEV₁/FVC (χ² = 4.84; P = .03). Parent-reported prevalence of asthma did not differ between groups. Logistic regression analysis suggested that the positive effects of DEX on pulmonary function at follow-up were mediated in part by shortened exposure to mechanical ventilation.

Conclusions Postnatal DEX exposure was associated with higher expiratory flow with no adverse effects on pulmonary outcomes at school age. The prevalences of asthma and impaired pulmonary function underscore the influence of neonatal illness on health at school age, and stress the importance of repeated follow-up examinations of these children. (J Pediatr 2007;150:345-50)
treatment of chronic lung disease,” and for additional long-term follow-up of well-designed, randomized, double-blind, controlled trials with no crossover or contamination of treatment.

To date, 3 studies have examined the long-term effects of postnatal corticosteroid treatment on pulmonary outcomes and growth in school-aged children and adolescents born prematurely with VLBW.24-26 The results are inconsistent, suggesting beneficial,24 adverse,25 and no24-26 effects of DEX on pulmonary outcomes compared with placebo, and no effects on somatic growth. But potential effects of DEX on pulmonary outcomes at school-age could have been masked by small sample sizes24,25 and open-label treatment with DEX in a large proportion24,26 of children in the placebo group after the initial study period. Furthermore, the early trials were conducted at a time when surfactant was not routinely available.

Consequently, the primary purpose of the present investigation was to examine the long-term effects of postnatal DEX exposure on pulmonary outcomes in a sample of school-aged children who, as VLBW neonates, received surfactant and participated in a double-blinded, randomized, controlled trial with no contamination of treatment (ie, no open-label DEX use).

METHODS

Participants

Participants were children age 8 to 11 years who as neonates participated in a randomized controlled trial of a 42-day tapering course of postnatal DEX to reduce the duration of ventilator dependency.27,28 The children were born between 1992 and 1995 and met the following eligibility criteria: (1) birth weight < 1501 g, (2) age 15 to 25 days, (3) ventilator dependence without improvement, (4) absence of clinical signs of sepsis, (5) no evidence of patent ductus arteriosus on echocardiography. The treatment group received DEX at an initial dose of 0.5 mg/kg/day that was tapered over 42 days.

Of the 118 infants who were randomized, 95 survived to age 1 year. Of these “long-term” survivors, 68 (38 DEX, 30 placebo) agreed to participate in this follow-up study. This study was approved by the institutional review boards of the Wake Forest University Baptist Medical Center and Forsyth Medical Center. Written informed consent was obtained from the parent or legal guardian, and verbal assent was obtained from the child.

Measurements and Procedures

Each child reported to the General Clinical Research Center at Wake Forest University Baptist Medical Center for height and weight measurements. Body mass index (BMI) (weight in kilograms divided by height in meters squared [kg/m²]) was calculated, and percentiles and z-scores based on age and sex were determined from the National Center for Health Statistics 2000 reference values.29 The child and parent reported that the child had asthma and/or used medications for asthma treatment. A subsample of children also underwent maximal progressive exercise testing on a cycle ergometer as part of the larger study. Spirometry was repeated immediately and 5 minutes postexercise, as well as 20 minutes after 3 puffs of albuterol delivered with a spacer. A 15% decrease in forced expiratory volume in 1 second (FEV1) from pre-exercise values was the criterion used to define exercise-induced bronchoconstriction.35 A 12% increase in FEV1 from pre-exercise levels was considered a positive bronchodilator response.35

Neonatal Characteristics

Neonatal data on birth weight, gestational age, antenatal steroid exposure, and number of days of mechanical ventilation and supplemental oxygen postrandomization were obtained from medical records by research nurses. Gestational age was based on the date of the mother’s last menstrual period, or if not available, the obstetrician’s estimate, or if no prenatal estimate was available, neonatal assessment. Birth weight z-values and percentiles (based on sex and gestational age) were derived from the reference data of Oken et al.37 An infant was considered small for gestational age (SGA) if birth weight was below the 10th percentile for gestational age. A diagnosis of CLD was made based on the use of supplemental oxygen at 36 weeks postmenstrual age.38 Treatment assignment (DEX or placebo) was obtained from the research database. All testers, children, and their parents were blinded to treatment assignment.

Data Analysis

Based on exploratory analyses, nonparametric statistics were used to describe central tendency and dispersion (ie, median, 5th and 95th percentiles). Mann-Whitney U and χ² tests were used to compare the DEX and placebo groups for continuous and categorical variables, respectively. Logistic
regression analysis was used to examine possible mediators of the effect of DEX on pulmonary function. Cochran-Mantel-Haenszel techniques were used with stratified analysis to develop common odds ratios and to test for conditional independence. The Breslow-Day test was used to test for homogeneity of stratum-specific odds ratios.

RESULTS

A total of 68 of the 95 surviving children (38 DEX, 30 placebo) underwent follow-up evaluation. Of the remaining 27 children, 12 children could not be located, 1 child declined participation, and 9 children agreed to participate but did not keep scheduled appointments (Figure). Neonatal characteristics in surviving participants and nonparticipants were compared, and no differences were found. The participants’ neonatal characteristics are presented in Table I. Extremely low birth weight (<1001 g) was present in 92% of the DEX group and 83% of the placebo group. Birth weight, birth weight z-value, and gestational age did not differ significantly between the 2 groups. No children in the DEX group and only 2 children in the placebo group were SGA.

Treatment (DEX or placebo) was initiated at a median age of 19 days in both groups. Subjects in the DEX group received mechanical ventilation and supplemental oxygen for significantly fewer days than those in the placebo group. CLD was diagnosed in 50% of the DEX group and 73% of the placebo group (P = .05). All children were treated with surfactant.

Participants’ characteristics at follow-up evaluation are presented in Table II. All children were between 8 and 10 years of age, except 1 child who had to be rescheduled shortly after he turned 11 years old. The DEX and placebo groups did not differ in terms of current age, weight, height, or BMI.

Pulmonary Function

Sixty-three of the 68 children (35 DEX, 28 placebo) were able to adequately perform spirometry. Five children (3 DEX, 2 placebo) were unable to adequately perform spirometry due to cognitive delay (n = 4) or physical limitations associated with cerebral palsy (n = 1). As shown in Table III, average values for FEV₁ and FVC were not statistically different between the 2 treatment groups. However, a significantly (P = .03) greater proportion of the children in the placebo group had an FEV₁ below normal (<5th percentile) compared with the DEX group (68% vs 40%). Below-normal FVC was found in 36% of the placebo group and 17% of the DEX group (P = .08). The ratio of FEV₁ to FVC did not differ between the 2 groups, with 34% of the DEX group and

| Table I. Neonatal characteristics expressed as median, (5th, 95th percentiles), or proportion |
|-----------------------------------------------|---------------|
| DEX | Placebo |
| n | 38 | 30 |
| Gestational age, weeks | 25.0 (23.0, 28.1) | 26.0 (23.6, 29.9) |
| Sex, % male | 44% | 53% |
| Race, % Caucasian | 65% | 47% |
| Birth weight, g | 743 (523, 1172) | 789 (557, 1293) |
| Birth weight, z-value | -0.14 (-1.15, 1.06) | -0.24 (-1.47, 0.66) |
| Mechanical ventilation, days†‡ | 10 (1, 45) | 24 (2, 68) |
| Supplemental oxygen, days†‡ | 45 (6, 315) | 99 (24, 549) |
| +CLD diagnosis, %§ | 50% | 73% |

†Days postrandomization. §P < .05.

| Table II. Participants’ characteristics at follow-up evaluation expressed as median (5th, 95th percentiles), or proportion |
|-----------------------------------------------|---------------|
| DEX | Placebo |
| Age, years | 9 (8, 10) | 9 (8, 10) |
| Weight, kg | 32.1 (19.7, 51.9) | 28.6 (19.8, 73.1) |
| Weight z-score | 0.31 (-2.63, 2.31) | -0.68 (-2.94, 3.04) |
| Height, cm | 136.7 (117.3, 147.2) | 134.4 (116.5, 152.9) |
| Height z-score | 0.05 (-2.45, 1.94) | -0.28 (-2.66, 2.57) |
| BMI, kg/m² | 17.2 (14.3, 25.2) | 15.5 (13.2, 32.6) |
| BMI z-score | 0.35 (-1.43, 2.16) | -0.55 (-2.42, 2.54) |
| Asthma by parent report, % | 29 | 33 |

All P values were nonsignificant.
Table III. Pulmonary function by treatment group expressed as median* (5th, 95th percentiles)

<table>
<thead>
<tr>
<th></th>
<th>DEX</th>
<th>Placebo</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>35</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>FVC, % predicted</td>
<td>94 (72, 124)</td>
<td>89 (65, 141)</td>
<td>.08</td>
</tr>
<tr>
<td>FEV₁, % predicted</td>
<td>86 (49, 112)</td>
<td>76 (52, 126)</td>
<td>.08</td>
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<tr>
<td>FEV₁/FVC, %</td>
<td>81 (63, 98)</td>
<td>80 (65, 100)</td>
<td>.71</td>
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<tr>
<td>FEF₂₅₋₇₅, % predicted</td>
<td>68 (23, 136)</td>
<td>61 (23, 96)</td>
<td>.49</td>
</tr>
<tr>
<td>Change in FEV₁</td>
<td>2 (–18, 18)</td>
<td>2 (–24, 16)</td>
<td>.51</td>
</tr>
<tr>
<td>postexercise, %†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in FEV₁</td>
<td>3 (–20, 39)</td>
<td>7 (–35, 40)</td>
<td>.96</td>
</tr>
<tr>
<td>postbronchodilator, %‡</td>
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</tbody>
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*The Mann-Whitney U test was used to compare groups.
‡n = 56.
§n = 53.

DISCUSSION

The results of this study suggest that postnatal DEX treatment in VLBW infants does not adversely affect pulmonary function at school age and in fact may impart some benefit. A greater proportion of children who received postnatal DEX exhibited normal spirometry compared with children who received placebo. These results are consistent with the reported short-term benefits of DEX on pulmonary outcomes in the neonate, including decreased inflammation,14-16 decreased duration of mechanical ventilation and supplemental oxygen dependence,11-13 and improved lung mechanics.40,41 The results of multivariate analysis suggest that the long-term beneficial effects of DEX on pulmonary function at follow-up were mediated, at least in part, by the acute beneficial effects on the neonatal lung (ie, decreased duration of ventilation and supplemental oxygen use) observed in DEX-treated children.

Comparisons with previous studies are difficult due to differences in study design and DEX exposure, including dose and duration. The randomized controlled trial in which our subjects participated as infants was modeled after the study of Cummings et al,42 which demonstrated significant improvements in pulmonary outcomes (ie, faster weaning from mechanical ventilation and supplemental oxygen) in infants receiving a 42-day tapering course of DEX compared with placebo. However, in contrast to our study, follow-up evaluation of their participants at age 15 years revealed no long-term differences in pulmonary function between the children treated with DEX (n = 9) and those treated with placebo (n = 4).25 The lack of group differences may be due to the limited statistical power associated with their small sample size.

Mieskonen et al24 found significantly higher FVC in 7.8- to 9.2-year-old children treated postnatally with a 1-week course of DEX (n = 8) compared with those given placebo (n = 8). However, no differences in forced expiratory flow rates were found between treatment groups, possibly due to limited statistical power. In the largest follow-up study to date, Jones26 evaluated pulmonary function in 142 children age 13 to 17 who participated as infants in the Collaborative Dexamethasone Trial,43 a multicenter postnatal randomized controlled trial of 1 week of DEX treatment. Even though beneficial effects of DEX were seen in the postnatal period (ie, fewer days of ventilator dependence),43 no differences between the DEX (n = 68) and placebo (n = 74) groups were noted at follow-up in terms of FVC, FEV₁, FEV₁/FVC, FEF₂₅₋₇₅, peak expiratory flow rate or bronchodilator responsiveness, or respiratory morbidity (ie, current asthma, wheezing, coughing, inhaler use). However, a large proportion of the placebo group in that study (39%), as well as in the study by Mieskonen et al24 (50%), were exposed to open-label DEX treatment after the initial study period, which may have attenuated possible treatment effects.

Differences in sample characteristics (both postnatal and current) also hinder comparisons of our present study with previous studies. Our subjects were born in the postsur-
factant era and were generally smaller and born more prematurely and had a higher incidence of CLD than the participants in the studies of Jones and Mieskonen. As neonates, all of our subjects were dependent on mechanical ventilation, compared with only 2/3 of subjects in the study by Jones. In general, these differences suggest that our subjects may have been at greater risk for poor pulmonary outcomes compared with those in the studies by Jones and Mieskonen and thus more likely to benefit from DEX treatment.

At follow-up evaluation, our participants were similar in age to those of Mieskonen et al. and younger than the adolescents studied by Jones and Gross. It is possible that treatment group differences observed in childhood may resolve with subsequent growth and maturation.

Although DEX was associated with improved pulmonary function, 40% of the DEX group (and 68% of the placebo group) had below-normal FEV1, suggesting larger airway obstruction. However, lack of significant group differences in FEV1/FVC suggests that the lower FEV1 values may be attributed to the somewhat lower FVC in the placebo group and possibly airway restriction. Unfortunately, we were unable to determine lung volumes in most of the subjects, and thus we do not know whether the reduced values are due to mild restriction or secondary to airway obstruction and air trapping. The finding that 50% of the placebo group and 34% of the DEX group had FEV1/FVC values < 5th percentile suggests that compromised larger airway function is the more likely explanation. The positive bronchodilator response exhibited by some subjects in both groups suggests some reversibility of airway obstruction. Furthermore, the large proportion of children in both groups with below-normal FEF25-75 predicted values implies smaller airway obstruction, but also may be due to reduced FVC and TLC.

The prevalence of asthma did not differ between the 2 treatment groups. Approximately 1/3 of the children in each group had asthma as reported by the parent. This finding is consistent with that reported by Jones in the Collaborative DEX Trial follow-up study. Reliance on parental reports may underestimate the prevalence of asthma or pulmonary limitations, as evidenced by the considerable number of children with below-normal pulmonary function. The positive bronchodilator response observed in about 20% of the children suggests that some of the reduced pulmonary function may be ameliorated by pharmacologic intervention. Further investigation is needed to substantiate the prevalence of asthma and the extent to which reduced pulmonary function is reversible with appropriate asthma therapy.

We were also not able to determine the potential effects of postnatal DEX on DLCO, because most of the children could not adequately perform the single-breath DLCO maneuver. In animal models, DEX has been shown to impair septation and alveolarization. Mitchell et al. reported impaired gas transfer at rest and during exercise as measured by DLCO in preterm children with bronchopulmonary dysplasia (BPD) but not in those without BPD. Consequently, the possible negative effects of DEX on alveolarization may be countered by its positive effects of reducing exposure to mechanical ventilation and supplemental oxygen, and decreasing the subsequent risk for BPD.

Neonatal DEX exposure has been associated with impaired somatic growth early in life, and it also may impair concomitant lung growth. A study by Yeh et al. suggested an association between long-term growth impairment and postnatal DEX exposure at age 6 years. In contrast, the results of the current study as well as other follow-up studies show no long-term differences between DEX-treated and placebo-treated subjects in terms of weight and height at childhood or adolescence. However, without measuring TLC at follow-up, it is not possible to determine whether somatic and lung growth were discordant.

Major strengths of the present study are the absence of crossover or contamination of treatment after the postnatal randomized controlled trial, as well as a fairly large sample size for a single-center study. However, we do recognize that with a follow-up rate of 72%, the potential exists for bias due to loss to follow-up. Our results also may have been affected by survivor bias due to a trend toward higher mortality in the placebo group and the expectation that infants who died would have had worse pulmonary function had they survived. In addition, we did not have information on maternal smoking or asthma during pregnancy, or on the children's current smoke exposure, all of which may affect pulmonary outcomes in these children, although we would expect these factors to be balanced by randomization. The generalizability of our study may be limited by the use of a single-center sample and a longer course of DEX than that reported in a recent cohort study of VLBW infants.

Despite these limitations, however, the results of this study may have clinical implications related to the use of corticosteroids to prevent CLD in premature infants. We found no adverse long-term effects of postnatal DEX exposure on FVC or FEV1, airway reactivity, and parental reports of asthma in 8- to 11-year-old children born prematurely with VLBW. Our finding that a large proportion of the children had below-normal pulmonary function and parent-reported asthma illustrates the influence of neonatal illness on health at school age, the importance of surveillance to identify affected children, and the need for the best possible interventions to prevent CLD. Future studies of children who participated in randomized trials of postnatal steroids should include examination at an older age when reliable measures of lung volumes and DLCO can be obtained, along with more focused evaluation for the presence of asthma.

We are grateful to Alice Scott, RN, the General Clinical Research Center nurses, the pulmonary function laboratory technicians, students in the Health & Exercise Science Undergraduate and Graduate programs, and the children and their families for making this project possible.

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Neonatal Hydrocortisone Treatment: Neurodevelopmental Outcome and MRI at School Age in Preterm-born Children

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Objective To investigate neurodevelopment at school age in preterm infants treated with hydrocortisone for bronchopulmonary dysplasia (BPD) in the neonatal period.

Study design Preterm infants (n = 226; gestational age ≤32 weeks and/or body weight ≤1500 grams) performed subtests of the Wechsler Intelligence Scale for Children-Revised, the Visual Motor Integration test, a 15-Word Memory Test and the Movement Assessment Battery for Children at school age. Conventional MRI of the brain was obtained. Sixty-two children who received hydrocortisone for BPD (starting dose, 5 mg/kg/day; median duration, 27.5 days) were compared with 164 nontreated neonates.

Results Hydrocortisone-treated infants were younger, lighter, and sicker than their non-steroid-treated counterparts. Adjustments for gestational age, body weight, sex, mechanical ventilation, and small for gestational age were made. Adjusted mean Intelligence Quotient, Visual Motor Integration test, and memory test results were the same in the hydrocortisone-treated group and the non-steroid-treated group (99 versus 101, \(P = .62\); 97 versus 99, \(P = .49\), 7.9 versus 7.5, \(P = .42\), respectively). Motor function and incidence of cerebral palsy in both groups was not different (11% versus 7%, \(P = .97\)). Occurrence of brain lesions on MRI was similar for the two groups.

Conclusions Neonatal hydrocortisone treatment for BPD had no long-term effects on neurodevelopment. (J Pediatr 2007;150:351-7)

Despite improved mechanical ventilatory strategies and administration of exogenous surfactant, bronchopulmonary dysplasia (BPD) remains a problem in neonatal intensive care. The prevalence of BPD is high, and the condition is a significant cause of mortality, morbidity, and prolonged hospitalization. Dexamethasone (DXM) is the primary drug used to prevent chronic lung disease and to treat BPD. Corticosteroids improve short-term respiratory function, leading to a reduction in supplemental oxygen requirements and earlier extubation. The choice of DXM was arbitrarily based on it being a potent anti-inflammatory drug; however, many short-term as well as long-term negative side effects of DXM therapy have been reported in preterm infants. Short-term adverse effects include impaired glucose tolerance, hypertension, increased risk of nosocomial infections, gastrointestinal hemorrhage, and impaired weight gain and head growth. These effects are generally transient and reversible after withdrawal of the DXM treatment. Because of these relatively mild reversible effects, the use of DXM increased greatly in the early 1990s.

In the late 1990s, concerns on the long-term neurodevelopmental consequences arose when follow-up of randomized, controlled trials indicated an increased risk of cerebral palsy (CP) after early postnatal DXM exposure. Since then, more reports on negative effects on long-term follow-up after neonatal DXM prescription were published and recently reviewed. In our unit, hydrocortisone, a much less potent glucocorticosteroid, was used for BPD.
ticoid than DXM, has been the steroid of choice to treat ventilator-dependent preterm infants with BPD.

The aim of the current study was to investigate the impact of neonatal hydrocortisone administration on long-term neurodevelopment in preterm infants, measured at school age.

A small subset of this cohort has been reported earlier in a different context; the current paper describes the entire group of children with more extensive outcome measures.

**METHODS**

The children studied are part of a cohort of consecutively admitted patients soon after birth over a period of 2 years to the neonatal intensive care unit (NICU) of the Wilhelmina Children’s Hospital, a tertiary referral center. All children, born between March 1, 1991, and March 1, 1993, with a gestational age (GA) ≤32 weeks (range, 25.0 to 33.0) and/or a birth weight (BW) ≤1500 grams, were subsequently enrolled in a long-term follow-up study. The original group consisted of 375 children. Sixty-four children (17%) died, and 28 (7.5%) were excluded from the present study because of (multiple) congenital anomalies and/or chromosomal disorders. At the age of 7 or 8 years (occasionally 9 and 10 years), the children were invited to the hospital for 1 day to have several tests. Of the remaining 283, 22 children (7.8%) could not be traced because of moving, and the parents of 25 children (8.8%) refused to participate in the follow-up, resulting in 236 children (83.4%) who participated. For the current study, children who had received DXM (n = 7: 6 before extubation, 1 during part of his treatment at another hospital) were excluded, as well as 2 children with a congenital abnormality on neonatal cranial ultrasound (cavum septum pellucidum agenesis) and 1 child who had an undiagnosed neuromuscular disorder. This resulted in a study population of 226 children, of whom 62 had been treated postnatally with hydrocortisone and 164 who had not been treated with steroids. Median age at follow-up was 8.1 years in both groups. The Medical Ethics Committee of the University Medical Center Utrecht approved of the study, and parental informed consent was obtained.

**Hydrocortisone Treatment**

In all 62 children, hydrocortisone treatment was given for BPD and consisted generally of a starting dose of 5 mg/kg per day, divided into 4 doses for 1 week, followed by a tapering course of 3, 2, and 1 dose(s) each for 5 days (total of 3.75, 2.5, and 1.25 mg/kg per day, respectively). In the absence of respiratory improvement or when respiratory deterioration occurred after reduction of the dose, steroid treatment was either prolonged or repeated at the discretion of the attending neonatologist.

**Cranial Ultrasound**

Cranial ultrasound in the neonatal period was performed within 6 hours of admission, at least 3 times during the first week of life, and subsequently once a week until discharge. Hemorrhages (intraventricular hemorrhage, IVH) were classified according to Papile and periventricular leukomalacia (PVL) according to De Vries. Cranial ultrasound findings were classified into three groups: normal when no or minor abnormalities such as germinal layer or plexus cysts, subependymal pseudocysts, or lenticulostriate vasculopathy as exclusive findings were present (group 1); mildly abnormal when an IVH grade I or II, PVL grade I, or germinal layer necrosis or a combination of these features were present (group 2); and severely abnormal when one or more of the following features were present: IVH grade III or IV, cystic PVL grade II or III, thalamic lesion, focal infarction, or hemorrhage at the convexity of the brain (group 3).

**Magnetic Resonance Imaging**

Magnetic resonance imaging at school age was performed on the same day as the developmental tests, without sedation. With the use of a mirror placed above the head, children had eye contact with one of their parents; hearing protection was provided by headphones. The children were all imaged on a 1.5-Tesla Philips Gyroscan ACS-NT system (Philips Medical Systems, Best, The Netherlands). Details of MRI acquisition were described in detail previously.

MRI findings were also classified into 3 groups: normal (group 1); mildly abnormal when mild gliosis, mild ventricular dilation, an irregular shape of the ventricles, thinning of the corpus callosum, or a combination of these features were present (group 2); and severely abnormal when extensive gliosis or gliosis in combination with marked ventricular dilation was present (group 3).

For calculation of the area of the corpus callosum, the midsagittal T1 SE image (TR, 512 ms; TE, 15 ms; slice thickness, 4 mm; interslice gap, 0.6 mm) that most clearly delineated both the rostral and caudal end of the corpus callosum was selected for measurement. Magnetic resonance data were transferred in digital format to an Easy Vision Workstation, where all images were first enlarged from 256 × 256 to a magnification, at which the contour of the corpus callosum could be easily manually traced with a mouse-controlled cursor.

**Intelligence Quotient, 15-Word Test, and Visual-Motor Integration**

The children performed five subtests of the Wechsler Intelligence Scale for Children–Revised (WISC-R, Dutch version): similarities, vocabulary, block design, picture arrangement, and digit span. They were supervised by a child psychologist who was unaware of the neonatal history of the child. By using the procedures and tables published by Kaufman, scaled scores were converted to an estimated Intelligence Quotient (IQ) score based on two subtests, which is within a 95% confidence interval of the full-scale IQ score, with a standard error of estimate of 6.3.

The 15-Word Test is a Dutch adaptation of Rey's Auditory Verbal Learning Test. It consists of a list of 15
unrelated, concrete nouns, which are presented over five learning trials, with immediate recall after each trial. After a delay interval of 20 minutes and without further presentation, delayed recall is assessed. The number of correctly recalled words after each trial and after the delayed recall provides the scores for the test. For this study, the delayed recall results after 20 minutes were used.

For the Developmental Test of Visual–Motor Integration (VMI), only the children without cerebral palsy (CP) were considered. Raw scores were converted to VMI standard scores with a mean of 100, based on the fourth revised edition norms.14

Motor Function

All children were seen by a pediatric physiotherapist who was blinded to the MRI findings. Children were examined for the presence of CP. To further evaluate motor function, those with CP were not considered because the Movement Assessment Battery for Children (ABC) is not suitable for these children.

Movement ABC age band 2 for 7 and 8 years was used.15 The test contains three domains: manual dexterity (placing pegs, threading a lace, drawing a flower trail), ball skills (one-hand bounce and catch, throwing a bean bag into a box), and static and dynamic balance (stork balance, jumping in squares, and heel-to-toe walking). Each item is scored from 0 (best score) to 5 (poorest score). The total impairment score (TIS) is the sum of the three subscores and varies therefore between 0 (best score) and 40 (poorest score). Raw subscale scores are converted to percentile scores and classified as follows: <p5 (definitely abnormal), p5–p15 (borderline), and >p15 (normal).

Data Analysis

Treatment group differences in continuous baseline characteristics were tested by using t tests or using Mann–Whitney tests; group differences in proportional values were tested by using χ² tests or Fisher exact tests when appropriate. Univariate general linear models were used for associating hydrocortisone use with binary or categorical outcome variables, yielding group differences and associated significance tests. We used (multinomial) logistic regression models for associating hydrocortisone use with binary or categorical outcome variables.

Because we used nonrandomized clinical data, we had to account for treatment group differences in prognosis, since prescribing hydrocortisone is itself expected to be an indicator of worse developmental outcome, irrespective of treatment effects (confounding by indication). Therefore, we used logistic regression to calculate for each child a propensity score,16 indicating the likelihood of hydrocortisone treatment as predicted by GA, BW, sex, mechanical ventilation, and small for gestational age. This propensity score, reflecting baseline prognostic differences, was used for adjusting associations between treatment and outcome.

Results are expressed as regression coefficients or as odds ratios with 95% confidence intervals and P values (α = 0.05). All analyses were performed with the use of SPSS version 12.0.2 for Windows.

RESULTS

Patient Characteristics

Mean GA of steroid-treated children was less and mean BW was lower compared with children who never received steroids (Table I). Treated children were also sicker, as shown by a significantly higher incidence of mechanical ventilation, need for surfactant and inotropes, and an increased incidence of patent ductus arteriosus (PDA). There was no difference in the proportion of mothers of steroid-exposed and non–steroid-exposed children who had received a complete course of antenatal betamethasone (2 × 5.7 mg im, repeated 24 hours later) for fetal lung maturation.

Generally, hydrocortisone was started when the postnatal age was >1 week of age and the child was ventilator-dependent with increasing oxygen requirements or needed reintubation, not explained by infection or a hemodynamic significant PDA. Hydrocortisone was initiated at a median age of 19 days, with an interquartile range (IQR) of 14 days. The mean age of the steroid group had advanced to 30.5 postmenstrual weeks at start of treatment, which was not significantly different from GA at birth of the nontreated group (P = .09). In 2 children, hydrocortisone was prescribed without having been ventilated to avoid mechanical ventilation. Median duration of hydrocortisone treatment was 27.5 days (IQR, 12 days). In 58 of the 62 hydrocortisone-treated patients, we could calculate the cumulative dose per kilogram mean weight (mean weight: weight at the end of the treatment plus weight at the start of the treatment divided by 2). Median cumulative hydrocortisone dose was 70 mg/kg mean weight (IQR, 21 mg/kg).

Magnetic Resonance Imaging

Magnetic resonance imaging failed as the result of anxiety in 10 children (4 hydrocortisone–treated and 6 nontreated children). There were no differences in brain lesions on MRI between the steroid-treated and non–steroid-treated groups (Table II). Also, after adjustment for the propensity score, odds ratios for the risk on MRI lesions were not significantly different between the two groups. Mean midsagittal corpus callosum area was significantly smaller in the hydrocortisone–treated group compared with the nontreated group (313 mm² versus 348 mm², P = .005), but this difference disappeared after adjustment (335 mm² versus 340 mm², P = .72).

Cognitive Outcome

The unadjusted mean IQ at school age in the hydrocortisone–treated group was 98, compared with 101 in the
nontreated group (Table III), which was close to statistical significance. However, this difference disappeared after adjustment. In line with the analysis of mean IQs, of the 62 children who received hydrocortisone, 14 (22.6%) had an IQ compared with 23 (14.0%) of the 164 children who did not receive hydrocortisone. Thus, the hydrocortisone-treated children were at a slightly increased risk of a future lower IQ (OR 1.8; 95% CI, 0.9, 3.8; P = .12). Again, this was completely explained by differences in other prognostic factors (OR adjusted for propensity score 1.2; 95% CI, 0.5, 2.9; P = .76). Maternal level of education was not different between the hydrocortisone-treated group and the nontreated group (P = .28).

Memory test results were the same in both groups. For the VMI test, the children with CP were excluded for obvious reasons; no difference was found between the hydrocortisone-treated and the nontreated groups. Adjustment did not change the results.

Motor Outcome

There was no significant difference in incidence of CP between the hydrocortisone-treated group and the non–hydrocortisone-treated group (11.3% versus 7.3%, respectively; OR, 1.6; OR adjusted for propensity score 1.0; P = .97). In the hydrocortisone-treated group, the median (minimum–maximum) values for the TIS, the total manual dexterity score, the total ball skills score, and the total balance score were 5.5 (0 to 34), 2.0 (0 to 15), 2.5 (0 to 10), and 0.5 (0 to 15), respectively. In the non–hydrocortisone-treated group, these values were 5.5 (0 to 36.5), 1.0 (0 to 15), 2.3 (0 to 10), and 1.0 (0 to 12.5), respectively. There was no association for any of

<table>
<thead>
<tr>
<th>Table I. Baseline characteristics of hydrocortisone-treated versus nontreated preterm neonates</th>
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<tbody>
<tr>
<td>Hydrocortisone (n = 62)</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>GA, wk, mean (SD, SEM)</td>
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<tr>
<td>BW, g, mean (SD, SEM)</td>
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<tr>
<td>Age, wk, start HC (SD, SEM)</td>
</tr>
<tr>
<td>Sex, boys/girls, n</td>
</tr>
<tr>
<td>SGA (&lt;p2.3), n</td>
</tr>
<tr>
<td>Singleton/twins/triplets, n</td>
</tr>
<tr>
<td>Mechanical ventilation, n (%)</td>
</tr>
<tr>
<td>Duration of ventilation days, median (min-max)</td>
</tr>
<tr>
<td>Surfactant, n</td>
</tr>
<tr>
<td>PDA, n</td>
</tr>
<tr>
<td>PDA ligation, n</td>
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<tr>
<td>Sepsis, n</td>
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<tr>
<td>NEC, n</td>
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<tr>
<td>NEC surgery, n</td>
</tr>
<tr>
<td>Apgar 1 min, median (min-max)</td>
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<td>Apgar 5 min, median (min-max)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Cerebral ultrasound group 1, n</th>
<th>19 (31.1%)</th>
<th>78 (47.9%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>group 2, n</td>
<td>34 (55.7%)</td>
<td>55 (33.7%)</td>
</tr>
<tr>
<td>group 3, n</td>
<td>8 (13.1%)</td>
<td>30 (18.4%)</td>
</tr>
</tbody>
</table>

GA, gestational age; HC, hydrocortisone; PDA, patent ductus arteriosus; NEC, necrotizing enterocolitis; SGA, small for gestational age. Cerebral ultrasound group 1: normal; group 2: minor lesions; group 3: major lesions.

<table>
<thead>
<tr>
<th>Table II. Relative risk for MRI lesions at school age in hydrocortisone-treated versus nontreated infants</th>
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</thead>
<tbody>
<tr>
<td>Hydrocortisone (n = 58)</td>
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<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>MRI group 1, n</td>
</tr>
<tr>
<td>MRI group 2, n</td>
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<tr>
<td>MRI group 3, n</td>
</tr>
</tbody>
</table>

MRI, Magnetic resonance imaging; group 1, normal MRI; group 2, minor lesions; group 3, major lesions; OR, odds ratio; CI, confidence interval; OR adjusted, odds ratio adjusted for gestational age, birth weight, sex, need for mechanical ventilation, and small for gestational age.
the scores between hydrocortisone treatment and the risk of a score above the overall median value (Table IV).

There was also no difference in number of motor-impaired children between the treated and nontreated children. Adjustment did not change the outcome.

### DISCUSSION

The findings of this cohort study of 226 preterm-born children followed up for 8 years do not demonstrate any unfavorable structural or functional effects of neonatal treatment with hydrocortisone on brain development at school age. To appreciate these results, some issues need to be addressed. We estimated the effects of treatment by using data obtained in routine care rather than conducting a randomized trial. Consequently, children who were treated had clinical indication for treatment. Indeed, treated children generally had more disease and therefore had a priori a more problematic prognosis than untreated ones. To minimize confounding by indication, we made a great effort to adjust for differences by using propensity score methods. Although some remaining confounding cannot be excluded, this is unlikely to affect the conclusions. If anything, given the virtual identical prognosis in the two groups, the true outcome data. This is the largest report until now on long-term neurodevelopmental outcome in children following neonatal hydrocortisone treatment.

We found no differences in neurocognitive and motor performance between hydrocortisone-treated and nontreated children. The incidence of brain lesions on conventional MRI was also similar. Midsagittal corpus callosum areas were the same after adjustment. Previously, we reported no differences in total intracranial, cerebral gray or white matter, cerebrospinal fluid, and hippocampal volume between the hydrocortisone-treated and the nontreated groups in a subset of this cohort. The first controlled trial published in 1972 of postnatal corticosteroid treatment investigated the ability of hydrocortisone to alter the outcome in infants with respiratory distress syndrome. The aim of the study was to prevent mechanical ventilation; however, no significant effect on need for assisted ventilation or survival between the two groups was demonstrated. Autopsy of seven hydrocortisone-treated and seven nontreated infants showed no difference in lung, liver, adrenal, thymus, heart, and spleen pathology attributable to steroid treatment but a statistically significant association between IVH and steroid treatment. Twenty-four survivors (12...
in each group) had the same mean developmental quotient (Griffiths Developmental Scale) at the age of 1 year, but analysis of the subtests showed a significant difference in the results for gross motor development with a lower mean score for the steroid group.\textsuperscript{19}

Initial trials in the 1980s showed short-term improvement in pulmonary function and weaning from the ventilator in preterm infants with BPD after administration of DXM,\textsuperscript{20,21} resulting in an increased use of DXM. One retrospective study examining the outcome of neonates with a BW between 500 and 749 grams found that 43\% of infants born from 1990 to 1992 received DXM, compared with as many as 84\% of those born from 1993 through 1995.\textsuperscript{22} This almost routine use of DXM continued until 1998, when Yeh et al\textsuperscript{3} demonstrated a significant increase in neurodevelopmental dysfunction in neonates treated with DXM. In 1999, O’Shea et al\textsuperscript{4} described an increased risk of CP in very low birth weight infants who received a 42-day tapering course of DXM, started at day 15 to day 25 of life.

Because of these alarming publications on DXM, several groups tried to find an alternative corticosteroid for treating preterm infants with BPD. In a commentary in 2001, Thebaud et al\textsuperscript{23} questioned the rationale for the exclusive use of DXM and advocated the use of alternative steroids. Andre et al\textsuperscript{24} published a study of 45 consecutive preterm infants at risk of chronic lung disease who were treated at a median postnatal age of 16 days with a tapering course of methylprednisolone. Methylprednisolone has less anti-inflammatory activity than DXM (still 5 times higher than hydrocortisone), a negligible mineralocorticoid effect, and, in contrast to DXM, no sulphites are used for preservation. Those treated with methylprednisolone had a higher rate of body weight gain and a lower incidence of glucose intolerance and cystic PVL compared with 45 consecutive historic cases treated with DXM. Decastro et al\textsuperscript{25} compared 28 DXM-treated, extremely low birth weight neonates with 20 betamethasone-treated neonates who could not be weaned from the ventilator. Betamethasone has the same anti-inflammatory activity as DXM but does not contain sulphites as preservative. A shorter course and lower dose of betamethasone was nearly as effective as DXM in weaning ventilatory support without the undesirable short-term side effects of DXM. However, no data on long-term follow-up were provided for either of these studies. In a retrospective study, Heide et al\textsuperscript{26} compared hydrocortisone or DXM-treated children, matched for GA and severity of disease, with nontreated children. Improvement in respiratory status was comparable after DXM or hydrocortisone administration. Neonatal DXM-treated children needed special school education significantly more often than control children, whereas hydrocortisone-treated children had the same outcome as control children. In our study, no difference in cognitive and motor outcomes were found between hydrocortisone-treated and nontreated children.

A pilot study in 40 extremely low birth weight infants showed increased survival without BPD in hydrocortisone-treated infants during their first week of life.\textsuperscript{27} The multi-center, randomized trial that followed was stopped after the enrollment of 360 patients because of an increase in spontaneous gastrointestinal perforation.\textsuperscript{28} There appeared to be an interaction with the simultaneous use of hydrocortisone and indomethacin. The indication for hydrocortisone treatment was to prevent BPD by substituting hydrocortisone early in life, based on work showing evidence of early adrenal insufficiency and increased lung inflammation in preterm infants who subsequently have development of BPD.\textsuperscript{29} In our study, hydrocortisone was prescribed at a later stage to treat BPD. Therefore, there was no important interaction with indomethacin, which in our unit is usually prescribed in the first week of life only to treat a hemodynamically significant PDA.

Our findings suggest that hydrocortisone is a safer corticosteroid for treating preterm infants with BPD than is DXM. There are several hypotheses as to why hydrocortisone might be less harmful. DXM is a synthetic glucocorticoid, which has a 25 to 30 times higher anti-inflammatory activity than hydrocortisone. The biological half-time of hydrocortisone is 8 to 12 hours, in contrast to the 36 to 72 hours of DXM, reducing the risk of accumulation. The preservative in DXM is sodium bisulphite. Exposure of a neuronal cell line (rat mesencephalic cells) to high levels of sulphite induced a time-dependent decrease in viability.\textsuperscript{30} Sulphites were shown to be toxic in vitro to cultures of neurons and in vivo to the brains of 3- to 5-day-old mouse pups.\textsuperscript{31}

The absence of a difference in outcome, despite that the hydrocortisone-treated children were younger and sicker, needs reflection. By the time hydrocortisone was started, the neonates had reached a mean postmenstrual age of 30.5 weeks. Perhaps the absence of deleterious effects of hydrocortisone was due to this increased age, when possibly a neurologically critical period has passed. Finer et al\textsuperscript{32} reported an increased use of hydrocortisone, very early in life, to treat refractory hypotension in very low birth weight infants. This is a different indication and age at start of treatment compared with the children described in this study. In comparison to contemporary NICU standards, our children were at a rather advanced age at the start of their steroid treatment. Most of the adverse sequelae of DXM on long-term neurodevelopment were described for use of DXM soon after birth.\textsuperscript{23,34} Timing of treatment may also be important.

In a recent review, Grier and Halliday\textsuperscript{35} recommended restricted use of DXM and appropriate long-term neurodevelopmental follow-up for all infants receiving corticosteroids in the neonatal period. They also state that larger trials are required before corticosteroids other than DXM can be recommended. The current study, although observational, shows that hydrocortisone might be a safer alternative than DXM for BPD.

In conclusion, the findings in this large, long-term follow-up study in a cohort of preterm children provide strong support to the view that neonatal hydrocortisone treatment for BPD does not adversely affect neurodevelopmental outcome or conventional MRI of the brain at school age. In the absence of confirmation from adequately sized, randomized
trials, these results suggest that hydrocortisone rather than DXM should be used in cases in which corticoid treatment for chronic lung disease is judged to be necessary.

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REFERENCES


Ontogeny of Salivary Epidermal Growth Factor and Necrotizing Enterocolitis

BARBARA B. WARNER, MD, ANN LADD RYAN, MD, KIMBERLY SEEGER, MD, ANTHONY C. LEONARD, PhD, CHRISTOPHER R. ERWIN, PhD, AND BRAD W. WARNER, MD

Objective  To examine the ontogeny of salivary epidermal growth factor (sEGF) in premature infants and to determine the relation of sEGF to the development of necrotizing enterocolitis (NEC).

Study design  Salivary EGF was prospectively measured in 327 infants with gestational ages from 23 weeks to term. Infants of ≤32 weeks’ gestation (n = 261) were followed with weekly sEGF measurements through 3 weeks of life. Multivariable regression analyses were used to determine variables significantly related to sEGF levels and to identify predictors of NEC.

Results  Over the first 3 weeks of life, sEGF increased across gestational age and postnatal age categories. In multivariable models, gestational age was a significant predictor of sEGF levels (P < .009). In a cohort of 27 infants who had NEC, gestational age, race, and changes in sEGF levels between weeks of life 1 and 2 were predictive of the development of NEC. These infants had lower sEGF at week 1 and greater increases from week 1 to week 2 compared with infants without NEC.

Conclusions  There is a positive relation between sEGF levels and gestational age. Patterns of sEGF levels over the first 2 weeks of life were significantly related to development of NEC in very low birth weight infants. (J Pediatr 2007;150:358-63)

Neonatal necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency encountered in neonates, and its cause is unknown. During a period of decreasing overall neonatal mortality, the incidence of death from NEC has actually increased. Since prematurity is the single most important risk factor, it is possible that absent or reduced levels of specific factors that are normally expressed during later periods of gestation may contribute to the development of this condition. As such, exogenous replacement of key factor(s) may be clinically valuable as a means to reduce the incidence of NEC.

Epidermal growth factor (EGF) is a recognized major trophic factor for the developing intestine. Tyrosine kinase activity for the EGF receptor (EGFR) has been identified on both the apical and basolateral surfaces of enterocytes, although predominantly in the latter. In utero, infusion of EGF has been demonstrated to accelerate the maturation of intestinal enzyme activity and to stimulate intestinal growth. The importance of EGF to gut development was highlighted by the observation that EGFR knockout mice die either in utero or early in the neonatal period with a hemorrhagic enteritis that is remarkably similar to human NEC.

EGF is normally found in many endogenous fluids bathing the developing intestine, including amniotic fluid, fetal urine, breast milk, bile, and saliva. In amniotic fluid, the concentration of EGF increases as gestation progresses. Although EGF is produced to some extent in duodenal Brunner glands and kidney, the vast majority of EGF is produced in the salivary glands. The significance of salivary-derived trophic factors to the intestine was revealed by a study in which pharmacologic stimulation of submandibular gland secretion with systemic isoproterenol was shown to have a trophic effect on the small intestine. Salivary EGF has subsequently been shown to play an important role in intestinal epithelial maintenance and cytoprotection.

In humans, the ontogeny of EGF expression is poorly understood. Data on urinary EGF levels suggest a positive relation with increasing gestational age. Information on
serum EGF levels over gestational ages is limited to umbilical cord blood levels.\textsuperscript{19,20} The pattern of expression of salivary EGF (s\textsuperscript{sEGF}) in infants over a range of postnatal ages and with varied gestational ages is entirely unknown.

The purpose of the current study was to examine the ontogeny of s\textsuperscript{sEGF} in premature infants. We specifically tested the hypothesis that deficient s\textsuperscript{sEGF} expression is associated with prematurity. We propose that deficient expression of this important intestinotrophic factor contributes to the development of NEC.

\section*{METHODS}

\subsection*{Study Design and Patient Population}

This was a prospective, observational study conducted at the three hospitals that provide level III neonatal intensive care within the Cincinnati region (Good Samaritan Hospital [GSH], University of Cincinnati Hospital [UH], and Cincinnati Children's Hospital Medical Center [CCHMC]) from September 2002 to December 2004. Institutional Review Board approval was granted at each site. Infants of greater than 23 weeks' gestation at birth were enrolled at the two maternity hospital (GSH, UH) within the first 72 hours of life, after parental consent was given. Infants were enrolled from either the neonatal intensive care unit or from the mother baby unit (healthy infants \leq 35 weeks). Exclusion criteria included the presence of major chromosomal or congenital anomalies, diagnosis of cystic fibrosis, or a medical condition judged by the attending neonatologist to be incompatible with survival beyond the first week of life. After enrollment, a saliva sample was obtained, along with demographic and clinical data. Infants of \leq 32 weeks' gestation were followed through 3 weeks of life or with once-weekly collection of saliva, as well as demographic and clinical data.

\subsection*{Saliva Sample Collection and Processing}

Saliva samples were collected with sterile cotton-tipped swabs placed in the mouth of the infant and saturated with saliva. Samples were collected by nursing or research personnel between 5:00 AM and 10:00 AM before feeding. Samples were frozen at \textasciitilde 80 °C. Saliva was extracted from the swab by removing the cotton portion of the swab, placing it in a 1 mL syringe, and eluting the contents with 250 \mu L of normal saline.

Salivary EGF content was determined with the use of the commercially available Quantikine Human EGF ELISA kit (R & D Systems, Inc, Minneapolis, Minn). Total protein content of the saliva was determined by using the Quantigold Assay (Diversified Biotech, Boston, Mass). EGF levels were normalized to salivary protein and are reported as picograms EGF/micrograms of total protein.

\subsection*{Variable Definitions}

Information was collected on variables that differed between subjects (ie, gestational age at birth) as well as on variables that also varied within one subject from week to week (ie, feeding type). Between-case variables included both maternal and infant data. Maternal data included antenatal exposure to antibiotics, steroids, or indomethacin in the week before delivery. Infant data included birth weight, gestational age, based on best obstetrical estimate, small for gestational age status, sex, race, site of delivery, and Apgar score. Within-case variables were collected at each weekly sample and included day of life (DOL), exposure since last sample to antibiotics, postnatal steroids, indomethacin, inotropic support, use of oral or nasal hardware (including feeding tubes, gastric suctioning tubes, endotracheal tubes, and CPAP devices), presence of a patent ductus arteriosus, feeding data, and development of NEC. Feeding data included DOL of first feed, last feeding type before sample collection (nothing per os [NPO], infant formula, or human milk), and DOL to full feeds. Full feedings were defined as 120 kcal/kg per day. Human milk feedings were defined as use of any human milk. NEC was defined as infants with clinical and radiographic presentation greater than or equal to Bell stage II classification\textsuperscript{21} within the first 4 weeks of life.

\subsection*{Statistical Analysis}

Original data were entered and maintained in an Access (Microsoft Corporation, Roselle, Ill) database and analyzed by using the SAS statistical software package (SAS Institute Inc, Cary, NC). Normalized sEGF values were converted to log values for all statistical analyses, when used as either a dependent or independent variable, as the log values formed a normal (gaussian) distribution. Significance testing for unadjusted differences between gestational age groups (23 to 25 weeks, 26 to 28 weeks, and 29 to 32 weeks) and postnatal week was done by using restricted maximum likelihood, assuming compound symmetric covariance structure, and between-within degrees of freedom. Models predicting the log sEGF values used ordinary least-squares methods when sEGF from only one time period was predicted. For models that predicted log sEGF across time, mixed (hierarchical) models were used, using SAS Proc Mixed, assuming a compound symmetric covariance between time points, and using between-within degrees of freedom. Models predicting the NEC diagnosis were logistic models. In these, when a change in sEGF across weeks was used as an independent variable, the change variable was the change in the log sEGF values. All multivariable models were constructed through use of backward selection of predictor variables, in which each remaining independent variable was a significant predictor when adjusted for the other variables remaining in the model.

The entire cohort (all infants 23 to 41 weeks) was included in the analysis of factors significantly related to sEGF levels at enrollment, labeled week 1 values. For multivariable regression analyses of factors related to sEGF over time and factors related to development of NEC, only infants \leq 32 weeks' gestational age at birth were included.
RESULTS

Cohort Characteristics

There were 327 infants enrolled, with gestational ages that ranged from 23 to 41 weeks. Two hundred sixty-one infants (79.8%) had gestational ages less than or equal to 32 weeks at birth and were followed over the first 3 weeks of life, with weekly saliva sampling. Information on between- and within-case variables for these infants is shown in Table I. The racial composition reflected that found within the local community, with 70% of infants being white, 28% African-American, and the remaining 2% of Asian or Hispanic descent. Because of the small number of infants in racial groups other than white or African-American, models including race as a factor made use of infants in those two race categories only. All infants were inborn, consistent with the near exclusive inborn population of participating hospitals. Results reported on within-case variables are reported cumulatively over the 3-week period. Any exposure, whether single or repeated, would be included as one positive exposure in Table I. The only exception to this is feeding data. Data on last feeding type at the week 3 time point is given rather than the cumulative data to better represent changes in feeds and use of human milk over this time period.

Salivary EGF Values

Salivary EGF levels stratified by gestational age for the entire cohort at enrollment (week 1) and by postnatal week of life for infants of ≤32 weeks’ gestation at birth are depicted in the Figure. For infants of less than 32 weeks’ gestation, sEGF values increased over time over the first 3 weeks of life within each gestational age category. During the first 2 weeks of life, the level of sEGF was related to gestational age, with the lowest values in the 23 to 25 weeks’ gestation infants. By 3 weeks of life, gestational age differences began to diminish. Significance testing for unadjusted differences between gestational age groups (23 to 25 weeks, 26 to 28 weeks, and 29 to 32 weeks) and postnatal age found significant differences exist between gestational age groups and ($P < .0006$) over postnatal week ($P < .001$, see Methods).

Multivariable Regression Analysis for sEGF

A multivariable regression analysis to predict sEGF level was done by using week 1 data and included all gestational age groups. Of the original 327 infants enrolled, 319 were included in multivariate regression analysis.

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Table I. Between- and within-case variables for infants ≤32 weeks’ gestation at birth

<table>
<thead>
<tr>
<th>Between-case variables</th>
<th>Within-case variables*</th>
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<tbody>
<tr>
<td>Maternal antibiotics (%)</td>
<td>67.3</td>
</tr>
<tr>
<td>Maternal steroids (%)</td>
<td>79.9</td>
</tr>
<tr>
<td>Maternal indomethacin (%)</td>
<td>20.0</td>
</tr>
<tr>
<td>Gestational age [mean (SD)], weeks</td>
<td>28.0 (2.6)</td>
</tr>
<tr>
<td>GA distribution, n (%)</td>
<td>23 to 25 weeks</td>
</tr>
<tr>
<td></td>
<td>26 to 28 weeks</td>
</tr>
<tr>
<td></td>
<td>29 to 32 weeks</td>
</tr>
<tr>
<td>Birth weight [mean (SD)], grams</td>
<td>1098 (375)</td>
</tr>
<tr>
<td>SGA (%)</td>
<td>11.5</td>
</tr>
<tr>
<td>Female (%)</td>
<td>52.5</td>
</tr>
<tr>
<td>Race, white (%)</td>
<td>70.3</td>
</tr>
<tr>
<td>5-Minute Apgar [Ave (SD)]</td>
<td>7.3 (2.0)</td>
</tr>
<tr>
<td>Oral hardware (%)</td>
<td>69.3</td>
</tr>
<tr>
<td>Nasal hardware (%)</td>
<td>95.0</td>
</tr>
<tr>
<td>Dopamine, dobutamine, or epinephrine (%)</td>
<td>8.8</td>
</tr>
<tr>
<td>PDA (%)</td>
<td>26.1</td>
</tr>
<tr>
<td>Indomethacin (%)</td>
<td>27.2</td>
</tr>
<tr>
<td>Antibiotics (%)</td>
<td>86.6</td>
</tr>
<tr>
<td>Steroids (%)</td>
<td>10.0</td>
</tr>
<tr>
<td>H2 blocker (%)</td>
<td>7.7</td>
</tr>
<tr>
<td>Feeding</td>
<td>Last feeding type (%)</td>
</tr>
<tr>
<td></td>
<td>NPO</td>
</tr>
<tr>
<td></td>
<td>Formula</td>
</tr>
<tr>
<td></td>
<td>Human milk</td>
</tr>
<tr>
<td></td>
<td>DOL to 1st feed (median, days)</td>
</tr>
<tr>
<td></td>
<td>DOL to full feeding (median, days)</td>
</tr>
</tbody>
</table>

*Within-case variables represent any exposure up to the time of sample collection. Exposures are reported cumulatively over the 3-week period with the exception of Last Feeding. †Type, which is reported for the week-3 time point only.
sis. One was excluded for missing data on the 5-minute Apgar score and 7 for race other than white or black. In the regression analysis, lower gestational age (parameter estimate 0.09/week, 0.03 standard deviation [SD], \( P = 0.009 \)) was significantly related to lower sEGF levels after controlling for demographic and clinical variables. White race, higher 5-minute Apgar, NPO status, formula feeds, presence of oral hardware, and receiving antibiotics or indomethacin were also significantly related to lower sEGF values.

To evaluate predictors of sEGF over time, a hierarchical (both between and within case variables) multivariable regression analysis was done for infants of \( \leq 32 \) weeks. Parameter estimates and probability values of significantly related variables are shown in Table II. Gestational age and postnatal age were both significantly related to sEGF levels. For infants of \( \leq 32 \) weeks’ gestational age, sEGF levels rose significantly with increasing days of life. Differences in the rate of postnatal sEGF rise between gestational age groups did not, however, reach statistical significance. Similar to week 1 results, white race, 5-minute Apgar, NPO status, and postnatal antibiotics or indomethacin were associated with lower sEGF levels. Presence of oral hardware was no longer significant, probably related to the high proportion of infants having it in place (Table I). Formula-fed infants tended toward lower levels than human milk–fed, although it did not reach statistical significance, and NPO status remained significantly related to lower levels. Female infants of \( \leq 32 \) weeks had significantly higher levels than their male counterparts.

### Multivariable Regression Analysis Predicting NEC

Twenty-seven infants had development of NEC during the study period, for an NEC rate of 10.3% in infants of less than 32 weeks’ gestation age at birth. The median time of onset was DOL 12 (DOL 9 and DOL 20 at the 25th and 75th percentiles, respectively). Infants who had NEC were more premature and smaller compared with infants without NEC, with a mean gestational age of 26.4 weeks (1.8 standard deviation [SD]) versus 28.2 weeks (2.5 SD) and a mean birth weight of 956 grams (297 SD) versus 1112 grams (368 SD) grams. Infants who had NEC had lower levels of sEGF during the first week of life (3.6 pg/\( \mu \)g; SD, 5.1) compared with non-NEC infants (4.9 pg/\( \mu \)g; SD, 8.2), with greater increases in subsequent weeks 2 and 3 (7.4 vs 6.0 pg/\( \mu \)g for week 2 and 9.2 vs 8.1 pg/\( \mu \)g for week 3 for NEC and non-NEC, respectively). To evaluate the temporal relation of sEGF levels to NEC, sEGF levels obtained before NEC diagnosis were compared with sEGF levels obtained within the week of diagnosis. The median increase in sEGF was 130% (range, +450% to −66%), an increase greater than that expected over a 1-week interval (Figure).

To determine the between-case or within-case variables significantly related to the development of NEC, a multivariable regression model using infants of \( \leq 32 \) weeks’ gestation was constructed. Included in the analysis was the weekly sEGF value as well as the change in value between weeks. Two hundred forty infants were included in the analysis. Twenty-one infants were excluded for missing sEGF values. Three variables significantly related to the development of NEC: gestational age at birth, race, and change in EGF level were associated with NEC. For every 1-point increase in gestational age, the odds of NEC decreased by a factor of 0.23 (OR, 0.77; CI, 0.63 to 0.93). The odds of NEC was decreased for white infants (OR, 0.27; CI, 0.11 to 0.65) compared with African-American infants. Although the week-1 value of sEGF alone was not significantly related to NEC, the absolute increase in sEGF from week 1 to week 2 was significantly related to NEC. For every 1-point increase in difference between the week-1 to week-2 log value of sEGF, the odds of NEC was increased 1.66-fold. Sex, medications, feeding type, or other measured demographic or clinical variables did not significantly predict NEC after adjusting for other variables in the model.

### DISCUSSION

We report a prospective analysis of the ontogeny of sEGF in premature infants and of the link between sEGF and the development of NEC. The findings from this study demonstrate that the concentration of EGF in the saliva of neonates is significantly related to gestational age as well as postnatal age. Infants at earlier gestational ages have lower levels in the first days of life, with postnatal increases occur-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter estimate* (SD)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (per week)</td>
<td>0.05 (0.02)</td>
<td>.046</td>
</tr>
<tr>
<td>Postnatal age in (per day of life)</td>
<td>0.04 (0.01)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>5-Minute Apgar</td>
<td>−0.06 (0.02)</td>
<td>.008</td>
</tr>
<tr>
<td>Race, white</td>
<td>−0.18 (0.10)</td>
<td>.075</td>
</tr>
<tr>
<td>Sex, female</td>
<td>0.21 (0.09)</td>
<td>.017</td>
</tr>
</tbody>
</table>

Table II. Predictors of sEGF levels for infants \( \leq 32 \) weeks’ gestation, over the first three weeks of life

<table>
<thead>
<tr>
<th>Variable associated with development of NEC</th>
<th>( P ) value</th>
<th>Odds ratios (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (1-week intervals)</td>
<td>.009</td>
<td>0.77 (0.64-0.94)</td>
</tr>
<tr>
<td>Race, white</td>
<td>.004</td>
<td>0.27 (0.11-0.66)</td>
</tr>
<tr>
<td>Change in log sEGF from week 1 to week 2 (1-point difference)</td>
<td>.011</td>
<td>1.67 (1.13-2.47)</td>
</tr>
</tbody>
</table>

Table III. Multivariable adjusted odds ratios for development of NEC in infants \( \leq 32 \) weeks’ gestation at birth

* Parameter estimate from multivariable regression analysis indicating direction and strength of relation (SD, standard deviation of parameter estimate).
† Human milk feeds are defined as any delivery of human milk.
In a neonatal rat model of necrotizing enterocolitis, demonstrate a phenotype similar to that found in human between EGF and NEC. EGF receptor knock-out mice cannot be ruled out. Through oral gastric tubes, contamination by refluxed milk ing, and infants of less than 32 weeks’ gestation are fed that human milk–derived EGF contaminated the saliva sam-

drved values demonstrated in earlier work. 22,23 Saliva is increasingly being used as a noninvasive sampling source in the pediatric populations, including preterm infants. 24,25

A positive relation between EGF and gestational age has been reported for EGF concentrations in other tissues and body fluids. These studies were either cross-sectional, 16,19,20 limited to the first week of life, 17,18 or had small study populations. 15,16 This study examined the effect of gestational age, while controlling for other clinical and demographic variables that may affect sEGF levels. Markers of illness severity such as NPO status, postnatal antibiotics, and indomethacin were associated with lower sEGF levels. Whether these variables are acting as markers of illness severity or have a direct effect is unclear. Our results differ from earlier studies that found increased urinary EGF levels with postnatal steroid use. 26 This may be due to the much lower doses and shorter duration of steroids in current use. We also found no relation of sEGF levels and small for gestational age status, although other studies varied in reporting decreased levels with intrauterine growth retardation 19,27 or no relation. 17 Our data are similar to those reported for sex, 16 with higher levels found in female infants. Compared with infants who receive human milk feedings, formula-fed infants had lower sEGF levels. EGF is present in significant amounts in human milk and is systemically absorbed. 5,28,29 Alternatively, it is possible that human milk–derived EGF contaminated the saliva samples. Although care was given to obtain samples before feeding, and infants of less than 32 weeks’ gestation are fed through oral gastric tubes, contamination by refluxed milk cannot be ruled out.

Several lines of evidence implicate an important link between EGF and NEC. EGF receptor knock-out mice demonstrate a phenotype similar to that found in human NEC. 9 In a neonatal rat model of necrotizing enterocolitis, enteral supplementation with EGF significantly reduced the incidence of NEC. 30 The mechanism by which EGF confers protection in NEC has recently been linked to the well-described role of EGF in altering the balance of proapoptotic and antiapoptotic proteins at sites of injury. 31 Exogenous administration of EGF has also been shown to reduce bacterial translocation in a number of animal injury models. 32-34 Indeed, perturbation of the normal intestinal mucosal barrier, allowing translocation of bacteria or their products systemically, has been implicated in the pathogenesis of NEC. 35,36

Human data linking sEGF and NEC were previously reported by this laboratory. 22,23 Infants with surgical NEC were found to have significantly lower serum and sEGF levels compared with gestational and postnatal age–matched control subjects. A case report and small series of patients have also been treated with exogenous EGF for NEC, with reported improvement after treatment. 37,38 This study found that infants with NEC trended toward lower sEGF levels to start, with a significantly greater increase between the first 2 weeks of life.

These results were consistent when sEGF samples obtained before onset of diagnosis were compared with those obtained within the week of NEC diagnosis. These results are similar to those reported by Scott et al 39 for urinary levels of EGF in NEC, with levels increasing after diagnosis. The significance of increased EGF levels during this early time is presently unclear but may be in response to a subtle injury within the gastrointestinal epithelium. Ulceration of the epithelium from various human diseases, including Crohn disease and chronic duodenal and gastric ulcers, has been associated with the development of a novel cell line that secretes immunoreactive EGF onto the ulcer surface, which is associated with subsequent ulcer healing. 40 Whether the rise of sEGF levels found in this study is in response to early signs of intestinal epithelial injury remains conjectural.

Data regarding the effect of race on NEC have been conflicting, with some studies reporting no effect from race 41 and others reporting a significant increase in risk for black infants compared with white. 2,42,43 Within our cohort, white infants had lower risk for NEC compared with black infants. White infants also had lower levels of sEGF compared with black infants. A number of possibilities exist for this discrepancy. It may be that the relation between sEGF and NEC may not apply similarly across races, that the EGF response to injury is more important than actual levels, or, finally, that sEGF is a surrogate for some other factor that varies by race. The number of NEC cases (n = 27) limits further analysis based on race.

Advances in prenatal and postnatal care have resulted in greater numbers of low and extremely low birth weight infants surviving the initial days of life. For infants who survive, morbidity is high. NEC is related to higher rates of developmental delay and is currently the single most common cause of the short gut syndrome in children, many of whom require a small bowel and/or liver transplant. 44,45 Despite improvement in other areas of neonatal care, there have been no significant advances made in the prevention, incidence, or mortality from NEC over the last several decades. These observations underscore the significant need for additional studies directed at promotion of gastrointestinal maturation and injury repair mechanisms.

The authors thank Jenni Mason, RN, and the Neonatal Intensive Care Unit nursing staff at both Good Samaritan Hospital and University Hospital, without whose help this study would not have been possible.
REFERENCES

Association between Inadequate Sleep and Insulin Resistance in Obese Children

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Objective To analyze the relationships between sleep duration, obstructive sleep apnea syndrome (OSAS), and markers of insulin resistance in obese children.

Study design Forty obese children were evaluated for sleep-related complaints. Each child underwent a polysomnogram, an oral glucose tolerance test (OGTT), and fasting lipid panel tests. Indices of insulin resistance (HOMA-IR and WBISI) and insulin secretion (IGI) were calculated based on the results of the OGTT. Markers of insulin resistance were compared among groups categorized according to polysomnogram results.

Results Subjects with shorter sleep duration had higher fasting insulin, peak insulin, and HOMA-IR levels and lower WBISI levels, findings suggestive of insulin resistance. In contrast, differences in body mass index z scores were not observed. Subjects with OSAS (32 of 40 children) had higher triglyceride levels and HOMA-IR values than those without OSAS, but did not differ in sleep duration. Multiple linear regression analysis revealed that HOMA-IR was significantly correlated with age, sleep duration, and percentage of rapid-eye-movement sleep.

Conclusions Insulin resistance in obese children is associated with short sleep duration and OSAS. (J Pediatr 2007;150:364-9)

Besides placing adults and children at risk for the metabolic syndrome, obesity is also a significant risk factor for sleep abnormalities. Several epidemiologic studies have found short sleep duration to be closely linked to obesity in subjects of all ages. In a case-control study of 5-year-old children in France, short sleep duration was found to be associated with increased risk of obesity, even after controlling for television watching and parental overweight.1 Gupta et al2 described a link between obesity and sleep duration in a cohort of adolescents, noting that for each hour of sleep lost, the odds of being obese increased by 80%. A 13-year prospective study found a negative correlation between sleep duration and obesity in young adults even after controlling for a number of factors, including family history of overweight and levels of physical activity.3

In addition, short sleep duration has been associated with insulin resistance and impaired glucose metabolism in healthy adult volunteers. Several studies have demonstrated an association between habitual short sleep duration and subsequent risk of developing type 2 diabetes mellitus in adults.4-8 However, to the best of our knowledge, no studies to date have evaluated the relationship among sleep duration, insulin resistance, and glucose metabolism in children.

In adults, obstructive sleep apnea syndrome (OSAS), independent of obesity, is associated with insulin resistance and the metabolic syndrome. Shinohara et al9 found that visceral fat accumulation (a marker of insulin resistance) rather than subcutaneous adiposity is most strongly associated with OSAS. Prospective epidemiologic studies have also shown that adults who snore are more likely to develop type 2 diabetes mellitus and cardiovascular morbidities.10,11 In addition, sleep apnea is more common in disorders associated with insulin resistance, such as polycystic ovary syndrome.12 The aim of the present study was to analyze the association between sleep duration, severity of OSAS, and biochemical markers of insulin resistance in a cohort of obese children with sleep-related complaints.

AHI Apnea/hypopnea index
BMI Body mass index
CPAP Continuous positive airway pressure
HOMA-IR Homeostasis Model Assessment of Insulin Resistance
IGI Insulimogenic index
IL Interleukin
OGTT Oral glucose tolerance test
OSAS Obstructive sleep apnea syndrome
REM Rapid eye movement
SWS Slow-wave sleep
TNF Tumor necrosis factor
WBISI Whole Body Insulin Sensitivity Index
METHODS

We reviewed the outpatient charts of 40 obese children (body mass index [BMI] > 95th percentile for age and sex) who had been followed in the Weight Management Center of the Section of Endocrinology and Diabetes at St Christopher’s Hospital for Children. Charts were included for review if patients were obese, had completed an oral glucose tolerance test (OGTT) and a fasting lipid panel, and, because of sleep-related complaints, had completed an overnight multichannel polysomnogram at the Sleep Center at St Christopher’s Hospital for Children. Sleep-related complaints included snoring, excessive daytime sleepiness, and nonrestful sleep. A chart was not included in the review if the patient had any neuromuscular or craniofacial abnormality affecting the airway or was taking a sedative or noninhaled steroid medication up to 4 weeks before the polysomnogram. Patients with diabetes mellitus (fasting blood glucose > 126 mg/dL or 2-hour blood glucose in the OGTT > 200 mg/dL) were excluded from the study. This study was approved by the St Christopher’s Hospital for Children Institutional Review Board.

As part of the evaluation at the Weight Management Center, body weight in light clothing was recorded to the nearest 0.1 kg using a digital scale, and height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. BMI was calculated according to the standard formula (weight [kg] ÷ height [m]²). BMI z scores were computed using national standards for age and sex. Tanner staging was determined by physical examination. For the purposes of the chart review, height and weight measurements and Tanner staging closest to the date of the overnight multichannel polysomnogram were included in the study.

The following variables were measured during the overnight multichannel polysomnogram: total time in bed, total sleep time, sleep latency, sleep efficiency, percent time spent in each sleep stage, time spent snoring (snore microphone), oxygen saturation, end-tidal CO₂, nasal airflow, and thoracic/abdominal motion. During the chart review, the following variables were recorded: duration of sleep; sleep efficiency; apnea/hypopnea index (AHI), defined as the average number of episodes of apnea and hypopnea per hour of sleep; minimum oxygen saturation; peak end-tidal CO₂; percent time spent in rapid-eye-movement (REM) sleep; and percent time spent in slow-wave sleep (SWS). OSAS was defined as an AHI > 1.5 events per hour, or a minimum oxygen saturation <92%. All polysomnograms were interpreted by one of the authors (SK), who was unaware of the subjects’ laboratory results.

To complete the OGTT, after fasting overnight, insulin and glucose levels were drawn before the ingestion of 1.75 g/kg body weight (up to a maximum of 75 g) of glucose. Insulin and glucose levels were drawn at 30, 60, 90, and 120 minutes after ingestion. The glucose values from the OGTT were used to identify patients with impaired glucose tolerance (glucose at 120 minutes ≥ 140 mg/dL but < 200 mg/dL) or diabetes mellitus (fasting glucose > 126 mg/dL or glucose at 120 minutes ≥ 200 mg/dL), as defined by the American Diabetes Association. In addition, all patients underwent a fasting lipid panel.

The results of the OGTT were used to assess insulin resistance and insulin secretion. To assess insulin resistance, two indices—Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and Whole-Body Insulin Sensitivity Index (WBISI)—were calculated using the results of the OGTT. The HOMA-IR was calculated by multiplying fasting glucose (mmol/L) by fasting insulin (μU/mL), then dividing this value by 22.5. The WBISI was calculated as follows:

\[ \sqrt{\text{fasting glucose} \times \text{fasting insulin}}(\text{mean glucose} \times \text{mean insulin}) \]

The index of insulin secretion, the insulinoenic index (IGI) was calculated by dividing the Δ insulin (0 to 30 minutes) by the Δ glucose (0 to 30 minutes).

Based on the results of the polysomnogram, subjects were first divided into 2 groups according to sleep duration (> 6 hours and < 6 hours), and age, BMI z score, measures of insulin resistance, insulin secretion, glucose tolerance, and lipid levels were compared. The sleep duration of 6 hours was chosen as the separation point because this value is outside of the normal distribution of sleep duration, even for older adolescents. Patients were also divided into 2 groups according to the presence or absence of OSAS, and the same variables compared. To determine whether pubertal status acted as a confounding factor, subjects were stratified according to Tanner stage, and sleep time and AHI index were compared. Using the backward-stepwise method, multiple linear regression analysis was applied to model the significant predictors of HOMA-IR. Age and BMI z scores were included in the regression model because of their potential confounding effects. IGI and AHI were log-transformed before the analysis to achieve normal distributions.

Patient characteristics, laboratory results, and polysomnogram results were compared using unpaired Student's t tests or analysis of variance. Kolmogorov–Smirnov tests were used to assess normality of distribution. Variables with a nonnormal distribution were log-transformed before analysis. Linear regression models were developed using the backward-stepwise method. Statistical calculations were performed using SPSS for Windows, version 13.0 (SPSS Inc, Chicago, IL). All tests were 2-tailed, and P values < .05 were considered statistically significant.

RESULTS

The charts of 40 obese children were reviewed (20 males; age range, 3.5 to 18.5 years; mean age, 12.3 years) (Table 1). The mean total sleep duration measured was 737.4 ± 73 minutes. Fourteen subjects slept for < 6 hours. One subject slept for 15.5 minutes (parenteral termination of the sleep study); this subject’s data were not included in this analysis. When we divided our cohort into 2 groups according to sleep duration, we found higher fasting insulin (P = .02),
peak insulin \((P = .02)\), and HOMA-IR \((P = .01)\) values and lower WBISI values \((P = .01)\) in the group of children with a sleep duration of <6 hours. In addition, children with shorter sleep duration spent a significantly lower percentage of time in REM sleep \((P = .02)\). In contrast, age, IGI, BMI \(z\) score, and measures of severity of OSAS did not differ between the 2 groups (Table II).

Of the 40 children studied, 32 were diagnosed with OSAS. Age, BMI \(z\) score, sleep duration, OGTT, and fasting lipid panel results were compared between the group with OSAS and the group without OSAS (Table III). Triglyceride levels and HOMA-IR values were significantly higher in the group with OSAS (Table III). Although there was a trend toward greater adiposity (higher BMI \(z\) scores) in the group without OSAS, the difference was not statistically significant. No significant differences in sleep duration or any of the other remaining variables were found between the 2 groups.

Subjects were then grouped according to Tanner stage, and total sleep time and AHI were compared. Analysis of variance revealed no significant differences in sleep duration or AHI among the groups (Table IV).

Using the backward-stepwise method, sleep duration, log-transformed AHI, percent time spent in SWS (%SWS), percent time spent in REM sleep (%REM), peak CO\(_2\) levels, and nadir pulse oxygen levels were entered as independent variables. Age and BMI \(z\) scores were also included in the analysis because of their status as potential confounding factors. According to the regression equation, HOMA-IR = \(0.816 \times \text{age} + 0.023 \times \text{sleep time} - 0.457 \times \%\text{REM} - 6.297\)

This model accounts for 44% of the variance (adjusted \(R^2 = 0.44; P = .01\)). Notably, sleep study variables, rather than a measure of adiposity (BMI \(z\) score), were found to be significant predictors of insulin resistance in this cohort of obese children.

**DISCUSSION**

Sleep loss is becoming increasingly prevalent in both adults and in children. The decline in sleep duration has paralleled a dramatic increase in the prevalence of obesity and diabetes. Recent data suggest that these two epidemics may have a mechanistic relationship. Our findings indicate a link between decreased sleep duration and increased insulin resistance in obese children. Children with short sleep duration have greater insulin resistance than children with normal sleep duration. In addition, they spend proportionally less time in REM sleep. Of note, studies conducted in normal subjects have demonstrated that glucose utilization during REM sleep is higher than during non-REM sleep,\(^{16,17}\) thus suggesting decreased glucose utilization (ie, increased insulin resistance) in the subset of our patient population with short sleep duration.

In addition, our findings indicate that the association between sleep duration and insulin resistance is not due to potential confounding factors such as puberty or obesity. After stratifying the data by Tanner stage, we found no association between pubertal status and sleep duration. Multiple linear regression analysis demonstrated that age was a significant independent predictor of insulin resistance, but adiposity was not.

Although there have been no previous studies of the relationship between sleep duration and insulin resistance in children, our results are consistent with similar studies conducted in the adult population. Two controlled studies in healthy young men submitted to acute sleep reduction demonstrated increased insulin resistance and decreased glucose tolerance and insulin release.\(^{18,19}\) Of note, when sleep reduction is chronic (at least 6 months), glucose tolerance tends to normalize at the expense of significantly higher insulin secretion, suggesting the development of insulin resistance. It has also been found that adults reporting short sleep duration are more likely to develop diabetes.\(^{4-8}\) In one study, more than 8000 men with difficulty maintaining sleep were at higher risk of developing type 2 diabetes mellitus after controlling for family history of diabetes and BMI.\(^{5}\) Another, more recent study found that men reporting short sleep duration (≤ 5 to 6 hours of sleep per night) were twice as likely to develop diabetes.\(^{7}\)

Our findings concur with those of de la Eva et al,\(^{20}\) who, in a cross-sectional study of a cohort of obese children with sleep-related complaints, found an independent association of the severity of OSAS with fasting insulin levels, even after accounting for age and BMI. In contrast, Tauman et al\(^{21}\)
studied a group of obese and nonobese pediatric patients referred for evaluation of sleep complaints and found that obesity rather than severity of sleep apnea was the major determinant of insulin resistance and dyslipidemia. The opposing results may be due in part to differences in the respective populations studied. Both obese and nonobese children were included in Tauman et al’s study population. In addition, the mean age of Tauman’s study population was 3 years younger than the mean age of our population and 2 years younger than the mean age of subjects studied by de la Eva et al. We postulate that these conflicting results may have arisen because in older children, insulin resistance and sleep abnormalities may have existed for a longer time, thus strengthening their association.

A number of biological mechanisms have been thought to link sleep abnormalities and insulin resistance. Sleep deprivation leads to increased secretion of cortisol and growth hormone, two counterregulatory hormones predisposing to insulin resistance. In addition, sleep reduction results in an increased sympathetic tone, which inhibits pancreatic β-cell function and insulin secretion.

Another possible mechanistic link between inadequate sleep and insulin resistance may involve the effects of cytokines. In individuals with insulin resistance, the serum levels of proinflammatory cytokines, such as tumor necrosis factor (TNF-α) and interleukin (IL)-6, are increased. Experimental evidence also indicates that TNF-α and IL-6 can cause insulin resistance. Interestingly, several studies have demonstrated that patients with OSAS have elevated plasma concentrations of these two inflammatory mediators. In addition, one study reported increased TNF-α and IL-6 levels after mild (2 hour) sleep curtailment. Vgontzas et al have shown that injections of etanercept (a TNF-α–neutralizing antibody) in patients with OSAS results in decreased AHI, suggesting a causative role for TNF-α. On the other hand, two other studies have described a reduction of TNF-α plasma levels in patients with OSAS treated with continuous positive airway pressure (CPAP) for 4 to 6 weeks.

It remains unclear whether inadequate sleep or insulin resistance is the primary pathological process. The increased prevalence of sleep-disordered breathing in subjects with polycystic ovary syndrome (a condition in which insulin resistance is a primary abnormality) suggests that insulin resistance is the underlying causative mechanism of OSAS. Conversely, although two studies have demonstrated that CPAP treatment improves insulin resistance, implying that OSAS is the underlying pathological process, two other studies were unable to replicate those results.

Our study has three main limitations. First, as in all cross-sectional studies, the direction of causation cannot be inferred from our findings. Although we cannot determine whether insulin resistance may worsen obstructive sleep apnea or vice versa, a recent article by Vgontzas et al described a “bi-directional, feed-forward, pernicious association” between sleep apnea and insulin resistance in the adult population. Larger, longitudinal studies are needed to determine whether the same is true in obese pediatric populations. In addition, it is well known that several factors affect the development of

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**Table II. Subject characteristics according to sleep time**

<table>
<thead>
<tr>
<th></th>
<th>&gt;6 hours</th>
<th></th>
<th>≤6 hours</th>
<th></th>
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<td></td>
<td>Mean SD</td>
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<td>Mean SD</td>
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<tr>
<td>N</td>
<td>25</td>
<td></td>
<td>14</td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>11.7</td>
<td>4.4</td>
<td>14.0</td>
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<td>Prepubertal (number of subjects)</td>
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<td></td>
<td>2</td>
<td></td>
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<tr>
<td>BMI z score</td>
<td>2.7</td>
<td>0.6</td>
<td>2.5</td>
<td>0.3</td>
<td>NS</td>
<td></td>
<td></td>
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<tr>
<td>Subjects diagnosed with OSAS</td>
<td>17</td>
<td></td>
<td>12</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>164.2</td>
<td>24.2</td>
<td>158.2</td>
<td>23.6</td>
<td>NS</td>
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<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>45.6</td>
<td>14.1</td>
<td>43.1</td>
<td>7.9</td>
<td>NS</td>
<td></td>
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<tr>
<td>Triglycerides (mg/dL)</td>
<td>79.7</td>
<td>30.7</td>
<td>83.1</td>
<td>27.4</td>
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<tr>
<td>Fasting glucose (mg/dL)</td>
<td>83.4</td>
<td>9.4</td>
<td>84.9</td>
<td>5.0</td>
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<td>Glucose, 120 min (mg/dL)</td>
<td>88.4</td>
<td>21.2</td>
<td>106.1</td>
<td>25.0</td>
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<tr>
<td>Fasting insulin (μU/mL)</td>
<td>16.0</td>
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<td>25.7</td>
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<td>.02</td>
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<td>Peak insulin (μU/mL)</td>
<td>113.6</td>
<td>93.5</td>
<td>226</td>
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<tr>
<td>HOMA–IR</td>
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<td>5.5</td>
<td>2.9</td>
<td>.01</td>
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<tr>
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<td>2.2</td>
<td>1.1</td>
<td>.01</td>
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<tr>
<td>IGI</td>
<td>5.1</td>
<td>8.3</td>
<td>3.5</td>
<td>1.8</td>
<td>NS</td>
<td></td>
<td></td>
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<tr>
<td>AHI</td>
<td>7.7</td>
<td>13.8</td>
<td>10.3</td>
<td>11.5</td>
<td>NS</td>
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<td>%SWS</td>
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<td>20.3</td>
<td>7.7</td>
<td>NS</td>
<td></td>
<td></td>
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<tr>
<td>%REM</td>
<td>18.6</td>
<td>5.7</td>
<td>13.5</td>
<td>5.8</td>
<td>.02</td>
<td></td>
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<tr>
<td>Highest ETCO₂ (mm Hg)</td>
<td>52.4</td>
<td>7.1</td>
<td>51.8</td>
<td>5.0</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nadir pulse ox (%)</td>
<td>87.4</td>
<td>8.6</td>
<td>87.1</td>
<td>6.6</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep time (minutes)</td>
<td>416.7</td>
<td>33.2</td>
<td>296.1</td>
<td>60.8</td>
<td>&lt;.01</td>
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<tr>
<td>Sleep efficiency (%)</td>
<td>90</td>
<td>6</td>
<td>69</td>
<td>16</td>
<td>&lt;.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS, not significant; HDL, high-density lipoprotein; ETCO₂, end-tidal carbon dioxide concentration. Boldface type indicates statistically significant differences between groups.
Table III. Subject characteristics according to diagnosis of OSAS

<table>
<thead>
<tr>
<th></th>
<th>With OSAS Mean (SD)</th>
<th>Without OSAS Mean (SD)</th>
<th>P value</th>
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<tr>
<td>n</td>
<td>32</td>
<td>8</td>
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<tr>
<td>Age (years)</td>
<td>12.5 (4.2)</td>
<td>11.4 (4.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Prepubertal (number of subjects)</td>
<td>9 (3)</td>
<td></td>
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<tr>
<td>BMI (kg/m²)</td>
<td>30.7 (5.2)</td>
<td>29.7 (5.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>82.0 (9.5)</td>
<td>83.3 (5.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose, 120 min (mg/dL)</td>
<td>96.9 (21.6)</td>
<td>89.9 (11.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting insulin (µU/mL)</td>
<td>20.2 (13.4)</td>
<td>14.6 (9.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Peak insulin (µU/mL)</td>
<td>181.1 (137.9)</td>
<td>107.1 (57.8)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>45 (13.4)</td>
<td>43.7 (8.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>165.5 (21.7)</td>
<td>148.8 (27.3)</td>
<td>NS</td>
</tr>
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<td>Triglycerides (mg/dL)</td>
<td>88.7 (47.2)</td>
<td>65 (20.1)</td>
<td>.04</td>
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<td>HOMA–IR</td>
<td>4.6 (4.2)</td>
<td>2.7 (2.5)</td>
<td>.04</td>
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<td>5.1 (5.1)</td>
<td>6.3 (6.4)</td>
<td>NS</td>
</tr>
<tr>
<td>IGI</td>
<td>3.4 (5.7)</td>
<td>6.1 (8.4)</td>
<td>NS</td>
</tr>
<tr>
<td>AHI</td>
<td>12.3 (16.1)</td>
<td>0.3 (0.5)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>%SVS</td>
<td>19.1 (8.5)</td>
<td>19.6 (9.0)</td>
<td>NS</td>
</tr>
<tr>
<td>%REM</td>
<td>16.2 (6.5)</td>
<td>14.8 (7.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Highest ETCO₂ (mm Hg)</td>
<td>52.6 (6.6)</td>
<td>50.9 (2.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Nadir pulse ox (%)</td>
<td>85.3 (8.5)</td>
<td>93.1 (2.6)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Sleep time (minutes)</td>
<td>369 (78)</td>
<td>390 (52)</td>
<td>NS</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>79 (15)</td>
<td>84 (12)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant; HDL, high-density lipoprotein; ETCO₂, end-tidal carbon dioxide concentration. Boldface type indicates statistically significant differences between groups.

Table IV. Sleep time and AHI stratified by Tanner stage

<table>
<thead>
<tr>
<th>Tanner stage</th>
<th>I</th>
<th>2–3</th>
<th>4–5</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>5</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Mean sleep time (minutes)</td>
<td>402.7 (372.1)</td>
<td>361.4 (NS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean AHI</td>
<td>9.4 (14.2)</td>
<td>9.2 (NS)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ANOVA, analysis of variance; NS, not significant.

insulin resistance and other components of the metabolic syndrome, including exercise habits and adipose tissue distribution. For example, in adults, visceral adiposity is associated with more severe obstructive sleep apnea. Our study is limited in our ability to assess these confounding factors, because information on exercise duration or distribution of body fat was not collected. Finally, our study may be subject to selection bias. In fact, the subjects included in our study were selected based on their history of snoring or other sleep-related complaints and were not randomly chosen among all of the children followed at our Weight Management Center. Despite these limitations, however, our findings demonstrate a clear association between inadequate sleep duration, OSAS, and insulin resistance in obese children, independent of the degree of adiposity and independent of pubertal status. A longitudinal study of a larger population sample of obese children (with and without sleep complaints) is warranted, to confirm our findings and to evaluate the effects of treatment on sleep disorders and/or insulin resistance.

REFERENCES


Decreased Adult Height in Survivors of Childhood Acute Lymphoblastic Leukemia: A Report from the Childhood Cancer Survivor Study

ERIC J. CHOW, MD, MPH, DEBRA L. FRIEDMAN, MD, MS, YUTAKA YASUI, PhD, JOHN A. WHITTON, MS, MARILYN STOVALL, PhD, LESLIE L. ROBISON, PhD, AND CHARLES A. SKLAR, MD

Objective To determine risk factors associated with reduced adult height in survivors of childhood acute lymphoblastic leukemia (ALL).

Study design This was a cross-sectional study. Attained adult height was determined among 2434 ALL survivors participating in the Childhood Cancer Survivor Study, a cohort of 5-year survivors of common pediatric cancers diagnosed from 1970 to 1986, and compared with 3009 siblings.

Results All survivor treatment exposure groups (chemotherapy alone, chemotherapy with cranial or craniospinal radiotherapy) had decreased adult height and an increased risk of adult short stature (height standard deviation score $< -2$) compared with siblings ($P < .001$). Compared with siblings, the risk of short stature for survivors treated with chemotherapy alone was elevated (OR, 3.4; 95% CI, 1.9, 6.0). Among survivors, significant risk factors for short stature included diagnosis of ALL before puberty, higher-dose cranial radiotherapy ($\geq 20$ Gy versus $< 20$ Gy), any radiotherapy to the spine, and female sex.

Conclusions Survivors of childhood ALL are at increased risk of adult short stature, including those treated with chemotherapy alone. Risk is highest for those treated with cranial and craniospinal radiotherapy at a young age. (J Pediatr 2007;150:370-5)

Cranial and craniospinal radiotherapy were commonly used in the 1970s and early 1980s to treat as well as to prevent the spread of acute lymphoblastic leukemia (ALL) to the central nervous system (CNS) in children. Although radiotherapy was effective, it was associated with adverse endocrine and neurocognitive outcomes.1 As a result, over the past three decades, radiotherapy doses have been reduced or eliminated in an attempt to decrease these adverse long-term outcomes and have been replaced by more intensive chemotherapy.

Several studies have examined growth in ALL survivors. Growth deficits have been reported consistently after doses of $\geq 24$ Gy cranial radiotherapy, but the data are less consistent for doses $< 20$ Gy.2-17 The effect on loss of stature was greater in children who also received radiotherapy to the spine, secondary to direct inhibition of vertebral growth.13 For most studies in which the impact of chemotherapy without radiotherapy was examined, growth suppression during treatment was followed by catch-up growth.2,3,10,11,15,18 However, catch-up growth has not been observed consistently across studies.6,14

We hypothesized that adult survivors of childhood ALL would have shorter adult heights than their siblings and that cranial or craniospinal radiotherapy would be a significant risk factor, in a dose-dependent manner. We also hypothesized that the risk for decreased adult height among ALL survivors treated with chemotherapy alone would be smaller than that conferred by cranial or craniospinal radiotherapy. Therefore, we compared the attained adult height of a large population of pediatric ALL survivors enrolled in the Childhood Cancer Survivor Study (CCSS) with a sibling cohort to determine more precisely the risk factors associated with adult short stature.
METHODS

Childhood Cancer Survivor Study Description

The CCSS is a resource cohort study that was established to evaluate hypotheses associated with long-term health-related outcomes in childhood cancer survivors. Specifics concerning the methodology and subject accrual for this cohort have been reported in detail. Briefly, the cohort was constructed from rosters of all children treated for most forms of childhood cancer at each of 26 institutions in the United States and Canada (see Appendix). Inclusion criteria included (1) diagnosis of one of the following forms of childhood cancer before 21 years of age: leukemia, Hodgkin and non-Hodgkin lymphoma, neuroblastoma, soft-tissue sarcoma, bone cancer, malignant CNS tumor, or kidney tumor; (2) initial treatment at one of the collaborating institutions between January 1, 1970, and December 31, 1986; and (3) survival for at least 5 years after diagnosis.

The Human Subjects Committee at each participating institution reviewed and approved the CCSS protocol. Beginning August 1, 1994, all cohort members (or parents of patients under 18 years of age) completed a baseline questionnaire that included information on demographic and socioeconomic characteristics, health conditions and health-related behaviors, family history of cancer, inherited conditions and congenital anomalies, and reproductive history. Two follow-up questionnaires have been sent since, to all participants as well. For those patients who survived for 5 years after the initial cancer diagnosis and subsequently died, a family member completed the baseline questionnaire. Medical records were reviewed and abstracted for cancer diagnosis and treatment data including chemotherapy and radiotherapy exposures, using a standardized protocol.

ALL Survivors and Sibling Control Subjects

Of 5814 ALL survivors eligible for the CCSS, 811 were lost to follow-up despite tracking, 801 declined participation, and 47 were pending contact at the time of analysis. Prior analysis found no significant differences between participants and nonparticipants with respect to sex, cancer diagnosis, age at diagnosis, age at contact, and type of cancer treatment. The rate of nonparticipation was significantly higher among next-of-kin of deceased as opposed to living patients. Survivors were excluded from this analysis if diagnosed after 17 years of age or if they had a recurrence of their primary leukemia, developed a second malignant neoplasm, or underwent hematopoietic stem cell transplant before 18 years of age, leaving 2990 survivors available for analysis. Those who lacked self-reported or proxy-reported adult height data (defined as the tallest height recorded at age ≥18 years) or had incomplete radiotherapy exposure data also were excluded, resulting in a final study cohort of 2434 (2384 alive at time of study enrollment).

A cohort of 5857 siblings was randomly selected from all eligible CCSS cases. At the time of this analysis, 3846 siblings had agreed to participate and were recruited to serve as a comparison group. If a cancer survivor had more than one sibling, the sibling of closest age was selected for participation. Among siblings, 3009 were ≥18 years of age at the time of study enrollment and had self-reported adult height data. Of these siblings, 818 were siblings of ALL survivors, with the remainder being related to other CCSS cases.

Exposure and Outcome Assessment

Cumulative chemotherapy doses were available for selected agents, which included anthracyclines (daunorubicin and doxorubicin summed), cyclophosphamide, cytarabine, epipodophyllotoxins (etoposide and teniposide summed), and methotrexate (intravenous, intramuscular, and intrathecal doses). Systemic and intrathecal doses were classified separately. To examine dose-response, each agent was categorized into none, low, medium, and high doses, based on tertiles or previously published cut-points when available. Although systemic doses were adjusted for the patient’s body surface area at the time of administration, intrathecal doses were not. For asparaginase, corticosteroids, oral methotrexate, mercaptopurine, thioguanine, and vincristine, dosage information was not available, only exposure recorded “yes” or “no.” Scores for overall chemotherapy intensity were created on the basis of the cumulative number of drugs received as well as their dose (if known), with higher scores assigned to patients exposed to higher levels of individual agents. All agents were analyzed individually and in combination, along with overall treatment duration (<2.5, 2.5 to 3.5, and >3.5 years) and measures of chemotherapy intensity.

CNS radiotherapy doses were abstracted in 5-Gy increments. Ninety-five percent of survivors (1511 of 1584) who received cranial radiotherapy were treated with doses between 15 to 29 Gy to the brain and 88% of survivors (180 of 204) who received spinal radiotherapy received doses between 10 to 24 Gy to the spine. Remaining patients received radiotherapy to the brain and spine outside these ranges, but their numbers were too small to allow meaningful stratification. As a result, CNS radiotherapy doses were categorized into <20 Gy and ≥20 Gy doses. Exclusion of survivors exposed to <15 Gy or ≥30 Gy did not affect reported estimates. Radiotherapy exposures occurring after age 17 were excluded from analysis.

Height was assessed both in absolute terms as well as by standard deviation scores (SDS). Raw heights were converted to SDS by Epi Info (version 3.3.2, Centers for Disease Control Year 2000 growth charts. In general, one SDS change in height was approximately 7 cm for both male and female subjects.

Statistical Analysis

For mean height comparisons between survivor subgroups, t tests were used. Multivariable linear regression was applied to simultaneously examine factors that contributed to changes in height SDS among survivors. Multivariable logis-
tic regression was used to examine risk of clinical short stature (height SDS, dichotomized at $-2$) among survivors. To assess potential contributions of puberty, a surrogate variable was created a priori in the absence of information on pubertal timing: pubertal status at diagnosis was dichotomized at age 8 for girls and age 10 for boys. The final models adjusted for sex, age at diagnosis, ethnicity, and were stratified by the surrogate variable for pubertal status at diagnosis, given clear evidence from an exploratory analysis that radiation effects on growth differed according to pubertal status. Year of diagnosis and year of birth did not affect the outcomes and were not included in the final models. When regression models were analyzed including survivors who had missing radiation exposures as a separate group, results did not change significantly. To account for potential within-family correlation between survivors and siblings, generalized estimating equations were used whenever survivors were compared with siblings and were adjusted for sex, ethnicity, and pubertal status at diagnosis. All reported estimates represent adjusted values, unless otherwise indicated. Analyses were done with the use of STATA (version 9, StataCorp, College Station, TX).

### RESULTS

Basic demographic and clinical characteristics of the survivor and sibling cohorts are summarized in Table I. Survivors who met inclusion criteria for this study but had missing adult height or radiation exposure data differed significantly from survivors with full exposure data in the percentage of women (46.4% versus 51.1%), representation of ethnic minorities (non-Caucasian, 21.6% versus 11.7%), and older age at diagnosis (>4 years, 56.6% versus 48.7%).

When mean adult heights and height SDS stratified by sex and treatment exposures were examined (Table II), all survivor treatment groups, including those treated with chemotherapy alone, had decreased adult height and height SDS compared with siblings ($P < .001$). There was no significant height difference between siblings related to ALL survivors and siblings related to other CCSS survivors (ie, diagnoses other than ALL).

The effects of radiotherapy on adult height SDS differed between survivors who were prepubertal versus postpubertal at diagnosis (Figure). Among survivors diagnosed before puberty, height SDS was decreased at all doses of cranial and craniospinal radiotherapy compared with survivors treated with chemotherapy alone in a dose-dependent fashion (trend, $P < .001$). For example, the differences in adult height SDS between survivors exposed to chemotherapy only versus those also exposed to <20 Gy cranial radiotherapy or ≥20 Gy craniospinal radiotherapy were $-0.71$ (95% CI, $-0.84, -0.58$) and $-1.80$ (95% CI, $-2.08, -1.53$), respectively. The difference in height SDS between survivors treated with <20 Gy versus ≥20 Gy cranial radiotherapy also was significant ($-0.31$; 95% CI, $-0.45, -0.17$). Although survivors exposed to any dose of craniospinal radiotherapy had similar levels of height loss, the height SDS of these survivors were on average $-0.88$ (95% CI, $-1.08, -0.68$) lower than those treated with cranial radiotherapy alone. Among survivors diagnosed after pubertal onset, a significant negative impact on height SDS was not seen at any cranial or craniospinal radiotherapy dose compared with chemotherapy alone. However, on average the adult height SDS of survivors treated after pubertal onset remained shorter than siblings ($-0.42$; 95% CI, $-0.51, -0.32$).

All survivor exposure groups were at significantly greater risk of adult clinical short stature (height SDS < $-2$)

### Table I. Selected characteristics of cases (5-year ALL survivors) and control subjects (siblings)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Survivors n = 2434</th>
<th>Siblings n = 3009</th>
</tr>
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<tbody>
<tr>
<td>Age at last follow-up, years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>27</td>
<td>31</td>
</tr>
<tr>
<td>Range</td>
<td>18.47</td>
<td>18.56</td>
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<tr>
<td>Female, %</td>
<td>51.1</td>
<td>52.7</td>
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<tr>
<td>Ethnicity, %*</td>
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</tr>
<tr>
<td>Caucasian, non-Hispanic</td>
<td>88.3</td>
<td>88.9</td>
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<tr>
<td>Black, non-Hispanic</td>
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<td>2.3</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
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<td>3.1</td>
</tr>
<tr>
<td>Asian/Native/Pacific Islander</td>
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<td>1.4</td>
</tr>
<tr>
<td>Other</td>
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<td>1.0</td>
</tr>
<tr>
<td>Diagnosis age, %</td>
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<td></td>
</tr>
<tr>
<td>0-4 years</td>
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</tr>
<tr>
<td>5-9 years</td>
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<td></td>
</tr>
<tr>
<td>10-14 years</td>
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</tr>
<tr>
<td>15-18 years</td>
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<tr>
<td>Year of diagnosis, %</td>
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<td>1970-1975</td>
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<td>1976-1980</td>
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</tr>
<tr>
<td>1981-1986</td>
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<tr>
<td>Chemotherapy, % exposed</td>
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<td>Asparaginase</td>
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<td>Cytarabine, systemic</td>
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<td>Cytarabine, intrathecal</td>
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<tr>
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<td>Thioguanine</td>
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<td>Vincristine</td>
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<td>Radiotherapy, % exposed</td>
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<tr>
<td>No exposure</td>
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<tr>
<td>Cranial &lt;20 Gy</td>
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<td>Cranial ≥20 Gy</td>
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<tr>
<td>Craniospinal ≥20 Gy</td>
<td>4.2</td>
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</table>

*Percentages do not add up to 100 because of missing values.

ALL, acute lymphoblastic leukemia.
treated with radiotherapy did not have an increased risk of adult brain alone. Survivors diagnosed after pubertal onset and associated with similar effects, regardless of the dose to the spinal dose versus cranial doses (2.0, 4.8). Different doses of craniospinal radiotherapy were significant for prepubertal survivors (craniospinal radiotherapy [CSRT]) was significant for prepubertal survivors versus chemotherapy alone, cranial radiotherapy [CRT], and chemotherapy with vincristine could not be assessed because exposure was available. However, when exposure (yes/no) to these agents was included in the overall analysis, no modification of effect was seen.

Last, there was an increased proportion of female survivors with adult short stature (12.5%; 155 of 1243) compared with male survivors (5.5%; 65 of 1191) and female siblings (1.1%; 18 of 1587). In the regression analyses, after adjusting for age and pubertal status at diagnosis, ethnicity, and radiotherapy exposures, female survivors had an increased risk of short stature compared with the chemotherapy only group, except for a borderline association among those treated with ≥20 Gy cranial radiotherapy (OR, 2.8; 95% CI, 0.9, 8.5).

No chemotherapy agent analyzed—anthracyclines, cyclophosphamide, cytarabine, epipodophyllotoxins, and intra-thecal methotrexate—showed a consistent dose-effect or a trend suggestive of a dose relation with adult height SDS or risk of short stature when analyzed individually, in combination, or according to overall treatment intensity and duration (Table IV; available at www.ipeds.com). The dose-effect on height outcome of asparaginase, corticosteroids, thiopurines, and vincristine could not be assessed because exposure was common among all survivors and cumulative doses were not available. However, when exposure (yes/no) to these agents was included in the overall analysis, no modification of effect was seen.

Risk of short stature also appeared to be greater for survivors diagnosed before puberty versus older patients (Table III). Among prepubertal survivors, risk was greater among those who received cranial radiotherapy doses ≥20 Gy versus <20 Gy (OR, 1.9; 95% CI, 1.3, 2.8) and any craniospinal dose versus cranial doses ≥20 Gy (OR, 3.1; 95% CI, 2.0, 4.8). Different doses of craniospinal radiotherapy were associated with similar effects, regardless of the dose to the brain alone. Survivors diagnosed after pubertal onset and treated with radiotherapy did not have an increased risk of adult short stature compared with the chemotherapy only group, except for a borderline association among those treated with ≥20 Gy cranial radiotherapy (OR, 2.8; 95% CI, 0.9, 8.5).

No chemotherapy agent analyzed—anthracyclines, cyclophosphamide, cytarabine, epipodophyllotoxins, and intra-thecal methotrexate—showed a consistent dose-effect or a trend suggestive of a dose relation with adult height SDS or risk of short stature when analyzed individually, in combination, or according to overall treatment intensity and duration (Table IV; available at www.ipeds.com). The dose-effect on height outcome of asparaginase, corticosteroids, thiopurines, and vincristine could not be assessed because exposure was common among all survivors and cumulative doses were not available. However, when exposure (yes/no) to these agents was included in the overall analysis, no modification of effect was seen.

Last, there was an increased proportion of female survivors with adult short stature (12.5%; 155 of 1243) compared with male survivors (5.5%; 65 of 1191) and female siblings (1.1%; 18 of 1587). In the regression analyses, after adjusting for age and pubertal status at diagnosis, ethnicity, and radiotherapy exposures, female survivors had an increased risk of short stature (OR, 3.0; 95% CI, 2.2, 4.2) as well as decreased height SDS (−0.27; 95% CI, −0.37, −0.18) compared with male survivors.

**DISCUSSION**

This report represents the largest cohort of childhood ALL survivors evaluated for adult height to date. Although the negative impact of ALL therapy on final height has been previously documented, we used this large population of survivors to determine more precisely risk factors associated with adult short stature. We found that as a group, these survivors drawn from the CCSS cohort were at increased risk of adult short stature compared with siblings. In particular, we found clear differences in height outcomes between survivors treated with higher doses of cranial radiotherapy (≥20 Gy versus <20 Gy), as well as between those treated with <20

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**Table II. Adult height (centimeters) and height SDS stratified by sex and treatment exposures**

<table>
<thead>
<tr>
<th>Exposure group</th>
<th>Male n</th>
<th>Height (cm) Mean ± SD</th>
<th>Female n</th>
<th>Height (cm) Mean ± SD</th>
<th>SDS Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siblings</td>
<td>1422</td>
<td>180.1 ± 6.9</td>
<td>1587</td>
<td>165.5 ± 7.2</td>
<td>0.33 ± 1.11</td>
</tr>
<tr>
<td>Survivors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemotherapy alone</td>
<td>379</td>
<td>178.5 ± 7.7†</td>
<td>471</td>
<td>164.1 ± 7.3†</td>
<td>0.12 ± 1.13†</td>
</tr>
<tr>
<td>Chemotherapy, cranial RT &lt;20 Gy</td>
<td>366</td>
<td>175.2 ± 8.1</td>
<td>339</td>
<td>160.4 ± 8.1</td>
<td>−0.45 ± 1.24</td>
</tr>
<tr>
<td>Chemotherapy, cranial RT ≥20 Gy</td>
<td>339</td>
<td>174.6 ± 7.9</td>
<td>336</td>
<td>157.3 ± 8.1</td>
<td>−0.93 ± 1.24</td>
</tr>
<tr>
<td>Chemotherapy, craniospinal RT &lt;20 Gy</td>
<td>21</td>
<td>171.5 ± 9.1</td>
<td>17</td>
<td>156.2 ± 8.8</td>
<td>−1.10 ± 1.36</td>
</tr>
<tr>
<td>Chemotherapy, craniospinal RT ≥20 Gy</td>
<td>31</td>
<td>168.9 ± 9.8</td>
<td>33</td>
<td>156.1 ± 9.2</td>
<td>−1.11 ± 1.42</td>
</tr>
<tr>
<td>Chemotherapy, craniospinal RT &lt;20 Gy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemotherapy, craniospinal RT ≥20 Gy</td>
<td>55</td>
<td>168.5 ± 10.0</td>
<td>47</td>
<td>155.7 ± 7.7</td>
<td>−1.17 ± 1.18</td>
</tr>
</tbody>
</table>

RT, radiotherapy; SD, standard deviation; SDS, standard deviation score.

*P < .001 between siblings and survivors treated with chemotherapy alone.

†P < .001 for all comparisons between survivors treated with chemotherapy alone and subgroups treated with radiotherapy.

---

**Figure.** Height standard deviation scores (mean ± SD) across exposure groups stratified by pubertal status at ALL diagnosis with siblings as comparison; t test for differences in height SDS between prepubertal and postpubertal survivors were significant (+P < .05; ++P < .001). Test of trend across treatment exposure groups (chemotherapy alone, chemotherapy with cranial radiotherapy [CRT], and chemotherapy with craniospinal radiotherapy [CSRT]) was significant for prepubertal survivors (P < .001) but not postpubertal ones (P = .37).
Gy cranial radiotherapy versus chemotherapy alone. Those who received any form of spinal radiotherapy had the shortest adult heights among all survivor groups. Prior studies had not been consistent in finding differences in final height between survivors treated with 18 Gy versus 24 Gy cranial radiotherapy or between 18 Gy and treatment with chemotherapy alone. These studies had relatively few subjects who reached adult height (n < 200), limiting their statistical power to detect differences.

The mechanism by which cranial radiotherapy results in short stature is not entirely clear. Although cranial radiotherapy may affect growth hormone secretion, especially at doses > 20 Gy, the evidence for growth hormone deficiency after 18 Gy doses has been inconsistent. The duration of the pubertal growth spurt and peak growth velocity may also be decreased after cranial radiotherapy as a result of growth hormone deficiency. However, the degree of hormone deficiency does not always correlate with the degree of growth retardation, suggesting the contribution of other causative mechanisms.

A second mechanism through which cranial radiotherapy may be causing short stature is its effects on pubertal timing. ALL patients exposed to cranial radiotherapy appear to be at increased risk of earlier puberty, particularly females treated at a young age. The combination of growth hormone insufficiency and earlier pubertal onset is associated with shorter adult stature. Consistent with these hypotheses, our study found that risk of short adult stature was greater among those treated with ALL at younger ages and that girls appeared to be affected more than boys.

Contrary to some but not all previous studies, we found that compared with siblings, patients treated with chemotherapy alone had mildly shorter mean adult heights and a 3-fold increased risk of short adult stature across age groups, pubertal status, and secular time periods, even after adjustment for possible demographic confounding variables. Differences in results between this study and others may stem from differences in the definition of “adult/final” heights and in comparison groups. For example, earlier termination of follow-up may obscure significant growth changes occurring in later adolescence and use of older population norms or midparental heights may not reflect secular trends toward increased height. Also, many earlier studies predominantly enrolled patients who were exposed to cranial radiotherapy, leaving relatively few unirradiated patients for analysis, thus diminishing power to detect differences.

Limitations of this study included the use of self or proxy-reported height data, lack of longitudinal growth information, and the use of surrogate pubertal status. Self-reported heights have been well-validated and correlate closely with measured heights. Any bias introduced by self-reported values tends to overestimate true height by at most 1 to 2 cm and therefore is unlikely to explain our study’s findings. Although we did not have longitudinal growth data that allowed us to compare height SDS at the beginning of treatment with subsequent values, we used conservative final height criteria and sampled a much larger number of adult survivors compared with earlier studies resulting in greater power to detect differences between exposure groups. The use of sibling control subjects also helped to validate the significant differences found. It also is unlikely that misclassification of pubertal status accounted for the differences found between younger and older age groups in this study, as the results did not change significantly when different age cut-offs were used.

Given that the treatment of ALL involves using multiple chemotherapeutic agents simultaneously, it has been difficult to separate the contribution of individual drugs to...
adult short stature. Studies that have analyzed chemotherapy exposures by dose “intensity”\textsuperscript{14} or protocol\textsuperscript{6,11,13} have not identified specific drugs associated with growth suppression. With the chemotherapy information available in this study, we could not isolate specific agents or factors, such as treatment duration or intensity, associated with short stature. As most patients with ALL are currently treated exclusively with chemotherapy, future analyses should focus on understanding better the relationships between adult height and chemotherapy doses, duration, and type.

This research used the CCSS, a resource supported by the National Cancer Institute, to promote and facilitate research on long-term survivors of cancer diagnosed in childhood and adolescence. Investigators may apply to use the CCSS by proposing an analysis of existing data or proposing new initiatives that would use the cohort (including specimens within the biorepository). Interested investigators are encouraged to visit the CCSS website at www.cancer.umn.edu/ccss to learn more about this unique resource.

REFERENCES

APPENDIX

CCSS Institutions and Investigators

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*Institutional Principal Investigator.
†Former Institutional Principal Investigator.
‡Member CCSS Steering Committee.

Table IV. Risk of short stature (adult height SDS <-2) among survivors treated with chemotherapy alone

<table>
<thead>
<tr>
<th>Exposure</th>
<th>OR*</th>
<th>95% CI</th>
<th>Trend, P value</th>
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</thead>
<tbody>
<tr>
<td>Anthracycline, mg/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.0</td>
<td>referent</td>
<td></td>
</tr>
<tr>
<td>1-100</td>
<td>0.9</td>
<td>0.2, 4.4</td>
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<tr>
<td>101-300</td>
<td>0.9</td>
<td>0.1, 7.6</td>
<td></td>
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<tr>
<td>&gt;300</td>
<td>0.7</td>
<td>0.1, 3.2</td>
<td></td>
</tr>
<tr>
<td>.63</td>
<td></td>
<td></td>
<td></td>
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<td>Cyclophosphamide, mg/m²</td>
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</tr>
<tr>
<td>1-2000</td>
<td>1.2</td>
<td>0.4, 3.9</td>
<td></td>
</tr>
<tr>
<td>2001-5000</td>
<td>1.7</td>
<td>0.4, 6.6</td>
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</tr>
<tr>
<td>&gt;5000</td>
<td>0.7</td>
<td>0.1, 3.3</td>
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<tr>
<td>.91</td>
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<td></td>
<td></td>
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<tr>
<td>Cytarabine, mg/m²</td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>1.0</td>
<td>referent</td>
<td></td>
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<tr>
<td>1-2500</td>
<td>0.6</td>
<td>0.1, 2.9</td>
<td></td>
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<tr>
<td>251-5000</td>
<td>0.5</td>
<td>0.1, 2.1</td>
<td></td>
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<tr>
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<td>1.1</td>
<td>0.3, 4.2</td>
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<tr>
<td>.64</td>
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<tr>
<td>Cytarabine-intrathecal, mg</td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>1.0</td>
<td>referent</td>
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<tr>
<td>1-250</td>
<td>0.6</td>
<td>0.1, 2.9</td>
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<td>251-5000</td>
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<tr>
<td>Epipodophyllotoxin, mg/m²</td>
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<td>Any</td>
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<td>Methotrexate-intrathecal, mg</td>
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<td>151-200</td>
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<td>&gt;200</td>
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<td>.24</td>
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<td>Treatment intensity score‡</td>
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<td>Low</td>
<td>1.0</td>
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<tr>
<td>Medium</td>
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<td>0.1, 1.2</td>
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<tr>
<td>High</td>
<td>0.6</td>
<td>0.2, 1.9</td>
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<tr>
<td>.35</td>
<td></td>
<td></td>
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<tr>
<td>Treatment duration, years</td>
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<tr>
<td>&lt;2.5</td>
<td>1.0</td>
<td>referent</td>
<td></td>
</tr>
<tr>
<td>2.5-3.5</td>
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<td>0.4, 3.4</td>
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<tr>
<td>&gt;3.5</td>
<td>1.7</td>
<td>0.3, 9.1</td>
<td></td>
</tr>
<tr>
<td>.56</td>
<td></td>
<td></td>
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</tbody>
</table>

*Adjusted for sex, age at diagnosis, and ethnicity.
†No observations with height SDS <-2.
‡Tertiles, based on the cumulative number of drugs received as well as their dose category (none, low, medium, or high) if known.
Clinical Effects and Safety of Rituximab for Treatment of Refractory Pediatric Autoimmune Diseases

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Objective To evaluate the safety, tolerability, and clinical effects of rituximab, an anti-CD20 monoclonal antibody, in the treatment of severe pediatric autoimmune diseases.

Study design We reviewed the records of 10 patients treated with rituximab for severe, refractory autoimmune diseases at a single tertiary care children’s hospital. Adverse events as well as treatment effects were recorded.

Results All patients received 4 weekly doses of rituximab at 375 mg/m² per dose. One patient died as the result of complications of her underlying systemic lupus erythematosus 7 weeks after rituximab therapy. Three patients had serious infections, all of which resolved with standard therapy. Rituximab led to transient or sustained improvement in clinical and laboratory parameters in nine subjects. At a median follow-up of 9 months, the median prednisone dose was reduced in the responders by 0.75 mg/kg per day (mean decrease of 63%), and four patients were able to discontinue corticosteroids entirely. With longer follow-up (median, 22 months), we found that 5 of 9 patients remained clinically stable after rituximab therapy, whereas 4 patients had recurrent or new features of their underlying autoimmune disorders requiring additional corticosteroids or other immunosuppressive medications.

Conclusions Rituximab had an acceptable toxicity profile in this group of patients with severe, refractory autoimmune diseases, although there were three serious infections and one patient death. Rituximab appears to be beneficial for patients with refractory autoimmune diseases and may reduce corticosteroid exposure. Although rituximab therapy provided a durable clinical benefit for some patients in this population, other patients had reemergence of their underlying autoimmune disease. (J Pediatr 2007;150:376-82)

Autoimmunity is a complex process that involves the interaction of multiple immune cell types. B-lymphocytes are thought to play a central role in the autoimmune disease process. In addition to serving as precursors to autoantibody-secreting plasma cells, B-cells also function as antigen-presenting cells and are capable of providing costimulatory signals required for T-cell activation.1-4 Thus, depletion of B-cells could interrupt several key aspects of an ongoing autoimmune response.

Treatment of patients with refractory and severe autoimmune diseases presents a difficult therapeutic challenge. Children with refractory forms of autoimmune disease have severe morbidity, not only from the disease but also from drug toxicity and opportunistic infections.5-8

Rituximab, a chimeric anti-CD20 monoclonal antibody, selectively depletes B-cells by complement-dependent cytotoxicity and antibody-dependent, cell-mediated cytotoxicity.9,10 CD20 is a B-lymphocyte–restricted surface antigen that is expressed on B-cells from the pre-B stage to the mature B stage but not on hematopoietic stem cells, normal plasma cells, or other normal tissues.11-14 B-cell depletion with the use of rituximab has emerged as a promising therapeutic approach to the treatment of autoimmune diseases in adults, including rheumatoid arthritis, Wegener granulomatosis, dermatomyositis, autoimmune cytopenias, and systemic lupus erythematosus.15-23

Preliminary results have revealed a favorable safety profile of rituximab. When used in combination with a variety of chemotherapeutics, the only significant increase in

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From the Department of Medicine, Programs in Rheumatology, Gastroenterology, and Hematology and Department of Orthopaedic Surgery, Children’s Hospital Boston, and Section on Immunology and Immunogenetics, Joslin Diabetes Center; Harvard Medical School, Boston, Massachusetts.

Drs El-Hallak and Binstadt contributed equally to this work.

Dr Sundel is a consultant for Genentech. Genentech had no involvement in any aspect of the preparation of this report or the decision to submit the report for publication. Genentech made no payment to anyone to produce this manuscript.

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10.1016/j.jpeds.2006.10.067
toxicity has been a higher rate of neutropenia, but without a corresponding increase in infection rate. Several cases of reactivation of hepatitis B infection after rituximab therapy have been reported in adults.

In the largest prospective pediatric safety series to date, 36 patients were treated with rituximab for chronic immune thrombocytopenic purpura; infusion-related events were common (47%) with the first dose, and serum sickness occurred in 2 subjects. No infectious risk was evident, however. The current study describes a long-term follow-up investigating the safety and tolerability of rituximab in pediatric patients with a variety of severe, refractory autoimmune diseases.

**METHODS**

We reviewed the charts of 10 patients with refractory autoimmune diseases treated with rituximab at the Center for Ambulatory Treatment and Clinical Research of Children’s Hospital, Boston. Patients in organized trials were excluded from this analysis. The diseases were considered refractory if the patient continued to have active disease by clinical or laboratory criteria despite the use of a variety of immunosuppressive medications. In each case, informed consent was obtained before administration of the medication. For each patient, data were compiled from the time of rituximab treatment until June 2006. Because of the diversity of underlying conditions and concurrent medications, patients were analyzed using themselves as control; events during the interval after treatment with rituximab were compared with the same time period before the administration of rituximab for each patient. Two patients included in our prior report were not included in the present study. One was treated at a different hospital and the other one was lost to follow-up and thus lacks sufficient data regarding long-term clinical effects and adverse events.

Each patient received 375 mg/m² rituximab intravenously once weekly, for four total doses according to the manufacturer’s infusion recommendations. Patients received acetaminophen and diphenhydramine immediately before treatment but did not receive corticosteroids. Laboratory results reviewed included complete blood counts, total immunoglobulin levels, and levels of complement components C3 and C4 in all patients. In addition, specific markers of disease activity were analyzed when relevant (for example, muscle enzymes in juvenile dermatomyositis). B-cell percentages were determined by CD19 expression on peripheral blood lymphocytes by using standard flow cytometric analysis. B-cell depletion was defined as a CD19+ cell percentage of <5% of the total circulating lymphocyte count. Medication toxicity was graded by using the National Cancer Institute’s Cancer Therapy Evaluation Program’s Common Terminology Criteria for Adverse Events. Intravenous immunoglobulin (IVIG) was not given routinely after rituximab therapy. When used (as detailed in the following sections), the dose of IVIG was 400 to 500 mg/kg per dose. The Institutional Review Board at Children’s Hospital, Boston, approved this chart review.

**Statistical Analysis**

Continuous data were tested for normality to determine whether parametric or nonparametric methods should be used to assess differences between pre-rituximab and post-rituximab treatment. The Wilcoxon signed-ranks test was used to assess changes in prednisone dose and in the number and grade of infections. The paired t test was used to evaluate changes in lymphocyte counts. In addition, the nonparametric Friedman test was used to evaluate changes in prednisone dose over three time points. Normally distributed variables are presented as means and standard deviations, and those variables not conforming to a gaussian bell-shaped distribution are summarized as the median and range. A power analysis indicated that 10 patients would provide 80% power to detect changes of 30% or more in each of the variables, assuming a standard deviation or variability of 20% (effect size = 1.5, α = 0.05, β = 0.20), using paired analysis (version 6.0, nQuery Advisor, Statistical Solutions, Saugus, Mass). Analysis of the data was performed with the SPSS statistical package (version 14.0, SPSS Inc, Chicago, Ill). Two-tailed values of P < .05 were considered statistically significant.

**RESULTS**

**Demographics and Baseline Characteristics of Patients**

The study included patients with polyarticular juvenile rheumatoid arthritis (n = 1), systemic lupus erythematosus (SLE) (n = 2), juvenile dermatomyositis (n = 1), vasculitis (n = 1), Evans syndrome (n = 3), and half-siblings with undefined multisystem autoimmune diseases (n = 2) (Table I). Shorter-term follow-up of these final two patients has been published previously. The mean age of onset of disease was 11 years (range, 5 to 20), and the mean duration of disease was 5.2 years (median, 3.1; range, 0.6 to 17.3). The mean age at treatment was 16 years (range, 9 to 25). Patients received multiple immunosuppressive agents both before and after receiving rituximab (Table I). The median duration of follow-up time was 22 months (mean, 26.6; range, 1 to 57 months).

**Tolerability: Adverse Events During Infusions**

No major adverse events were observed during the rituximab infusions, and all patients received the planned four doses. Three patients had mild infusion-related symptoms including hypotension, headache, dizziness, dyspnea, and throat irritation. These primarily occurred during the first infusion and resolved after decreasing the infusion rate or administering additional diphenhydramine and acetaminophen.

**Safety: B-Cell Depletion and Recovery, Serum Immunoglobulin Levels**

As expected, rituximab treatment was associated with B-cell depletion in each of the nine patients whose B-cells were measured after treatment. We observed a negligible decrease in absolute lymphocyte counts following rituximab therapy (data not shown).
<table>
<thead>
<tr>
<th>Sex</th>
<th>Diagnoses</th>
<th>Major complaints/Rituximab indication</th>
<th>Age at diagnosis (y)</th>
<th>Age at treatment (y)</th>
<th>Disease duration (mo)*</th>
<th>Prior immunosuppressive medications</th>
<th>Concomitant treatment†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1¶</td>
<td>M IDDM, atrophic gastritis with dysplasia, enteritis, colitis (half-sibling of patient 2)</td>
<td>Abdominal pain, demyelination</td>
<td>7</td>
<td>9</td>
<td>20</td>
<td>Mesalamine</td>
<td>Mesalamine</td>
</tr>
<tr>
<td>2¶</td>
<td>F Enteritis, encephalopathy, thyroiditis, BOOP</td>
<td>Chest pain, abdominal pain, rashes</td>
<td>12</td>
<td>16</td>
<td>48</td>
<td>CYC, 6-MP, IVMP, Pred, IVIG</td>
<td>6-MP, Pred, IVIG, mesalamine</td>
</tr>
<tr>
<td>3</td>
<td>F SLE</td>
<td>Fever, rashes, chest pain, arthritis, alopecia</td>
<td>7</td>
<td>12</td>
<td>63</td>
<td>AZA, HCQ, IVMP, MMF, Pred</td>
<td>HCQ, MMF, Pred</td>
</tr>
<tr>
<td>4</td>
<td>F SLE</td>
<td>Chest pain, rashes, anemia</td>
<td>20</td>
<td>21</td>
<td>17</td>
<td>AZA, Infliximab, IVIG, MMF, Pred</td>
<td>IVIG, MMF, Pred‡</td>
</tr>
<tr>
<td>5</td>
<td>F Vasculitis, glomerulonephritis, hemolytic anemia, bronchiectasis</td>
<td>Morning stiffness, joint pain, fatigue</td>
<td>5</td>
<td>7</td>
<td>147</td>
<td>Etanercept, HCQ, MTX, Pred</td>
<td>HCQ, MTX, Pred</td>
</tr>
<tr>
<td>6</td>
<td>F Polyarticular JRA</td>
<td>Muscle weakness, rashes, calcification</td>
<td>8</td>
<td>14</td>
<td>71</td>
<td>CYC, Cyclosporine, IVIG, IVMP, MTX, Pred</td>
<td>CYC, IVIG, MTX</td>
</tr>
<tr>
<td>7</td>
<td>F JDMS</td>
<td>Bruising, splenomegaly, deterioration of reticulocyte count and falling platelet count and persistently positive Coombs</td>
<td>8</td>
<td>10</td>
<td>26</td>
<td>Pred</td>
<td>Pred</td>
</tr>
<tr>
<td>8¶</td>
<td>M Evans syndrome</td>
<td>Acute hemolytic crises with syncope, dyspnea, weakness, jaundice</td>
<td>19</td>
<td>20</td>
<td>15</td>
<td>Pred, IVMP, Splenectomy, RBC transfusion</td>
<td>Pred</td>
</tr>
<tr>
<td>9</td>
<td>M Evans syndrome</td>
<td>Deterioration of reticulocyte count, platelet count, persistently positive Coombs test</td>
<td>16</td>
<td>16</td>
<td>7</td>
<td>IVIG, IVMP, Pred, Plt/RBC transfusion</td>
<td>Pred</td>
</tr>
</tbody>
</table>

AZA, azathioprine; BOOP, bronchiolitis obliterans organizing pneumonia; CYC, cyclophosphamide; HCQ, hydroxychloroquine; IDDM, insulin-dependent diabetes mellitus; IVIG, intravenous immunoglobulin; IVMP, intravenous methylprednisolone; JDMS, juvenile dermatomyositis; JRA, juvenile rheumatoid arthritis; MMF, mycophenolate mofetil; 6-MP, 6-mercaptopurine; MTX, methotrexate; Pred, prednisone.

*Before treatment with rituximab.
†At the time of rituximab therapy.
¶Patients 1, 2, and 8 have been previously reported (Reference 27 and 37).
The rate at which B-cell numbers recovered varied among the patients. Seven of 9 patients achieved B-cell recovery at a median of 7 months after rituximab therapy (range, 6 to 12 months) (Table II). Two patients (2 and 8) continued to have <5% peripheral B-cells at 60 and 174 months, respectively, after their most recent rituximab dose. The overall median time to B-cell repopulation was 8 months. Five patients (1, 4, 5, and 7, and 8) were retreated with rituximab at a median of 17 months after the initial course. These patients again achieved B-cell depletion. Retreatment with rituximab was associated with continued clinical benefit in patients 5 and 7, but there was no appreciable improvement in patients 1, 4, or 8. Decisions regarding whether or not to retreat with rituximab, and the number of doses to administer, were made by each patient’s physicians.

Immunoglobulin (Ig)G and IgM levels remained within normal ranges for most of the follow-up period both in patients who received IVIG and those who did not. Patient 1 had moderately low IgG and IgM before rituximab, but maintained the same IgG level after treatment. She regained normal levels 30 months after rituximab therapy, which coincided with B-cell repopulation. Five patients (patients 2, 3, 5, 7, and 10) received IVIG as an immunomodulatory therapy for their underlying disorder before treatment with rituximab. Four continued to receive IVIG after B-cell depletion, whereas IVIG was discontinued in one patient after rituximab therapy (patient 10). One patient (patient 2) developed low IgM and IgG levels after rituximab therapy despite IVIG therapy. One patient (patient 8) with Evans syndrome had recurrent immune thrombocytopenia after rituximab therapy and was treated with IVIG.

**Adverse Events: Infections**

The overall rate of all infections was 2.8 events per patient-year after rituximab therapy, whereas the rate of infections before rituximab therapy was 2.4 events per patient-year (difference not significant by paired t test). Grade 3 infections are defined as those requiring intravenous antimicrobial therapy or interventional radiologic or operative therapy; grade 4 infections are those associated with life-threatening consequences such as sepsis. Patient 1 had grade 3 Staphylococcus aureus infection of a central venous line after rituximab therapy. Patient 2 had grade 3 Candidemia before rituximab therapy and grade 4 S aureus sepsis after rituximab. Patient 5 was hospitalized twice for grade 3 pulmonary infections and once for grade 3 herpes infection before rituximab; after rituximab therapy, this patient had two episodes of grade 3 pneumonia and one episode of grade 3 herpes keratitis. All infections responded rapidly to standard therapy.

**Clinical Effects**

All patients were assessed during outpatient clinic visits or admission to the hospital. Nine of the 10 patients demonstrated both clinical and laboratory improvement in markers of disease activity (Table II). Patient 3 did not improve after rituximab therapy and died as the result of preexisting aortic valve incompetence related to SLE 7 weeks after completing anti–B-cell therapy. Rituximab therapy allowed a reduction in daily prednisone dosing for 8 of 9 patients; patient 1 was not initially receiving prednisone but was receiving 2 mg daily 12 months after rituximab. The daily prednisone dose decreased significantly by 81% from the start of therapy (median, 0.75 mg/kg per day; interquartile range, 0.37 to 0.91) to the 9-month follow-up (median, 0.14 mg/kg per day; interquartile range, 0 to 0.20) (Figure). Four patients were able to discontinue oral prednisone entirely.

As indicated in Table II, with a longer median follow-up of 22 months, 5 patients remained clinically stable. However, 4 of 9 patients had new or recurrent features of their severe autoimmune diseases or have required the reintroduction or initiation of additional immunosuppressive therapy, including prednisone in some patients (Figure 1). Thus, at the 22-month median follow-up, the median daily prednisone dose was not significantly different from the start of rituximab therapy (median, 0.13 mg/kg per day; interquartile range, 0.02 to 1.0).

**DISCUSSION**

Recent data indicate that rituximab is effective and safe in the treatment of certain adult autoimmune diseases. Published reports regarding a potential role in the treatment of pediatric autoimmune diseases, however, have been comparatively limited, with trials largely restricted to hematologic disorders. In the current study, we report favorable clinical effects and safety data with the use of rituximab in the treatment of a variety of severe and refractory pediatric autoimmune diseases. Nine of 10 patients demonstrated clinical improvement after rituximab therapy, and treatment was associated with a decrease in mean corticosteroid dose. The duration of clinical effects after rituximab therapy was variable, with some patients achieving sustained benefit and others having reemergence of autoimmune disorders.

Additionally, rituximab appeared to be well tolerated in this population. The number and severity of infections did not change significantly after B-cell depletion, consistent with previous reports involving rituximab treatment of autoimmune diseases. There were five serious infections in three patients after rituximab therapy. These patients recovered fully with standard therapy. A potential association between rituximab and an increased risk of infections cannot be excluded, based on the small number of patients in this study and in view of the confounding factor that all of these patients were chronically immunosuppressed with other therapeutic agents. Further studies are necessary to determine the infection risk in heavily immunosuppressed patients treated with rituximab.

The absence of serious infusion-related reactions is consistent with the experience in adults with rheumatoid arthritis and SLE. Such reactions appear to be less frequent in patients with autoimmune conditions than in those with malignancies. In non–Hodgkins lymphoma, for example, tumor lysis
<table>
<thead>
<tr>
<th>Follow-up (mo)</th>
<th>Cycles of rituximab,* (time between cycles)</th>
<th>Initial response to rituximab</th>
<th>Time to B-cell return (mo)</th>
<th>IVIG</th>
<th>Current immunosuppressive regimen†</th>
<th>Active clinical issues</th>
<th>Time to relapse (mo)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1¶ 48</td>
<td>2 (32 mo)</td>
<td>Reduced enteritis flares and pulmonary lesions</td>
<td>6</td>
<td>No</td>
<td>Tacrolimus</td>
<td>Trilineage cytopenias</td>
<td>31.5</td>
</tr>
<tr>
<td>2¶ 57</td>
<td>1</td>
<td>Improved gastrointestinal and CNS symptoms</td>
<td>Remains &lt;5%</td>
<td>Yes</td>
<td>Tacrolimus, Pred</td>
<td>Profuse diarrhea, Pure red cell aplasia (transient)</td>
<td>49.5</td>
</tr>
<tr>
<td>3 1</td>
<td></td>
<td>1</td>
<td>Failed to respond to treatment. Died</td>
<td>NA</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4 25</td>
<td>2 (17 mo)</td>
<td>Improved arthritis. No fever. Improved rash and cytopenias</td>
<td>8</td>
<td>No</td>
<td>MMF, MTX, HCQ, Pred</td>
<td>New class III lupus nephritis</td>
<td>24.5</td>
</tr>
<tr>
<td>5 40</td>
<td>2 (15 mo)</td>
<td>Reduced fever, rash, and respiratory symptoms. No further hemolysis</td>
<td>7</td>
<td>Yes</td>
<td>Infliximab, Pred</td>
<td>Stable</td>
<td>NA</td>
</tr>
<tr>
<td>6 19</td>
<td>1</td>
<td>Decreased stiffness, pain, and fatigue</td>
<td>7</td>
<td>No</td>
<td>MTX, HCQ, Pred</td>
<td>Stable</td>
<td>NA</td>
</tr>
<tr>
<td>7 23</td>
<td>2 (23 mo)</td>
<td>Improved muscle strength, muscle enzymes, decreased fatigue</td>
<td>7</td>
<td>Yes</td>
<td>MTX, Pred</td>
<td>Stable</td>
<td>NA</td>
</tr>
<tr>
<td>8¶ 21</td>
<td>2 (17 mo)</td>
<td>Decreased episodes of petechiae</td>
<td>Remains &lt;5%</td>
<td>Yes</td>
<td>MMF, Pred 6-MP§</td>
<td>BOOP and recurrent ITP</td>
<td>11</td>
</tr>
<tr>
<td>9 17</td>
<td>1</td>
<td>No further jaundice or hemolytic crises</td>
<td>10</td>
<td>No</td>
<td>None</td>
<td>Stable</td>
<td>NA</td>
</tr>
<tr>
<td>10 15</td>
<td>1</td>
<td>Improved cytopenias, decreased bruising</td>
<td>12</td>
<td>No</td>
<td>None</td>
<td>Stable</td>
<td>NA</td>
</tr>
</tbody>
</table>

*First cycle of rituximab = 4 weekly doses of 375 mg/m² per dose; subsequent cycles occasionally consisted of fewer doses at the discretion of the treating physician.
†Immunosuppressive agents started after rituximab therapy are underlined.
‡Time after first dose of rituximab to relapse of clinical symptoms requiring additional immunosuppression.
§6-MP was started following rituximab therapy but subsequently discontinued.
||Deceased, so current status is not applicable (NA).
¶Patients 1, 2, and 8 have been previously reported (Reference 27 and 37), and a report regarding patient 8 is in press (Reference 37). Abbreviations are as per Table I; ITP, immune thrombocytopenia.
may contribute to the mostly mild-to-moderate transient infusion reactions seen in this group, the frequency of which is greater during the first infusion than during subsequent infusions. 33,34 In contrast to two prior studies in pediatric patients, 25,31 we observed no cases of serum sickness.

Initial B-cell depletion was complete in all of our patients. Relapse of clinical disease paralleled B-cell repopulation in two of four patients. This pattern is consistent with the notion that disease perpetuation depends on a restricted population of pathogenic B-cell clones and that relapse requires the reemergence of such clones. 15 Similar to studies of adult patients treated with rituximab, 36 serum immunoglobulin levels tended to remain in the normal range after rituximab therapy, although 4 of 10 of our pediatric patients were receiving IVIG therapy before treatment with rituximab and continued to receive IVIG after rituximab. Conversely, no patient required initiation of IVIG replacement for hypogammaglobulinemia after rituximab therapy in this series, similar to the experience in adult patients. 36 Thus, IVIG does not need to be given routinely after rituximab therapy. The development of recurrent infections or evidence of impaired specific immunity after rituximab would be reasonable clinical indications for the initiation of IVIG replacement therapy in select patients. In addition, IVIG therapy may be indicated for the treatment of the underlying autoimmune diseases, as in our patient 8, who received IVIG after rituximab therapy to treat recurrent immune thrombocytopenia.

Although our study is limited by the small number of patients, disease heterogeneity, and its retrospective nature, our findings suggest that rituximab therapy is beneficial and has an acceptable safety profile for pediatric patients with severe, refractory autoimmune diseases. In this population, some patients receive long-term clinical benefit from rituximab therapy, whereas others have recurrence of their autoimmune disorders. Our experience suggests that the use of rituximab for retreatment of recurrent severe autoimmune manifestations might be less effective than the initial course of rituximab therapy.

REFERENCES


In 1957, M. G. Peterman reported two sets of siblings presenting in the neonatal period with cholestasis. With the limited available therapy, there was progression of disease leading to death. Common to all the cases was the presence of giant cell transformation of liver cells on post-mortem examination resulting in the diagnosis of neonatal hepatitis. Peterman described the diagnosis as a “general all-inclusive term, probably as satisfactory as any,” recognizing that the etiology was ill-defined.

Over the last 50 years, cases of familial neonatal cholestasis have served as models to elucidate hepatic physiology, to identify specific genetic and molecular defects, and to expand the differential diagnosis of intrahepatic cholestasis presenting in the newborn period. This, in turn, has lead to improved diagnostic and therapeutic modalities.

Many of the known genetic forms of intrahepatic cholestasis fall into broad categories of defects in embryogenesis, bile acid metabolism, and canalicular transport. Other causes of intrahepatic cholestasis include multi-organ disorders, metabolic diseases, and other clinically definable syndromes.1

Evaluation of infants presenting with conjugated hyperbilirubinemia has evolved to include recognition of the immature physiology of the newborn liver, evaluation for infection and metabolic disease, radiographic study of the biliary anatomy, and differentiation of intrahepatic cholestatic syndromes through a combination of clinical features, serum bile acid level, serum gamma glutamyl transpeptidase level, and liver biopsy. Diagnostic studies of the future include development of a gene chip, biomarkers of genetic defects, and targeted testing of molecular defects.

Continued understanding of liver physiology and molecular pathophysiology of cholestatic disease has resulted in therapy to reduce symptoms, improve nutritional status, and delay progression of disease. These measures encompass medical therapy as well as surgical intervention including liver transplantation. The goal, however, should be to avoid the need for liver transplantation by defining and treating the underlying disease.

Despite the progress over the last 50 years in diagnosis and treatment of neonatal hepatitis, approximately 15% of patients continue to be diagnosed with idiopathic disease.1 Clearer definition of phenotype, precise nomenclature of known diseases, and multi-center registries will be critical in improving diagnostic and treatment strategies in those with defined disease and in understanding the etiology of disease in those yet undiagnosed cases.

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10.1016/j.jpeds.2006.11.014

REFERENCE

Objective  We studied the relationship between % body fat (%BF), cardiovascular fitness (CVF), and insulin resistance (IR) in overweight middle-school children.

Study design  Middle school children (n = 106, body mass index [BMI] > 95th percentile for age) underwent evaluation of body composition, maximal volume of oxygen utilization (VO₂) uptake/kg lean body mass (VO₂max/kgLBM), and fasting glucose and insulin (FI) concentrations and derived homeostasis model assessment index (HOMAIR).

Results  Both %BF (r = .33, P < .001) and VO₂max/kgLBM (r = −0.42, P < .0001) were significantly correlated with FI. Bivariate regression analysis revealed %BF (P = .008 vs FI, P = .035 vs HOMAIR) and VO₂max/kgLBM (P < .001 vs FI, P = .009 vs HOMAIR) to be independent predictors of insulin sensitivity. In males, VO₂max/kgLBM was a better predictor of FI and HOMAIR than %BF.

Conclusions  In obese middle-school children, both %BF and VO₂max/kgLBM are independent predictors of FI levels. The relationship between CVF and FI levels was significant in both sexes but was particularly profound and stronger than %BF in males. Efforts to reduce risk of type 2 diabetes mellitus in an increasingly obese child population should include exercise intervention sustained enough to improve CVF. (J Pediatr 2007;150:383-7)

An increasingly pervasive environment of reduced physical activity coupled with easy access to calories is spawning an epidemic of poor cardiovascular fitness (CVF), obesity, insulin resistance (IR), type 2 diabetes mellitus, blood lipid abnormalities, and hypertension in our youth. Studies in adults have shown IR to be an independent predictor for the development of hypertension, coronary heart disease, stroke, cancer, and type 2 diabetes, and greater insulin sensitivity a protective factor against all of these clinical events. These data serve as a strong stimulus to devise effective public health strategies to improve insulin sensitivity in children and adolescents. The degree to which such a strategy should emphasize improving fitness or reducing fatness in children remains unresolved.

Although obesity clearly increases the risk of insulin resistance, type 2 diabetes mellitus, and other cardiovascular morbidities, it has been shown in adults that level of fitness is a more accurate predictor of cardiovascular and all-cause death than weight status. It is presumed that the beneficial effect of improved CVF on insulin sensitivity reflect combined effects of increased lean mass and reduced fat mass; however, physical activity alters insulin sensitivity independent of changes in weight and body composition in adults and in children. If CVF is shown to have a more profound effect on insulin sensitivity than percent body fat, efforts to improve insulin sensitivity in children may be best focused on increasing physical activity rather than simply restricting calories to achieve weight control. In this study, we report the relationships between insulin sensitivity (assessed by fasting insulin [FI] levels and HOMAIR), CVF (measured by maximal oxygen uptake/kilogram lean body mass [LBM]), and percent body fat (measured by dual-energy x-ray absorptiometry [DXA]) in a cohort of overweight middle school children.
METHODS

Children (n = 106) at a single middle school with a body mass index above the 95th percentile for age participated in this study. Over a 24-month period, each participant underwent testing at the University of Wisconsin Exercise Science Laboratory before and at the conclusion of the 9-month school year during a single visit after an overnight fast, supervised by the same investigators. The procedures were approved by the Human Subjects Committee, and informed written consent was obtained before initiating the testing protocol. Testing included a physical examination, blood work to determine fasting glucose and FI levels, baseline body composition, and CVF assessment before beginning the program. Subjects with evidence for glucose intolerance or overt type 2 diabetes were excluded. Height was measured on a wall-mounted stadiometer to the nearest 0.5 cm. Weight was measured on a calibrated beam balance platform scale to the nearest 0.1 kg.

Percent body fat and LBM were measured by DXA. Whole body scans were performed with the Norland XR-36 whole body bone densitometer (Norland Corporation, Ft. Atkinson, WI) and tissue masses were analyzed with software version 3.7.4/2.1.0. Each scan session was preceded by a calibration routine with multiple quality control phantoms that simulate soft tissue and bone. Based on 18 scans of 6 subjects with the XR-36 whole body procedures, the total body coefficients of variation are as follow: soft tissue mass 0.2%, total body mass 0.2%, lean body mass 1.0%, fat mass 2.5%, percent fat 2.4%, and total BMC 0.9%.12

Children underwent a 4-minute submaximal treadmill walk test at 5% grade and subsequent measurement of maximal oxygen consumption (VO2max) performed by open circuit spirometry with a progressive treadmill walking protocol to volitional fatigue with a Medical Graphics CPX-D (St. Paul, MN). Specifically, the speed of the treadmill is set initially per the subject’s comfort, starting at 0% grade and increasing 2% every minute. Requirements to strictly define whether subjects reached their maximal oxygen consumption by this protocol included at least 2 of the following 4 criteria: (1) visible evidence of exhaustion; (2) maximal heart rate >200 beats/min; (3) respiratory exchange ratio (VCO2/VO2) >1.0; and (4) a plateau in oxygen consumption (Fig 1). The plateau in oxygen consumption was defined as a change of < 2 mL/kg/min in O2 consumptions over the last 60 seconds of the test. All subjects tested to exhaustion, 98.5% had respiratory exchange ratio (RER) > 1.0 and fulfilled 2 criteria, and 78% of participants achieved 3 or all 4 of the criteria. Analysis of LBM by DXA was used to calculate VO2max/kgLBM. Since the major influence of body weight on VO2max is explained by fat free mass (FFM) and fat mass itself has minimal effect on VO2max, CVF was expressed as VO2max/kgLBM.

A 10-mL fasting blood sample for insulin and glucose was obtained from an antecubital vein. Samples were separated by low-speed centrifugation 4000g for 10 minutes.

Figure 1. Graphical representation of maximal exercise testing in child demonstrates successful fulfillment of criteria for VO2max measurement.
Fasting insulin concentration was determined with the chemiluminescent assay (Esoterix, Callabasas Hills, CA), and glucose concentration was determined by an enzymatic method (Beckman Diagnostics, Fullerton, CA). HOMAIR was calculated with the formula: Fasting insulin (uU/mL) × Fasting glucose (mmol/L)/22.5, assuming that normal young subjects have an insulin resistance of 1.0.13

During the 9-month school periods within the 24-month testing period, children participated in physical education classes 5 times every 2 weeks for a 45-minute class period. Three subjects dropped out from this study; 2 moved away during the school year, and one student stopped coming to school because of non-medical reasons. None of their data were included in these analyses, and none of these data were significantly different from the rest of the cohort at baseline. Tests were repeated in participating children at the conclusion of and beginning of each school year during the 24-month period, resulting in 222 observations in 106 different children.

Statistical Methods

Body composition, cardiovascular fitness, and insulin sensitivity measurements were summarized by standard descriptive statistics in terms of means and standard deviations. The bivariate associations between body composition, cardiovascular fitness and fasting insulin concentration were examined with the Spearman rank correlation analysis. Univariate and multiple regression models with subject specific random effects were used to predict fasting insulin concentration from body composition and cardiovascular fitness measurements. Fasting insulin concentration was log-transformed in the regression analyses to meet the assumption of normality. Since the study design involved repeated tests in subjects, an autoregressive correlation structure was used to account for correlations between repeated measurements within a subject. Statistical inference was performed with maximum likelihood estimation. For the cross-validation studies, univariate regression models, with %BF or VO2max/kgLBM as predictor variables, were fitted using data from a subset of k (k = 50 and 100) randomly selected subjects.14,15 The fasting insulin concentrations of the subjects/observations not selected for the regression analysis were compared to the predicted fasting insulin concentration from the regression equations. This process was repeated 100 times, and the mean squared errors of the predicted versus observed fasting insulin concentrations were computed. A smaller mean squared error represents a better fit. All statistical analyses were performed with SAS software (version 8.2; SAS Institute, Cary, NC). All P values were 2-sided, and P values ≤ .05 were considered statistically significant.

RESULTS

Patient characteristics are presented in the Table as mean ± SD. At study enrollment, mean age of the study participants was 12.8 ± 1.4 years, and 55% of the subjects were female. The mean body mass index (BMI) was 30.8 ± 5.9 (males 31.7 ± 6.9, females 30.1 ± 5.0; P = .237), and mean percent body fat (%BF) was 35.8 ± 5.5 (males 36.0 ± 6.3, females 35.6 ± 4.9; P = .462). Mean measurement of VO2max/kgLBM at study initiation was 62.0 ± 16.4 mL/kg/min for females, 68.3 ± 16.4 mL/kg/min for males (P = .215), and 64.8 ± 21.8 mL/kg/min for the combined group. Mean fasting insulin was 24.3 ± 16.1 μIU/mL (males 23.9 ± 17.1 μIU/mL, females 24.6 ± 15.4 μIU/mL, P = .665; normal values 4-24 μIU/mL). Mean HOMAIR was 5.1 ± 2.3 for females and 4.9 ± 2.7 for males (P = .872), and 5.0 ± 2.5 for the combined group.

For all subjects, univariate analysis revealed highly significant correlations between FI and VO2max/kgLBM (r = −0.42, P < .001; Fig 2) and FI and %BF (r = .33, P < .001; Fig 3), indicating that lower CVF and higher body fat had a highly significant association with higher fasting insulin levels. When HOMAIR was used as the indicator of insulin sensitivity, the correlation with VO2max/kgLBM remained significant (r = −0.27, P = .001), but the correlation with %BF was less robust (r = .18, P = .07).

Among all subjects, %BF and VO2max/kgLBM were strongly and negatively correlated with each other (r = −0.57 (P < .001). Consequently, bivariate regression analysis was performed to correct for this interaction. With this model, for males and females combined, both %BF (P = .008 vs FI, P = .035 vs HOMAIR) and VO2max/kgLBM (P < .001 vs FI, P = .009 vs HOMAIR) were significant independent predictors of

**Table. Study subject characteristics (n = 106 subjects)**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Males</th>
<th>Females</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>106</td>
<td>48 (45%)</td>
<td>58 (55%)</td>
<td>.943*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>12.8 ± 1.4</td>
<td>12.7 ± 0.9</td>
<td>12.9 ± 1.7</td>
<td>.731†</td>
</tr>
<tr>
<td>BMI</td>
<td>30.8 ± 5.9</td>
<td>31.7 ± 6.9</td>
<td>30.1 ± 5.0</td>
<td>.237†</td>
</tr>
<tr>
<td>VO2max/kgLBM (mL/kg/min)</td>
<td>64.8 ± 21.8</td>
<td>68.3 ± 16.4</td>
<td>62.0 ± 16.4</td>
<td>.251†</td>
</tr>
<tr>
<td>%Body fat</td>
<td>35.8 ± 5.5</td>
<td>36.0 ± 6.3</td>
<td>35.6 ± 4.9</td>
<td>.462†</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>51.33 ± 11.29</td>
<td>53.35 ± 19</td>
<td>49.66 ± 8.26</td>
<td>.336†</td>
</tr>
<tr>
<td>Fasting insulin (μIU/mL)</td>
<td>24.3 ± 16.1</td>
<td>23.9 ± 17.1</td>
<td>24.6 ± 15.4</td>
<td>.665†</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>86.0 ± 6.6</td>
<td>85.9 ± 6.5</td>
<td>86.0 ± 6.8</td>
<td>.947†</td>
</tr>
<tr>
<td>HOMAIR</td>
<td>5.0 ± 2.5</td>
<td>4.9 ± 2.7</td>
<td>5.1 ± 2.3</td>
<td>.872†</td>
</tr>
</tbody>
</table>

* Chi-square test. † Wilcoxon rank sum test.
FI levels and insulin sensitivity by HOMAIR. Thus in this group of male and female children combined, VO2max/kgLBM and %BF were independent predictors of insulin sensitivity, and VO2max/kgLBM was a better predictor of insulin sensitivity than %BF. Cross-validation studies confirmed a smaller mean squared error of predicted versus observed FI values for VO2max/kgLBM (12.99 μIU/mL) than for %BF (14.51 μIU/mL), indicating that in this study group VO2max/kgLBM was a better predictor of fasting insulin levels than %BF.

When males (n = 48 subjects, 103 observations) and females (n = 58 subjects, 119 observations) were analyzed separately, the sex-specific effect of %BF on FI (r = .37, P = .005 for females; r = .30, P = .031 for males) was significant for both sexes. In males, VO2max/kgLBM had a clearly stronger relationship to FI (r = −.52, P < .001) and HOMAIR (r = −.417, P = .0032) than %BF (r = .29). For females, VO2max/kgLBM retained a highly significant inverse correlation with FI (r = −.38, P = .004) equal in strength to that of %BF. However, there was no correlation between %BF and HOMAIR (P = .2294) or between VO2max/kgLBM and HOMAIR (P = .35) in females. Further, when VO2max over total body mass (rather than LBM) was used, multivariate regression analysis (ie, regression model of FI levels on VO2max/kg total body mass or %BF) showed the association with FI to be significant for males (P = .001) but not for females (P = .214).

DISCUSSION

This study shows that, like adults, cardiovascular fitness is an equal, perhaps even more important predictor of insulin sensitivity than is fatness in children. Differences in the interactions between CVF and %BF with sex have been noted previously. Our data suggest that the adverse effects on FI levels were most profound in males with high %BF and low CVF. In one prior study of older adolescents, when CVF (assessed by sub-maximal VO2 consumption) and %BF were analyzed together, for boys, both CVF and %BF retained predictive value for FI levels, whereas for girls, only %BF was significantly predictive of FI levels. In our study of younger children, obese females also showed statistically more robust correlations between %BF and FI levels than between VO2max/kgLBM and FI, and less robust (but still significant) correlations than males between VO2max/kgLBM and FI. The preservation of CVF as a predictor of FI in females in our study could be related to the use of VO2max/kgLBM as a more precise measurement of CVF than VO2max/kg total body mass. Studies of children whose fitness was assessed by submaximal aerobic capacity show that the major influence of body weight on VO2max is explained by LBM, that fat mass does not have any effect on VO2max, and that fitness and VO2max should be considered independent entities. Expressing VO2max per total body mass, rather than LBM, confounds the expression of fitness, especially when discussing metabolic activity, by incorporating fatness, and thereby relatively inert tissue, into the calculation of VO2max. The slightly lower VO2max/kgLBM in combination with a comparable %BF in females may explain why the association of FI with VO2max/kg total body mass was not statistically significant, in contrast to males. And whereas there were relatively small differences in body composition (ie, either %BF, kg of LBM, or the ratio of %BF to LBM) between boys and girls in this group of prepubertal and early pubertal children, greater discrepancies in %BF with advancing puberty could be expected to further confound the expression of fitness as VO2max/total body mass.

It is important to note particular characteristics of the study group and limitations of this report. First, even though all children had BMI >95th percentile, only mild elevations in fasting insulin levels were observed. On the one hand, it is noteworthy that significant effects of CVF on FI were still evident within a fairly narrow range of observed FI levels. On the other hand, it is unknown whether these mild elevations...
in FI levels can be confidently associated with the morbidities of hyperinsulinemia cited above. Second, the sample size of the study (106 subjects) was still fairly small, and we used repeated measurements in the same subjects. Finally, we relied primarily on FI levels and, to a lesser extent, calculated HOMA$_{IR}$ as indicators of insulin sensitivity. Although not as diagnostic for insulin resistance as frequently sampled testing of glucose and insulin, both fasting insulin levels and HOMA$_{IR}$ have been validated as strongly correlated with frequently sampled intravenous glucose tolerance test in obese children and adolescents. The lower strength of correlation between %BF and VO$_{2\text{max}}$ and HOMA$_{IR}$ compared with FI levels in this study was unexpected. However, FI levels have been found to have greater precision than HOMA$_{IR}$ alone in some studies.\textsuperscript{19}

Improved physical fitness is clearly effective in improving insulin sensitivity in adults,\textsuperscript{20} but most adults do not achieve the Surgeon General’s recommended 30 minutes of moderate physical activity on most days of the week.\textsuperscript{21} Childhood is a critical period for nurturing lifetime activity behavior\textsuperscript{22,23} and an attractive starting point for collaborative effort is the school setting, where both active and passive decisions regarding physical activity, food choices, and attendance can be reasonably controlled and programmatically altered. Still, skepticism about the importance and feasibility of changing fitness levels in children has limited acceptance and application of policies and programs required to achieve this goal. We and others have shown that school-based programs can significantly improve cardiovascular fitness and reduce fasting insulin levels in overweight children.\textsuperscript{14,24,25}

Special thanks to Robert Hanssen and Nancy Crassweller, MS, the administration and the volunteer students of River Bluff Middle School for their assistance in carrying out this project.

REFERENCES

Relationship of Physical Activity, Fitness, and Fatness with Clustered Metabolic Risk in Children and Adolescents: The European Youth Heart Study

NICO S. RIZZO, MSc, JONATAN R. RUIZ, BSc, ANITA HURTIG-WENNLÖF, PhD, FRANCISCO B. ORTEGA, BSc, AND MICHAEL SJÖSTRÖM, MD, PhD

Objectives To examine the associations of physical activity (PA) at different levels and intensities and cardiorespiratory fitness (CRF) with a clustering of metabolic risk factors in children and adolescents with special consideration of body fat.

Study design Total PA and intensity levels were measured by accelerometry in children (9 years, n = 273) and adolescents (15 years, n = 256). CRF was measured with a maximal ergometer bike test. Measured outcomes included fasting insulin, glucose, triglycerides, total and high-density lipoprotein cholesterol, blood pressure, and body fat. A metabolic risk score (MRS) was computed as the mean of the standardized outcome scores. A “non-obesity-MRS” was computed omitting body fat from the MRS. Analysis of variance and multiple regressions were used in the analysis.

Results Total and vigorous PA was inversely significantly associated with MRS in adolescent girls, the group with lowest PA, becoming insignificant when CRF was introduced in the analysis. Significant regression coefficients of total PA and CRF on non-obesity–MRS diminished when body fat was entered in the analysis.

Conclusions CRF is more strongly correlated to metabolic risk than total PA, whereas body fat appears to have a pivotal role in the association of CRF with metabolic risk. (J Pediatr 2007;150:388-94)

Prevalence of the metabolic syndrome has increased dramatically in the last two decades. Clustering of metabolic risk factors has been found in pediatric populations. Large population studies have shown that even though the prevalence of the metabolic syndrome in children and adolescents is relatively low (~4%) when compared to the adult population (~24%), there is a high prevalence of the metabolic syndrome in overweight and obese adolescents (29%).

Low physical activity (PA) levels and cardiorespiratory fitness (CRF) have been associated with a higher clustering of metabolic risk factors in young people and adults. Similarly, observational studies have shown that childhood adiposity is associated with an unfavorable metabolic profile, which continues into adulthood. Because of the strong inverse correlation between CRF and fatness, it is possible that deleterious consequences ascribed to adiposity may be partially due to the influence of low CRF. The positive influence of PA on both fitness and fatness has also been recently shown.

Studies examining associations between PA, CRF, and metabolic risk factors are limited and generally confined to questionnaire-based assessments of PA, which often lack the necessary accuracy, especially in children. Recently it has been shown that there might be an independent, inverse relationship between objectively measured PA and metabolic risk factors after adjustment for CRF in children. Yet, the influence of the intensity level of PA on the relationship was not analyzed in that study. For preventive purposes it is of interest to understand the relative importance of total PA and its intensity levels. Additionally, it is of importance to understand the influence of sex and age. Therefore the purpose of this study was to examine the effects of PA at different levels and intensities and CRF on metabolic risk factors in Swedish children and adolescents, with special consideration of body fat.
METHODS

Study Design

This was a school-based, cross-sectional study of a representative sample of 273 healthy Swedish children (9.6 ± 0.4 years) and 256 adolescents (15.6 ± 0.4 years). Study design, selection criteria and sample calculations have been reported elsewhere.16,17 The local ethical committees approved the study protocols (Örebro City Council case no. 680/98 and Huddinge University Hospital case no. 474/98). The children and their families received written information about the purpose and the content of the study. Written informed consent was obtained from one of the parents or a legal guardian for all the participants, and additional written informed consent was also obtained from the 15 year old participants.

Data Collection

Physical Examination. Body mass index (BMI) was calculated as weight/height squared (kg/m2). Skinfold thicknesses were measured with a Harpenden caliper at the biceps, triceps, subscapular, suprailliac, and triceps surae on the left side of the body according to the criteria described by Lohman et al.18 These measures have been shown to correlate highly with dual-energy X-ray absorptiometry-measured body fat percentage in children with similar ages.19,20 In this analysis, the sum of 5 skinfolds was used as an indicator of body fat since it has been suggested that BMI may not be the best indicator of body fat in children,21,22 and fatness rather than weight is associated with poor health.23 Nevertheless, for the purpose of comparing the results with previous publications, the subjects were also categorized as normal-weight, overweight, and obese applying the cutoff points published by Trost et al.29 The sum of moderate and vigorous PA (≥3 METs) was computed as a new PA intensity variable. Each minute over the specific cut-off was summarized in the corresponding intensity level group. Validation studies examining the accelerometer used in this study and the construction of summary variables for intensity of movement suggest that it is a valid and reliable measure of children’s PA.30,31

Cardiorespiratory Fitness. CRF was assessed according to the Hansen protocol32 with an electronically braked Monark bike ergometer (model 839E, Varberg, Sweden). Briefly, the workload was preprogrammed on a computerized cycle ergometer (Monark 829E Ergomedic, Vansbro, Sweden) to increase every third minute until exhaustion. Heart rate was registered continuously by telemetry (Polar Sport Tester, Kempele, Finland). Criteria for exhaustion were a heart rate ≥185 beats/min, failure to maintain a pedaling frequency of at least 30 revolutions/min, and a subjective judgment by the observer that the child could no longer keep up, even after vocal encouragement.

The power output was calculated as \( W = W_1 + (W_2 \times t/180) \), where \( W_2 \) is a work rate at fully completed stage, \( W_2 \) is the work rate increment at final incomplete stage, and \( t \) is time in seconds at final incomplete stage. The “Hansen formula” for calculated maximal oxygen consumption in milliliters per minute was \( = 12 \times \text{calculated power output} + 5 \times \text{body weight in kilograms}.32 \) CRF was expressed as maximal oxygen consumption per kilogram of body mass (mL/kg/min).

Socioeconomic Status and Other Factors. Maternal education, smoking habits, and ethnic background were assessed by means of a questionnaire. Low educational level was defined as 9 years of compulsory education or less, medium level as completed upper secondary school education or equivalent, and high level was defined as a completed university degree. Use of maternal educational status as the socioeconomic sta-
Intolerance, obesity, hypertension, and dyslipidemia, but they agree on the essential components such as glucose metabolism, insulin resistance, hyperinsulinemia, dyslipidemia, and HDL-C levels.

Definition of the Metabolic Risk Score

Several health care organizations have proposed clinical criteria for the metabolic syndrome in adults. These definitions agree on the essential components such as glucose intolerance, obesity, hypertension, and dyslipidemia, but they differ in detail and inclusion criteria. None of the cutoff points, though, apply specifically to children. Smoking every day or nearly every day by either parent was coded as smoking.

Statistical Analysis

All variables were checked for normality and appropriately transformed where necessary. Values for triglycerides and HDL-C were logarithmically transformed after being multiplied by a factor of 100. Insulin values were square rooted after being multiplied by a factor of 100. The sum of 5 skinfolds and total PA were logarithmically transformed.

Table I. Descriptive characteristics of the study sample

<table>
<thead>
<tr>
<th></th>
<th>Girls (n = 132)</th>
<th>Boys (n = 141)</th>
<th>P = for sex</th>
<th>Girls (n = 133)</th>
<th>Boys (n = 123)</th>
<th>P = for age groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>9.6 ± 0.4</td>
<td>9.5 ± 0.3</td>
<td>.044</td>
<td>15.6 ± 0.4</td>
<td>15.7 ± 0.4</td>
<td>.380</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>134 ± 7</td>
<td>139 ± 6</td>
<td>.305</td>
<td>166 ± 6</td>
<td>175 ± 8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>34.1 ± 6.3</td>
<td>32.8 ± 5.7</td>
<td>.086</td>
<td>57.8 ± 9.7</td>
<td>63.4 ± 10.7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Sum of 5 skinfolds (mm)</td>
<td>49.2 ± 18.9</td>
<td>37.5 ± 15.5</td>
<td>&lt;.001</td>
<td>63.2 ± 22.1</td>
<td>39.9 ± 17.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>17.3 ± 2.2</td>
<td>16.9 ± 2.3</td>
<td>.145</td>
<td>21.0 ± 3.0</td>
<td>20.5 ± 2.7</td>
<td>.185</td>
</tr>
<tr>
<td>Overweight and obesity (%)</td>
<td>.063</td>
<td></td>
<td></td>
<td>.876</td>
<td>.632</td>
<td>.228</td>
</tr>
<tr>
<td>Obese (%)</td>
<td>13</td>
<td>6</td>
<td></td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>6.1 ± 3.1</td>
<td>4.4 ± 2.4</td>
<td>&lt;.001</td>
<td>9.4 ± 3.8</td>
<td>8.3 ± 3.4</td>
<td>.016</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.9 ± 0.3</td>
<td>5.0 ± 0.3</td>
<td>.059</td>
<td>4.9 ± 0.4</td>
<td>5.1 ± 0.4</td>
<td>.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.8 ± 0.3</td>
<td>0.6 ± 0.2</td>
<td>&lt;.001</td>
<td>0.9 ± 0.4</td>
<td>0.8 ± 0.4</td>
<td>.005</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.5 ± 0.7</td>
<td>4.4 ± 0.6</td>
<td>.272</td>
<td>4.3 ± 0.7</td>
<td>3.8 ± 0.7</td>
<td>.004</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.4 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td>.002</td>
<td>1.4 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>.005</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>105 ± 7</td>
<td>104 ± 6</td>
<td>.793</td>
<td>109 ± 8</td>
<td>117 ± 10</td>
<td>&lt;.001</td>
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<tr>
<td>Cardiorespiratory fitness (mL/kg/min)</td>
<td>37.1 ± 5.0</td>
<td>42.6 ± 6.8</td>
<td>&lt;.001</td>
<td>40.4 ± 6.2</td>
<td>51.5 ± 6.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Total PA (counts/min)</td>
<td>663 ± 194</td>
<td>801 ± 273</td>
<td>&lt;.001</td>
<td>484 ± 155</td>
<td>561 ± 204</td>
<td>.001</td>
</tr>
<tr>
<td>Moderate and high PA (min/d)</td>
<td>181 ± 53</td>
<td>217 ± 65</td>
<td>&lt;.001</td>
<td>68 ± 28</td>
<td>82 ± 39</td>
<td>.001</td>
</tr>
<tr>
<td>High PA (min/d)</td>
<td>24 ± 16</td>
<td>35 ± 23</td>
<td>&lt;.001</td>
<td>12 ± 11</td>
<td>16 ± 13</td>
<td>.002</td>
</tr>
<tr>
<td>Socioeconomic status (%)</td>
<td>.483</td>
<td></td>
<td></td>
<td>.504</td>
<td>.021</td>
<td>.030</td>
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<tr>
<td>SES I</td>
<td>18.9</td>
<td>13.5</td>
<td></td>
<td>22</td>
<td>23.6</td>
<td></td>
</tr>
<tr>
<td>SES II</td>
<td>52.3</td>
<td>56</td>
<td></td>
<td>35.6</td>
<td>41.5</td>
<td></td>
</tr>
<tr>
<td>SES III</td>
<td>28.8</td>
<td>30.5</td>
<td></td>
<td>42.4</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Parental smoking (%)</td>
<td>42.4</td>
<td>39.7</td>
<td>.712</td>
<td>40.6</td>
<td>31.7</td>
<td>.155</td>
</tr>
<tr>
<td>Pubertal maturation (%)</td>
<td></td>
<td></td>
<td></td>
<td>.805</td>
<td>.198</td>
<td></td>
</tr>
<tr>
<td>Tanner Stage I</td>
<td>56.8</td>
<td>99.3</td>
<td></td>
<td>0</td>
<td>0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Tanner Stage II</td>
<td>38.6</td>
<td>0.7</td>
<td></td>
<td>0</td>
<td>1.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Tanner Stage III</td>
<td>4.5</td>
<td>0</td>
<td></td>
<td>4.5</td>
<td>3.3</td>
<td>&lt;.001</td>
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<tr>
<td>Tanner Stage IV</td>
<td>0</td>
<td>0</td>
<td></td>
<td>40.4</td>
<td>17.1</td>
<td></td>
</tr>
<tr>
<td>Tanner Stage V</td>
<td>0</td>
<td>0</td>
<td></td>
<td>45.1</td>
<td>78.0</td>
<td></td>
</tr>
</tbody>
</table>

SES, Socioeconomic status.
*Cutoff points for children according to Cole et al.24
Table II. Standardized regression coefficients (β), confidence interval (95% CI) and standardized coefficient of determination (R²) examining the association of physical activity (PA) and cardiorespiratory fitness (CRF) with metabolic risk score after controlling for pubertal maturation, height, socioeconomic status, and parental smoking

<table>
<thead>
<tr>
<th>Model</th>
<th>R²</th>
<th>β</th>
<th>P</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-year-old girls (n = 132)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model A</td>
<td>0.200</td>
<td>-0.125</td>
<td>.129</td>
<td>-0.001-0.000</td>
</tr>
<tr>
<td>Model B</td>
<td>0.240</td>
<td>-0.076</td>
<td>.360</td>
<td>-0.001-0.000</td>
</tr>
<tr>
<td>CRF</td>
<td>-0.220</td>
<td>.011</td>
<td>-0.037-0.005</td>
<td></td>
</tr>
<tr>
<td>15-year-old girls (n = 133)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model A</td>
<td>0.13</td>
<td>-0.214</td>
<td>.018</td>
<td>-0.001-0.000</td>
</tr>
<tr>
<td>Model B</td>
<td>0.21</td>
<td>-0.133</td>
<td>.187</td>
<td>-0.001-0.000</td>
</tr>
<tr>
<td>CRF</td>
<td>-0.322</td>
<td>.001</td>
<td>-0.042-0.012</td>
<td></td>
</tr>
<tr>
<td>9-year-old boys (n = 141)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model A</td>
<td>0.010</td>
<td>-0.082</td>
<td>.354</td>
<td>0.000-0.000</td>
</tr>
<tr>
<td>Model B</td>
<td>0.080</td>
<td>-0.017</td>
<td>.850</td>
<td>0.000-0.000</td>
</tr>
<tr>
<td>CRF</td>
<td>-0.270</td>
<td>.002</td>
<td>-0.032-0.007</td>
<td></td>
</tr>
<tr>
<td>15-year-old boys (n = 123)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model A</td>
<td>0.08</td>
<td>-0.049</td>
<td>.592</td>
<td>-0.001-0.000</td>
</tr>
<tr>
<td>Model B</td>
<td>0.26</td>
<td>-0.011</td>
<td>.896</td>
<td>-0.001-0.000</td>
</tr>
<tr>
<td>CRF</td>
<td>-0.447</td>
<td>&lt;.001</td>
<td>-0.062-0.028</td>
<td></td>
</tr>
</tbody>
</table>

In Model A, total PA is shown as a predicting factor in the regression model. In Model B, CRF is additionally entered in the regression equation.

Independent t tests, with a confidence interval (CI) set at 95%, and for nominal and ordinal data χ² test with Φ and Cramer’s V with an exact test according to Monte Carlo with a CI set at 99% were used. General linear models with Bonferroni’s adjustments for multiple comparisons were used to examine the differences in the MRS among CRF quintiles.

Multiple linear regression analysis was used to determine the degree by which the variance in the MRS was explained by the single factors. Several models were tested for each sex and age group: Model A represents the effect of PA on the MRS after controlling for pubertal status, body height, socioeconomic status, and parental smoking. In Model B, CRF is introduced in the regression analysis, showing the effect of both PA and CRF on the MRS. The same models were tested with the non-obesity-MRS (Model A’ and B’), and an additional model (Model C’) to examine the effect of body fat on the non-obesity-MRS and its influence on the effect of PA and CRF on the non-obesity-MRS was also performed. All analysis was performed with the statistical software package SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA), and the level of significance was set at α = 0.05.

RESULTS

The descriptive characteristics of the study sample are shown in Table I. Total PA had a significant effect on the MRS in 15-year-old girls, after controlling for confounders (Table II, Model A). No significant effect of total PA on MRS was observed when both total PA and CRF were entered in the regression analysis (Model B). CRF had a significant effect on the MRS in both age and sex groups with and without the inclusion of total PA in the regression analysis. Total PA was significantly associated with CRF in 9-year-old boys and girls and in 15-year-old girls (all P ≤ .006). Moderate PA was significantly associated with CRF in girls of both age groups (all P ≤ .036), and borderline significant in 9-year-old boys (P = .06), and vigorous PA was significantly associated with CRF in both age and sex groups (all P ≤ .047).

When controlling for confounders and total PA, a significant difference (P < .05) was seen in MRS between the lowest and the highest CRF quintiles in all age and sex groups (Figure) that became borderline significant in 9-year-old girls (P = .054). Significant differences were also seen between the sexes in both age groups (all P < .05). No significant differences were observed in MRS between the lowest and the highest quintiles of total PA.

Total PA had a significant effect on the non-obesity-MRS in 15-year-old girls (Table III, Model A’). No significant effect of total PA on the non-obesity-MRS was observed when both total PA and CRF were entered in the regression analysis (Model B’). CRF had a significant effect on the non-obesity-MRS in 15 year old girls and boys (Table III). No significant effect of total PA and CRF on the non-obesity-MRS was observed in any age and sex group when total PA, CRF and body fat were entered together in
the regression analysis (Table III, Model C'). Body fat had an effect on the non-obesity-MRS in children and adolescents, except for 15-year-old girls (Table III). When examining the interaction term of CRF × body fat, a significant interaction in 15-year-old girls (P = .014) was observed. The introduction of the interaction term rendered body fat significant (P = .009) and CRF nonsignificant (P = .168) in the regression analysis.

The influence of PA intensity level on the respective models was tested by using different PA intensity levels (ie, moderate, vigorous, moderate-vigorous) instead of the total PA. Vigorous PA intensity was associated with non-obesity-MRS in 15-year-old subjects, but became insignificant when controlling for CRF. The results remained unaltered when using moderate PA or moderate-vigorous PA intensity level.

Both MRS and non-obesity-MRS regression models were further tested for modifications by possible confounders and composition criteria. Excluding height as a confounder did not alter the results. Using HDL-C as a single risk factor instead of the ratio of HDL-C/TC in the computation of MRS or MRS' did not alter the outcome. Adjusting for BMI instead of the sum of 5 skinfolds did not change the results except in 15-year-old boys where the effect of CRF on the non-obesity-MRS remained significant when BMI was introduced in the regression equation (β = −0.231, P = .021, 95% CI = −0.045 to −0.004). The results remained unaltered when including only the Z-score of diastolic, systolic or the mean arterial blood pressure as defined by Gauer.39

<table>
<thead>
<tr>
<th>Model</th>
<th>R²</th>
<th>β</th>
<th>P</th>
<th>95% CI</th>
<th>R²</th>
<th>β</th>
<th>P</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>9 year Girls (n=132)</td>
<td></td>
<td></td>
<td>15 year Girls (n=133)</td>
<td></td>
<td></td>
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<tr>
<td>PA</td>
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<td>.116</td>
<td>−0.001-0.000</td>
<td>0.08</td>
<td>−0.042</td>
<td>.649</td>
<td>−0.001-0.000</td>
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<tr>
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<td>−0.101</td>
<td>.230</td>
<td>−0.001-0.000</td>
<td>0.19</td>
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<td>.895</td>
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<td>−0.130</td>
<td>.146</td>
<td>−0.029-0.004</td>
<td>0.354</td>
<td>&lt;.001</td>
<td>.001-0.000</td>
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<tr>
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<tr>
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<td>.123</td>
<td>−0.001-0.000</td>
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<td>.909</td>
<td>−0.001-0.000</td>
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<tr>
<td>CRF</td>
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<td>.866</td>
<td>−0.017-0.019</td>
<td>0.091</td>
<td>.400</td>
<td>−0.032-0.013</td>
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<td>&lt;.001</td>
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<tr>
<td>Body fat</td>
<td>−0.346</td>
<td>.001</td>
<td>−0.005-0.018</td>
<td></td>
<td></td>
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</tbody>
</table>

In Model A', total PA is shown as a predicting factor in the regression model. In Model B', CRF is additionally entered in the regression equation. In Model C', body fat (expressed as sum of five skinfolds) is entered in the regression equation.

DISCUSSION

The results of this study suggest that total PA does not have an independent effect on metabolic risk factors in children and adolescents and that CRF is more strongly correlated to metabolic risk than total PA. Body fat seems to play a pivotal role in the association of CRF with metabolic profile. In this context total PA, as well as CRF, should be viewed as interactive partners.

The associations of CRF and total PA with the MRS were generally stronger in the 15-year-old than in the 9-year-old subjects, with 15-year-old girls having the strongest response. These variations could be explained by possible metabolic differences in the age and sex groups and the significant differences in the level of PA and CRF between the various groups.

It has been suggested that in adults the level of CRF may have an impact on the association between objectively measured PA and a computed metabolic syndrome score with stronger associations between PA and the metabolic syndrome score being seen in subjects with the lowest CRF.42 Similarly, we found that the strongest association between PA and metabolic risk was seen in the age and sex groups with the lowest level of PA overall, and the lowest level of CRF in their specific age group (ie, 15-year-old girls). Several studies on adolescents have shown that CRF is strongly correlated to body fat and that CRF has a stronger impact on features of the metabolic syndrome than PA.43,44
In a study with Danish children, a significant effect of both total PA and CRF on MRS in 9-year-old children was observed. However, the sex groups were not analyzed separately, so that possible differences in the response between boys and girls may not have surfaced. Since the study groups and the methods used were similar, further studies are needed to reach more conclusive statements on the independent effect of PA in children and adolescents.

Higher levels of CRF and PA are associated with a reduced incidence of metabolic-related diseases in adults. These findings seem to persist even after controlling for body composition. In children though, the results are less clear. A partial explanation for these differences could be the fact that children are generally more physically active and that changes in fitness levels due to regular PA would be more marked in the more sedentary adult population. These differences together with the physiological differences between children and adults might render body fat more predictive in its effect on metabolic risk factors than CRF in the pediatric population.

The association of vigorous PA with the MRS may indicate the beneficial effects of higher PA intensity levels in respect to metabolic risk. We have recently found that the intensity of PA, especially vigorous PA, but not total PA was negatively related to body fatness, whereas both amount and intensity of PA were positively associated with CRF in children aged 9 to 10 years.

It is known that aerobic exercise results in physiological adaptations of skeletal muscle cells in adults. Some of the physiological adaptations that might furnish protective mechanisms in relation to metabolic syndrome factors include an increase in capillary supply to skeletal muscles, increase in the activities of enzymes of the mitochondrial electron transport chain, and a concomitant increase in mitochondrial volume and density. Additionally, an increased substrate use with a decrease of carbohydrate oxidation, as well as heightened insulin sensitivity, may play a part in the protective adaptations triggered by PA on metabolic risk factors.

The observations of this study are limited by the cross-sectional design. Prospective studies are required to draw more robust conclusions on the determining effect of PA and CRF on metabolic risk. The study was strengthened by the use of objectively measured exposure variables. Even though the cross-sectional design limits a conclusive statement on the direction of causality, the pivotal role of body fat for the positive effects of CRF and PA on the metabolic risk are physiologically plausible and in agreement with the literature.

REFERENCES
Six-Minute Walk Test in Children and Adolescents
RALF GEIGER, MD, ALEXANDER STRASAK, MD, BENEDIKT TREML, MD, KLAUS GASSER, MD, AXEL KLEINSASSER, PHD, VICTORIA FISCHER, MD, HARALD GEIGER, MD, ALEXANDER LOECKINGER, PHD, AND JOERG I. STEIN, PHD

Objective To evaluate the 6-minute walking distance (6MWD) for healthy Caucasian children and adolescents of a population-based sample from the age of 3 to 18 years.

Study design Two hundred and eighty boys and 248 girls completed a modified test, using a measuring wheel as incentive device.

Results Median 6MWD increased from the age of 3 to 11 years in boys and girls alike and increased further with increasing age in boys (from 667.3 m to 727.6 m), whereas it essentially plateaued in girls (655.8 m to 660.9 m). After adjusting for age, height (P < .001 in boys and P < .001 in girls) remained independently correlated with the 6MWD. In the best fitting and most efficient linear and quadratic regression models, the variables age and height explained about 49% of the variability of the 6MWD in boys and 50% in girls.

Conclusion This modified 6-minute walk test (6MWT) proved to be safe, easy to perform, and highly acceptable to children. It provides a simple and inexpensive means to measure functional exercise capacity in children, even of young age, and might be of value when conducting comparable studies. (J Pediatr 2007;150:395-9)

Currently, the self-paced 6-minute walk test (6MWT) is classified to represent the most suitable method to assess the submaximal level of functional exercise capacity in adults.1 This test measures the distance that a patient or participant can quickly walk on a flat, hard surface in a period of 6 minutes. Recently, the American Thoracic Society (ATS) published guidelines for performing 6MWTs in adults in clinical settings.2 Despite its usefulness in adults the 6MWT is not widely used in the pediatric population, mainly because of lack of standardized protocols, established reference values, and reference equations in healthy children for the 6-minute walking distance (6MWD). Previous studies using the 6MWT in children were either limited to patients with a given disease3-7 or included only small numbers of participants.8 Lack of motivation and understanding for the need of a 6MWT may affect performance more in children than in adults. The motivational aspects during the 6MWT have not been assessed and no qualifying criteria for a 6MWT in children have been defined.

We attempted to overcome those shortcomings in children by a modified 6MWT, providing a measuring wheel that displays the instantaneous walking distance. The aim of this study was to establish reference values for the 6MWD in healthy children and adolescents performing the modified 6MWT. Further, we determined the correlates of the total 6MWD in a population sample of children and adolescents.

METHOD

Subjects of this study were children of Caucasian ethnicity. The study was approved by the local university ethics committee and the institutional boards of the participating local schools and kindergartens. Informed consent was obtained from parents and subjects. The measuring wheels were commercially available (Nedo GmbH + Co. KG, Dornstetten, Germany). The handling bar of the measuring wheel was customized to three interchangeable different lengths (240 mm, 370 mm, 560 mm) to fit optimally to the children's height. The shortest handlebar (for the smallest children) came with a two-hand grip to ensure stability of the wheel during the test for those who could not manage or felt unsecure to steer the wheel one-handed (Figure 1; available at www.jpeds.com).

From the Department of Pediatrics, Clinical Division of Cardiology, Pulmology, Allergology, and Cystic Fibrosis (R.G., K.G., V.F., J.I.S.), the Department of Medical Statistics, Informatics, Health Economics (A.S.), and the Clinical Division of Anesthesiology and Critical Care Medicine (B.T., A.K., A.L.), Innsbruck Medical University, Innsbruck, Austria; and the Department of Pediatrics, District Hospital, Dornbirn, Austria (H.G.). Supported in part by an unrestricted grant from Actelion Pharmaceuticals, Austria. Submitted for publication Jul 27, 2006; last revision received Oct 16, 2006; accepted Dec 22, 2006. Reprint requests: Dr Ralf Geiger, Clinical Division of Pediatric Cardiology, Innsbruck Medical University, Anichstrasse 35, 6020 Innsbruck/Austria. E-mail: ralf.geiger@i-med.ac.at.

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| 6MWD | 6-minute walking distance |
| 6MWT | Forced expiratory volume in 1 second |
| ATS  | PEF |
| FEV1  | Peak expiratory flow |
| PEF   | SEE |
| SEE   | Standard error of the estimate |
Each participant was required to choose a measuring wheel with the most appropriate length of the handlebar for convenient handling. Adequate size of the measuring wheel was assumed when the participant held the device tightly, arms slightly bent, the handlebar extending with minimal angulation from the forearm, when standing in upright position.

All children participated in the 6MWT voluntarily. The tests were conducted at school or kindergarten between 8:00 AM and 2:00 PM. Participants were told not to exercise vigorously 2 hours before the test. On the day of the test all children were given a general physical examination for their state of health. Children who were on medication, with chronic or acute disease, or with unsigned informed consent were excluded from testing and analysis. Before the start of the 6MWT the participants’ weight and height, wearing light clothes but without shoes were determined using an electronic scale and a wall assembled stadiometer. Leg length was determined by measuring the distance from the upper edge of the symphysis to the ground in strict upright position.

Blood pressure was measured sphygmomanometrically by using either the Digital Blood Pressure Monitor ESP2000 (Terumo Corporation, Tokyo, Japan) or the Colin Press-Mate (Colin Electronics Co., Ltd., Japan). Transcutaneous oxygen saturation was measured and heart rate was recorded before and immediately after the 6MWT by using a finger pulse oximeter (Nonin Flight Stat, Aeromedix, Jackson Hole, Wyo). Forced expiratory volume in 1 second (FEV1) of expiration and peak expiratory flow (PEF) were measured in standing position without noseclip using an electronic peak flow/FEV1 meter (PiKo-1, Puluminary Data Services, Inc., Louisville, Colo). Each participant performed three tests and the best of three satisfactory efforts was recorded. Values were compared with reference values matched for height and sex and expressed as percentage of predicted.9

For a subjective rating of intensity of perceived exertion by the child after exercising a visual analog scale was used. Immediately after the test children >5 years of age were asked to judge the degree of fatigue reached from the test by adjusting a scale bar on a open scale that corresponded to a 100-mm scale on the back. The bar positioned at the left end of the scale (0 mm) meant: “not exhausted/out of breath at all,” and the bar at the right end (100 mm) meant “very exhausted/out of breath.” The visual analog scale was not used in children 3 to 5 years of age because of the uncertainty of their cognitive abilities to rate perceived exertion.

The tests were performed on up to three separately located courses, depending on the facilities at the location, to avoid competition among the children. Two flagpoles were positioned on a straight course spaced at a distance of 20 m. Each child had a personal instructor during the test. Participants were instructed in a standardized way according to the recommendations of the ATS (with the exception of the role of the measuring wheel) as follows: “The object of this test is to walk as far as possible in 6 minutes, which means to score as many meters on the scale as possible. You will be walking back and forth around the poles. You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while resting, but resume walking as soon as you are able to. Are you ready to do that? Remember that the object is to score as many meters as possible in 6 minutes, but without jogging or running. Start now!” An exception was made in the children who were 3 to 4 years of age because in pilot tests we had noticed that many of those simply could not accomplish the task of strictly walking. Thus children of this particular age group were allowed to walk or run or jog as they liked to “score points.” We did not extend this concession to the entire study population for safety reasons.

After each minute, participants were told in even tones the following standardized phrases: “You are doing well. You have 5 minutes to go.” After the second minute: “Keep up the good work. You have 4 minutes to go.” After three minutes: “You are doing well. You are halfway done.” After four minutes: “Keep up the good work. You have only 2 minutes left.” After five minutes: “You are doing well. You have only 1 minute to go.” No other words of encouragement or body language were used to speed up the participant. The instructor did not walk with the participant but stood close to the course during the test period to keep control of the test person. At 6 minutes on the stopwatch the participant was told to stop. The distance on the scale was recorded, transcutaneous saturation as well as heart rate was measured by finger tip pulse oximetry and the degree of perceived exhaustion of the participant was acquired by visual analogue scale.

Continuous values normally distributed are presented as means ± SD. For skewed data medians with ranges are indicated. “Exact” 95% confidence intervals for the mean 6MWD according to sex and age group were calculated using the method of Clopper/Pearson.10 Bivariate and partial correlation analyses were performed to evaluate outcomes in the 6MWD in relation to demographic and physical characteristics by means of nonparametric Spearman correlation. R2 values for multiple linear and quadratic regression models were calculated according to forward and backward stepwise selection of possible independent predictors of the 6MWD simultaneously for boys and girls with the use of standard regression techniques. However, only the best and most efficient models are presented. Due to multiple testing Bonferroni correction was applied to reduce the risk of Type I error. Only two-sided P values equalling to .001 or less were considered to indicate statistical significance. All statistical analyses were performed using the Statistical Package for the Social Sciences version 12.0 (SPSS Inc. Chicago, Ill).

RESULTS

Of the 640 intended participants, 280 boys and 248 girls completed the test (82.5%). The response rate varied from 39% in the group 3 to 5 years of age to 93% in 6 to 8 years, 99% in 9 to 11 years, 94% in 12 to 15 years, and 83% in ≥16 years of age. On the day of the test, three children had to be excluded after the physical examination because of acute
Six-Minute Walk Test in Children and Adolescents

In the group of the youngest children, where only 39% eventually completed the test, most of them were either sick or not sent to kindergarten by their parents on the day of the test, explaining the second lowest response rate of 83% in this age group.

As this test was voluntary, obese children might thus have refused to participate. Because of this potential bias this study group might not represent a “true” population sample. We also have incomplete data on those children who did not show up on the day of the test although informed consent was given. In the group of the youngest children, where only 39% eventually completed the test, most of them were either sick or not sent to kindergarten by their parents on the day of the test for fear of contagion because of an endemic acute infection. In the group of the oldest children, candidates had changed their mind and refused to cooperate on the day of the test. The group of the oldest children, candidates had changed their mind and refused to cooperate on the day of the test. The group of the oldest children, candidates had changed their mind and refused to cooperate on the day of the test.

DISCUSSION

Weight correlates to the 6MWD in adults but not in the children investigated. This might relate to the fact that the percentage of overweight children, according to age and sex-based BMI in our study sample was considerably lower (3.6%-5%) than the expected prevalence of 7% to 12%.11-13 As this test was voluntary, obese children might thus have refused to participate. Because of this potential bias this study group might not represent a “true” population sample. We also have incomplete data on those children who did not show up on the day of the test although informed consent was given. In the group of the youngest children, where only 39% eventually completed the test, most of them were either sick or not sent to kindergarten by their parents on the day of the test for fear of contagion because of an endemic acute infection. In the group of the oldest children, candidates had changed their mind and refused to cooperate on the day of the test, explaining the second lowest response rate of 83% in this particular age group.

During a self-paced test, motivation of the individual child is of paramount importance. By implementing the measuring wheel we sought to give “a task” to the children that should serve as an intrinsic motivational factor, which could not be influenced by the instructor. The study was not designed to measure the validity of this method. As expected, the measuring wheel proved very useful for focusing the concentration of the children on the test. The group of youngest children posed some methodical challenges. When allowed to run, most of the 3- to 4-year-old children switch between jogging and walking frequently during the 6 minutes. Within the test's concept of minimal external interference, these data are likely to reflect their capabilities more accu-
rately. In addition, running in the very young children did not seem to affect 6MWD to a great extent, given the lowest values of mean 6MWD in the youngest age group. As a self-paced test the 6MWT with measuring wheel reflects submaximal functional capacity of healthy children, which might represent more closely the patterns of their daily activities.3

It is most valuable for those who are moderately or severely impaired and whom a full cardiopulmonary exercise test would put at risk or would not be applicable. In those sick children where the 6MWT almost represents a maximal exercise test, it could help to grade their level of impairment. Repeated testing could aid to assess disease progression, the effects of medical intervention, or the need for additional or altered treatment. To allow for future direct comparisons among studies and to reduce the sources of variability caused by the test procedure itself, we used a standardized protocol that strictly followed the guidelines for the 6MWT in a clinical setting, prepared by the ATS, in all but one aspect.

We found it particularly difficult to select a “straight, enclosed, longer than 30 m corridor with a plane hard surface that is seldom travelled” (as stated in the guidelines) in any of the buildings where the tests were performed. For practical reasons, therefore, we chose 20 m as the distance between the two poles. The theoretical drawback of reducing the 6MWD by requiring probands to take more time to reverse directions more often becomes irrelevant when using a measuring wheel because the distance of the turns is also recorded by the meter. Moreover, a recent multicenter study found no significant effect of the length of straight courses ranging from 50 to 164 ft.2 Particularly in the very young children, the 20-m course seems to be advantageous as it allows the children to focus on the task more closely, and not “get lost” on the course.

We are not aware of any published reference values of the 6MWD in healthy children and adolescents with which we could compare our data. Thus, we cannot state whether the modified 6MWT would result in longer or shorter 6MWDs compared with those of a “normal 6MWT.” 6MWDs were in the range of those from healthy adults and adolescents1,2,8 and were positively correlated with age in children, which is opposite to that in adults, where 6MWD diminishes with growing age.14 By implementing age as a quadratic term, our regression models explain up to 50% of the variability of the 6MWD in healthy volunteers (R² values). Half of the variability of the 6MWD in children relates to other than anthropometric factors, such as physical fitness, coordination, and motor skills of the participants. Moreover, in children of all age groups we could observe some participants who were highly motivated and others in whom the degree of motivation could not easily be judged from their attitude toward the test. The results of our tests apply to the children’s first 6-minute walk. A “learning curve” might have to be anticipated, especially in very young children, when performing subsequent tests. We did not intend to evaluate the magnitude of this assumed learning effect, but it is known from the literature in adults that it would range from 0 to 17% of the 6MWD.2,15 Excellent

<table>
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<tr>
<th>Sex</th>
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<th>Median (range)</th>
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<th>95% CI</th>
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<td>3 to 5 y</td>
<td>22</td>
<td>544.3 (318.0-680.6)</td>
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<td>6 to 8 y</td>
<td>66</td>
<td>584.0 (455.0-692.0)</td>
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<td>9 to 11 y</td>
<td>57</td>
<td>667.3 (540.2-828.0)</td>
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<td>672.8 ± 61.6</td>
<td>656.5-689.2</td>
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<td>12 to 15 y</td>
<td>80</td>
<td>701.1 (276.1-861.0)</td>
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<td>727.6 (569.0-865.3)</td>
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<td>62</td>
<td>655.8 (548.0-818.0)</td>
<td>572.0-760.5</td>
<td>661.9 ± 56.7</td>
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<td>12 to 15 y</td>
<td>71</td>
<td>657.6 (485.5-785.0)</td>
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<td>663.0 ± 50.8</td>
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<td>16 y or older</td>
<td>44</td>
<td>660.9 (557.0-774.3)</td>
<td>571.2-756.2</td>
<td>664.3 ± 49.5</td>
<td>649.3-679.3</td>
</tr>
</tbody>
</table>

Figure 2. Mean ± SD 6-minute walk distance in healthy children and adolescents.

Table III. Six-minute walk distances in meters according to sex and age category in healthy children and adolescents
test-retest reliability of the 6MWT was recently reported in adolescents with a bias (mean difference between the two paired means) of 15 m (intraclass coefficient 0.94) at two separately held tests within 3 weeks. It seems reasonable to dismiss the possibility of a practice or training effect when recruiting cross-sectional data, but it might be of relevance when analysing subsequent interventional or treatment effects on 6MWD.

REFERENCES

AGENESIS OF THE CORPUS CALLOSUM: REPORT OF EIGHT CASES IN INFANCY
Koch FP, Doyle PJ. J Pediatr 1957;50:345-51

Today it is not uncommon for a pediatrician to encounter at least one child with hypoplasia or agenesis of the corpus callosum found by using magnetic resonance imaging (MRI). However, 50 years ago, Koch and Doyle surveyed 8 infants with agenesis of the corpus callosum, diagnosed by means of autopsy or pneumoencephalography, and added to the only 100 cases that had been described since the first was described by Reil in 1812. Koch and Doyle “hoped that pediatricians may become alerted to the possibility of diagnosing agenesis of the corpus callosum” when faced with a child with macrocephaly, developmental delays, cerebral palsy, or other neurologic problems. Thanks to MRI, their wish has come true, but do we know much beyond their accurate report of the development and function of the corpus callosum?

As the “information superhighway” between the hemispheres, the corpus callosum is formed between the ninth and 20th weeks of gestation. An abnormality in this intrahemispheric connection is rarely found in isolation of other symptoms or signs and may provide a clue to the etiology of a child’s underlying diagnosis. Agenesis of the corpus callosum can be associated with conditions as diverse as Dandy-Walker malformation, Chiari malformation, trisomies 13 and 18, holoprosencephaly, X-linked hydrocephalus, Aicardi syndrome, non-ketotic hyperglycemia, methystalmonic acidemia, mitochondrial defects, and fetal alcohol syndrome. We know, as Koch and Doyle did, that there are some children with agenesis or hypoplasia who are clinically unaffected or whose agenesis or hypoplasia are detectable only with rigorous neuropsychological testing.

As prenatal ultrasound scanning and MRI and other not-yet-invented imaging technologies become standard for obstetrical and neurological care, the spectrum of cerebral anomalies and variants found will doubtlessly increase. Among these findings, agenesis or hypoplasia of the corpus callosum will become more of a finding than a diagnosis, and we should heed Koch and Doyle’s advice. Like a simian crease or multiple café-au-lait spots, the pediatrician should use agenesis or hypoplasia as just one finding on the diagnostic journey for which he or she first obtained the MRI. Consideration of an ophthalmology evaluation is likely wise, and thought should be given to the possibility of an underlying genetic or metabolic disorder. In rare cases, the finding may just be incidental.

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10.1016/j.jpeds.2006.10.015
Figure 1. Measuring wheel with interchangeable size-adjusted handling bars.
### Table II. Six-minute walk test in healthy children and adolescents, pre and post measures

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age category</th>
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<th>Pre 6MWT</th>
<th>Post 6MWT</th>
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<td></td>
<td>tcSAT*</td>
<td>HR†</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3 to 5 y</td>
<td>22</td>
<td>98 (97, 100)</td>
<td>96.6 ± 10.8</td>
<td>146.9 ± 20.6</td>
</tr>
<tr>
<td></td>
<td>6 to 8 y</td>
<td>66</td>
<td>98 (96, 100)</td>
<td>88.1 ± 12.7</td>
<td>133.0 ± 19.1</td>
</tr>
<tr>
<td></td>
<td>9 to 11 y</td>
<td>57</td>
<td>98 (96, 99)</td>
<td>86.8 ± 15.9</td>
<td>135.5 ± 18.9</td>
</tr>
<tr>
<td></td>
<td>12 to 15 y</td>
<td>80</td>
<td>98 (97, 99)</td>
<td>85.3 ± 14.7</td>
<td>138.9 ± 21.5</td>
</tr>
<tr>
<td></td>
<td>16 y or older</td>
<td>55</td>
<td>98 (96, 100)</td>
<td>76.6 ± 15.8</td>
<td>130.4 ± 29.4</td>
</tr>
<tr>
<td>Female</td>
<td>3 to 5 y</td>
<td>25</td>
<td>99 (97, 100)</td>
<td>97.1 ± 11.0</td>
<td>150.0 ± 24.9</td>
</tr>
<tr>
<td></td>
<td>6 to 8 y</td>
<td>46</td>
<td>98 (96, 100)</td>
<td>96.0 ± 14.1</td>
<td>146.1 ± 16.6</td>
</tr>
<tr>
<td></td>
<td>9 to 11 y</td>
<td>62</td>
<td>99 (97, 100)</td>
<td>84.6 ± 12.3</td>
<td>144.1 ± 20.8</td>
</tr>
<tr>
<td></td>
<td>12 to 15 y</td>
<td>71</td>
<td>99 (98, 100)</td>
<td>88.0 ± 15.0</td>
<td>149.4 ± 20.2</td>
</tr>
<tr>
<td></td>
<td>16 y or older</td>
<td>44</td>
<td>98 (96, 100)</td>
<td>89.0 ± 19.6</td>
<td>141.9 ± 24.3</td>
</tr>
</tbody>
</table>

6MWT, 6-minute walk test; HR, heart rate (beats/minute); tcSAT, transcutaneous oxygen saturation (%); VAS, visual analog scale (mm).

*Values presented as medians (95% reference range).
†Values presented as mean ± SD.
‡Not measured.

### Table IV. Six-minute walk distance correlates in healthy children and adolescents, corrected for age*

<table>
<thead>
<tr>
<th>Height</th>
<th>Weight</th>
<th>BMI</th>
<th>tcSAT*</th>
<th>SBP</th>
<th>DBP</th>
<th>HR</th>
<th>PEF</th>
<th>FEV1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male δ</td>
<td>0.20</td>
<td>−0.09</td>
<td>−0.15</td>
<td>0.02</td>
<td>−0.04</td>
<td>−0.09</td>
<td>0.11</td>
<td>0.08</td>
</tr>
<tr>
<td>P value (two-tailed)</td>
<td>.001</td>
<td>.17</td>
<td>.02</td>
<td>.81</td>
<td>.53</td>
<td>.14</td>
<td>.08</td>
<td>.23</td>
</tr>
<tr>
<td>Female δ</td>
<td>0.25</td>
<td>0.00</td>
<td>−0.07</td>
<td>0.06</td>
<td>0.04</td>
<td>−0.02</td>
<td>−0.01</td>
<td>0.21</td>
</tr>
<tr>
<td>P value (two-tailed)</td>
<td>&lt;.001</td>
<td>.96</td>
<td>.28</td>
<td>.40</td>
<td>.56</td>
<td>.81</td>
<td>.16</td>
<td>.02</td>
</tr>
</tbody>
</table>

Due to multiple comparisons the level of significance is set at $P < .001$.

BMI, body mass index; DBP, diastolic blood pressure (pre-test); FEV1, forced expiratory volume in 1 second; HR, heart rate (pre-test); PEF, peak expiratory flow; SBP, systolic blood pressure (pre-test); tcSAT, transcutaneous oxygen saturation (pre-test).

*Non-parametric Spearman correlation (male n = 280, female n = 248).
Impaired Endothelial Function in Healthy African-American Adolescents Compared with Caucasians

MARY M. DUCK, BS, AND ROBERT P. HOFFMAN, MD

Objective  To determine whether African-American adolescents have endothelial dysfunction compared with Caucasians and whether differences are a result of differences in insulin sensitivity calculated from total glucose (SI) or secretion.

Study design  Thirty-three Caucasian (13.6 ± 2.6 years of age; body mass index [BMI] 21.6 ± 4.4 kg/m² mean ± SD) and 25 African-American (13.3 ± 2.9 years of age; BMI 24.0 ± 4.4 kg/m²) adolescents were studied. Forearm blood flow (FBF: plethysmography) was measured before and after 5 minutes of arterial occlusion. SI and acute insulin response to glucose (AIRG) were measured using intravenous glucose tolerance tests and minimal modeling.

Results  Baseline FBF did not differ between races. Postocclusion FBF was lower in African-Americans (17.2 ± 1.2 vs 22.6 ± 1.2 mL/dL/minute, P = .006). AIRG was higher in African-Americans (6050 ± 940 vs 2410 ± 30 U minute/mL, P = .001). Pubertal stage had no effect. SI did not differ by race or pubertal stage. In African-Americans, percent fall in FVR following arterial occlusion correlated (r = 0.67, P = .001) with log AIRG. No relationships were found between percent fall in FVR and SI in either race.

Conclusion  African-American adolescents have decreased endothelial function. This may be a result of increased insulin secretion. Endothelial dysfunction in African-American adolescents may predispose to cardiovascular and type II diabetes. (J Pediatr 2007;150:400-6)

African-American adults have increased morbidity and mortality from most of the diseases associated with the metabolic syndrome, including myocardial infarction at younger ages,1-4 stroke,5-6 hypertension,3 and in both adults and adolescents type II diabetes.7,8 Multiple differences have been demonstrated between African-Americans and Caucasians in the underlying pathophysiologic features of the metabolic syndrome. African-American adults have impaired endothelial function compared with Caucasian adults; specifically, endothelin 1, a potent vasoconstrictor, is increased9 and flow mediated brachial artery vasodilation10 is diminished. Hinderliter et al11,12 found higher minimum forearm vascular resistance (FVR) following vascular occlusion in African-American young adults compared with similar-aged Caucasian subjects. Endothelial dysfunction is an early predictor for both cardiovascular disease and type II diabetes.13 African-American adults have increased carotid artery intima- medial thickness.14 Adolescent African-Americans with a family history of essential hypertension have increased basal and stress-stimulated endothelin 1 levels compared with similarly selected, Caucasian adolescents.15

Mechanistically, there is a close link between endothelial function and insulin sensitivity in both normotensive and hypertensive subjects.16,17 This is important because African-American adolescents are more frequently insulin resistant compared with Caucasians.18,19 We therefore hypothesized that: (1) because insulin sensitivity is decreased in African-American adolescents endothelial function would also be lower in African-American adolescents; (2) because insulin sensitivity is lower in pubertal than in prepubertal and postpubertal subjects20 endothelial function would also be lower in pubertal subjects than in prepubertal or postpubertal subjects; and (3) endothelial function would be positively associated with muscle insulin sensitivity.

AIRG  Acute insulin response to glucose
ANOVA  Analysis of variance
BMI  Body mass index
FBF  Forearm blood flow
FVR  Forearm vascular resistance
HRR  Hepatic insulin resistance
LDL  Low-density lipoprotein
SI  Insulin sensitivity calculated from total glucose
SI*  Insulin sensitivity calculated from labeled glucose

From the University of Cincinnati College of Medicine (M.M.D.), and the Division of Pediatric Endocrinology, the Department of Pediatrics (R.P.H.), and the Clinical Research Center of the Ohio State University College of Medicine and Public Health and the Children’s Research Institute (R.P.H.), Columbus, Ohio.

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METHODS

Subjects
Healthy, adolescent volunteers between 8 and 18 years of age were recruited for participation. They were taking no medications and were free from chronic and acute disease at the time of study. Pregnancy tests were done on all females and all were negative. Subjects were recruited into one of the following groups: prepubertal (Tanner stage 1 breast or genital development depending on sex; African-American, n = 3; Caucasian, n = 11), pubertal (Tanner stage 2, 3, or 4 breast or genital development; African-American, n = 11; Caucasian, n = 11) or postpubertal (Tanner stage 5 breast or genital development; African-American, n = 11; Caucasian, n = 11). Tanner staging was done by the principal investigator (RPH), an experienced pediatric endocrinologist. The study was approved by the Ohio State University Office of Responsible Research. Informed consent was obtained from a parent or legal guardian and assent was obtained from each subject.

Protocol
Subjects were admitted to the Clinical Research Center of the Ohio State University at 8 AM. They were instructed not to eat or drink anything except water after 10 PM the evening before. Height and weight were measured and endothelial function was tested, as described below. Endothelial function of the resistance vessels was assessed by measuring forearm blood flow (FBF) before and after upper arm arterial occlusion.21-24 The stable isotope labeled frequently sampled intravenous glucose tolerance test and minimal modeling were used to assess insulin secretion and secretion.25

ENDOTHELIAL FUNCTION. FBF was measured in the dominant arm using venous-occlusion plethysmography with an indium-in-silastic strain gauge and an Hokanson EC6 plethysmograph (DE Hokanson Inc., Bellevue, Wash.). Sphygomonometric cuffs were placed on the arm at the wrist and on the upper arm. During measurement the wrist cuff was inflated to 200 mmHg to occlude flow to the hand, which is primarily skin blood flow, and the upper arm cuff was inflated to 40 mmHg for 10 out of every 15 seconds to occlude venous return. Data were recorded using PowerLab and Chart 4.0 (AD Instruments, Grand Junction, Colo) on a Power Mac G4 (Apple, Cupertino, Calif). FBF is expressed as milliliters of blood per deciliter of arm tissue per minute. Arterial blood pressure was continuously monitored by arterial tonometry in the nondominant arm (Model 7000, Colin Medical Instruments, San Antonio, Tex). FVR was calculated by dividing mean arterial pressure by FBF. For each subject, 2 minutes of FBF were recorded and then the upper arm cuff was inflated to 200 mmHg pressure for 5 minutes to occlude flow to the arm. It was then released, and FBF was again measured over the next minute. All studies were scored twice by a single observer (RPH) on separate occasions, and the mean value was used for analysis. Mean intra-observer coefficient of variation for FBF before upper arm occlusion was 5.1%, and it was 7.4% after upper arm occlusion. To confirm differences found between races in FBF postvascular occlusion, we also assessed the percent change in FVR following upper occlusion.

INTRAVENOUS GLUCOSE TOLERANCE TEST. After completion of the test of endothelial function, intravenous catheters were placed in each arm for the frequently sampled intravenous glucose tolerance test and to obtain the fasting blood sample for measurement of the fasting lipid profile. A bolus of 25% dextrose in water with approximately 13% [6-6]D2 glucose was given. The total glucose dose was 250 mg/kg. Three-milliliter blood samples were taken at −10, 0, 2, 4, 6, 8, 12, 14, 16, 19, 22, 27, 32, 42, 52, 62, 72, 82, 92, 102, 122, 142, 162, and 182 minutes relative to a glucose bolus for measurement of plasma glucose, insulin, and [6,6]D2 glucose concentrations.

Total body insulin sensitivity calculated from total glucose (S_T) and peripheral insulin sensitivity calculated from labeled glucose (S_L) were calculated using the one compartment minimal model for total and labeled glucose concentrations, respectively, using the program Minmod (Minmod, Inc., Los Angeles, Calif).25,26 The program was also used to calculate the acute insulin response to glucose (AIRG) over the first 19 minutes of the test. Hepatic glucose production was calculated through the use of Steele’s equations over the last hour of the study when the system had returned to a quasi-steady-state. To adjust for differing insulin levels between subjects during the time period when hepatic glucose production was determined, hepatic insulin resistance (HIR) was calculated by multiplying hepatic glucose production by the mean insulin level over the same hour because higher insulin levels should suppress hepatic glucose production.25,26

ASSAYS
Plasma glucose and insulin concentrations were measured in the CORE lab of the Clinical Research Center of the Ohio State University, and mole percent excess of [6,6]D2 glucose was measured by Metabolic Solutions (Cambridge, Mass). Plasma lipids were measured in the Clinical Laboratory of the Ohio State University Hospital.

Statistical Analysis
Analysis of variance (ANOVA) and ANOVA of repeated measures were used to determine differences in FBF and FVR responses to across-race and pubertal status. Two-tailed, planned contrasts were used for individual group comparisons. Pearson’s correlation coefficients and multiple linear regression were used to assess the relationships between reactive hyperemia and insulin sensitivity, secretion, BMI, and lipids. Systat 10 (Systat Software Inc., Point Richmond, Calif) was be used to perform all statistical analysis. Data are expressed as mean ± SE. Differences were considered significant at P < .05, and tendencies are mentioned as P < .1.
RESULTS

Subject Characteristics

BMI significantly differed by pubertal group (Table I; \( P < .001 \)) but did not differ between the races. Systolic blood pressure tended to be higher in African-American than Caucasian subjects (\( P = .056 \)), although no differences were seen for diastolic pressure. Neither systolic nor diastolic pressure varied by pubertal group. For plasma lipid levels no differences were seen between races or pubertal groups except for a trend toward higher low-density lipoprotein (LDL) levels in African-Americans (\( P = .071 \)). The race by group interaction approached significance (\( P = .064 \)) indicating the effect of pubertal stage on LDL cholesterol may vary between the races. Specifically, LDL cholesterol was increased in prepubertal African-American subjects. Fasting glucose and insulin levels did not differ between races or pubertal stages.

Endothelial Function and Insulin Sensitivity Differences

Baseline FBF was not different between the races. However, postocclusion FBF was significantly lower in African-Americans (Figure 1; \( P = .006 \)). The FBF increase following upper arm occlusion in Caucasian adolescents was greater than in African-American adolescents (time × race interaction, \( P = .004 \)). The time by pubertal stage effect (\( P = .10 \)) and time by pubertal stage by race effects (\( P = .28 \)) were
Impaired Endothelial Function in Healthy African-American Adolescents Compared with Caucasians

not significant, indicating that pubertal stage did not alter postocclusive FBF or the effect of race.

The greater postocclusion vasodilation in Caucasian adolescents was confirmed by the significant differences in percent change in FVR between the races (Table II; *P* = .018). The pubertal stage effect and race by pubertal stage interaction were not significant. When the subjects were analyzed by pubertal stage, there was a trend toward significant racial difference in the postpubertal subjects (*P* = .057).

AIRG was significantly greater (*P* = .001) and *S*ₚ was significantly lower (*P* = .023) in African-Americans than in Caucasians. There were no differences in *S*₁ or HIR between the races. When the subjects were compared within each pubertal stage group, the difference in AIRG was significant only in the postpubertal subjects (*P* = .001), although the differences in the prepubertal (*P* = .064) and pubertal (*P* = .051) groups approached significance. For *S*¹*, the difference between African-Americans and Caucasians was significant only in the prepubertal group (*P* = .004). No pubertal stage effects were seen.

Because of the small number of African-American prepubertal subjects and because these subjects had an increased BMI, separate analysis was done on just the pubertal and postpubertal subjects. Baseline FBF did not differ by race pubertal stage. Postocclusion FBF was lower in African-Americans (*P* = .033) and the time by race interaction for FBF was again significant (*P* = .014), indicating the increase in FBF was lower in African-Americans. This was confirmed by assessing the postocclusion percent fall in FBF (*P* = .041). Interestingly, with prepubertal subjects excluded, the postocclusion FBF was significantly lower in postpubertal than pubertal subjects (*P* = .009) and the time by puberty interaction was also significant (*P* = .023). The pubertal differences in percent fall FBF did not quite reach statistical significance (*P* = .084). Results from the intravenous glucose tolerance tests again revealed only a significant racial difference in AIRG.

Relationship of Endothelial Function to Insulin Secretion, Sensitivity, and Lipids

In the group as a whole no correlations were present between percent change in FVR and any of the plasma lipid measurement, *S*ᵢ, *S*ᵢ*, or HIR. There was a correlation between percent change in FVR and log AIRG (*r* = 0.41, *P* = .004). The percent change in FVR also correlated with BMI (*r* = 0.31, *P* = .016), and log plasma triglyceride levels (*r* = 0.49, *P* = .016), and log AIRG (*r* = 0.67, *P* = .001). No relationships were found between percent change in FVR and fasting glucose, fasting insulin, *S*ᵢ, *S*ᵢ*, or HIR in either race.

Multiple linear regression with percent change in FVR as the dependent variable and BMI, log plasma triglycerides, and log AIRG as independent variables was also performed in each race separately. In African-Americans the relationships of percent change in FVR to BMI and triglycerides were no

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**Table II. Endothelial function and stable labeled frequently sampled intravenous glucose tolerance test results in African-American and Caucasian adolescents**

<table>
<thead>
<tr>
<th></th>
<th>Prepubertal</th>
<th>Pubertal</th>
<th>Postpubertal</th>
<th>Race effect</th>
<th>Puberty effect</th>
<th>Interaction effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in FVR (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>-74.1 ± 3.1 (n = 3)</td>
<td>-79.5 ± 1.9 (n = 11)</td>
<td>-74.2 ± 2.3 (n = 11)</td>
<td>.018</td>
<td>.17</td>
<td>.76</td>
</tr>
<tr>
<td>Caucasian</td>
<td>-80.2 ± 2.7 (n = 11)</td>
<td>-82.4 ± 2.2 (n = 11)</td>
<td>-80.0 ± 1.6 (n = 11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIRG (pmol min/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>5880 ± 610</td>
<td>4240 ± 770</td>
<td>8350 ± 1900*</td>
<td>.001</td>
<td>.36</td>
<td>.60</td>
</tr>
<tr>
<td>Caucasian</td>
<td>2080 ± 770</td>
<td>2472 ± 550</td>
<td>2380 ± 430</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S</em>₁ (L/pmol min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>0.608 ± 0.213</td>
<td>1.49 ± 0.85</td>
<td>0.902 ± 0.258</td>
<td>.32</td>
<td>.60</td>
<td>.28</td>
</tr>
<tr>
<td>Caucasian</td>
<td>3.60 ± 1.00</td>
<td>1.91 ± 0.98</td>
<td>1.22 ± 0.38</td>
<td>.023</td>
<td>.19</td>
<td>.027</td>
</tr>
<tr>
<td><em>S</em>¹* (L/mU min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>0.330 ± 0.37*</td>
<td>1.06 ± 0.54</td>
<td>0.847 ± 0.192</td>
<td>.023</td>
<td>.19</td>
<td>.027</td>
</tr>
<tr>
<td>Caucasian</td>
<td>1.57 ± 0.60</td>
<td>1.01 ± 0.24</td>
<td>0.903 ± 0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIR (mmol mol/kg min L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>0.637 ± 0.19</td>
<td>0.282 ± 0.043</td>
<td>0.266 ± 0.80</td>
<td>.58</td>
<td>.47</td>
<td>.081</td>
</tr>
<tr>
<td>Caucasian</td>
<td>0.244 ± 0.124</td>
<td>0.026 ± 0.047</td>
<td>0.583 ± 280</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SE.

*P < .05 vs Caucasians in same pubertal group.
longer significant. The relationship to AIRG remained significant ($P = .012$). This relationship retained significance if BMI or triglycerides were included, independently. No relationships were found in Caucasians.

**DISCUSSION**

These results demonstrate that impaired endothelial function is present in African-American adolescents. Specifically, we found diminished maximal postocclusion FBF compared with pubertal stage–matched Caucasian subjects, which was confirmed by the greater fall in FVR in Caucasians. The percent change in FVR in the Caucasian adolescents in our study was nearly identical to that in nondiabetic adolescents found by Newkumett et al.$^{28}$ We believe that this impairment in endothelial function may predispose African-American adolescents to future cardiovascular complications of the metabolic syndrome as adults.

In adults, there is an interrelationship between endothelial function and insulin sensitivity.$^{16,17}$ Insulin-induced vasodilation$^{29-31}$ is mediated by endothelial nitric oxide release.$^{32}$ Diminished endothelial function, thus, decreases insulin-induced vasodilation and glucose delivery to and usage by muscle tissue. We, however, did not find the hypothesized relationship between percent change in FVR and insulin sensitivity.
sensitivity. These results confirm the findings of Singhal et al, using more rigorous measures of insulin sensitivity than that derived from fasting glucose and insulin levels using the homeostatic method in their study.

We did find a significant relationship between percent change in FVR and AIRG in the all subjects and in the African-Americans alone. The positive correlations indicate increased insulin secretion is associated with decreased endothelial function because percent change in FVR is negative. In the group as whole the racial difference in percent change in FVR was eliminated by controlling for increased insulin secretion. Interestingly however, this effect was primarily present only in the African-Americans, indicating hyperinsulinism may have differing effects in the two races or there may be a threshold effect with manifestation in the African-American adolescents alone as a result of their higher insulin secretion. Because insulin itself causes endothelial-induced vasodilation, persistent exposure to higher insulin levels may exhaust nitric oxide availability. On the other hand, it is also possible that the reverse is true and that endothelial dysfunction is the cause of the increased insulin secretion in African-American adolescents. Diminished insulin-induced endothelial vasodilation could lead to decreased glucose delivery and insulin resistance with compensatory increases in insulin secretion, as described above. This is less likely because insulin sensitivity did not differ between the races in this study.

We also found positive relationships between percent change in FVR and BMI and triglyceride levels in African-American adolescents compared with whites. Circulation 2000;101:1109-14.

Evidence of insulin resistance in African-American adolescents includes higher insulin to glucose ratios following oral glucose compared to Caucasians in all Tanner stages and in both sexes and increased glucose requirements during insulin clamp in African-American subjects. American adolescents. Diminished insulin-induced endothelial vasodilation may have differing effects in the two races or there may be a threshold effect with manifestation in the African-American adolescents alone as a result of their higher insulin secretion. Because insulin itself causes endothelial-induced vasodilation, persistent exposure to higher insulin levels may exhaust nitric oxide availability. On the other hand, it is also possible that the reverse is true and that endothelial dysfunction is the cause of the increased insulin secretion in African-American adolescents. Diminished insulin-induced endothelial vasodilation could lead to decreased glucose delivery and insulin resistance with compensatory increases in insulin secretion, as described above. This is less likely because insulin sensitivity did not differ between the races in this study.

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Evidence of insulin resistance in African-American adolescents includes higher insulin to glucose ratios following oral glucose compared to Caucasians in all Tanner stages and in both sexes and increased glucose requirements during insulin clamp in African-American pubertal and prepubertal children. The differences are not explained by differences in body composition, although obesity in African-American children worsens insulin resistance more than in Caucasians. African-American children compensate for the decreased insulin sensitivity by both increasing insulin secretion and decreasing insulin clearance.

In contrast to these studies that suggest that the increased insulin secretion in African-American adolescents is because of insulin resistance, Preeyasombat et al suggested that increased insulin secretion may be the primary defect and insulin resistance secondary, at least in obese African-American adolescents compared with obese Caucasian adolescents. This was based on findings from oral glucose tolerance tests that demonstrated that insulin sensitivity was not different between the two groups when corrected for insulin secretion, but the latter was increased in African-Americans. Our findings support this hypothesis. We found markedly increased AIRG in all pubertal stages but a decrease in S1 only in prepubertal African-American adolescents.

The study has two significant limitations. The first is the inclusion of subjects of all weights. Because of this we cannot categorically say that our results would be present in just lean adolescents. However, when BMI was included as a covariate, the race effect remained significant. Second, we did not study subjects by sex. Differences in endothelial function and insulin sensitivity have been reported between males and females. Each of our groups contained nearly equal numbers of boys and girls. Including sex in the ANOVA, however, did not eliminate the racial difference (data not shown).

Lastly, it would be useful to confirm our findings with other methods of measuring endothelial function, specifically, flow-mediated vasodilation of the brachial artery as measured by ultrasonography. The two techniques measure endothelial function in different types of blood vessels. The technique we used examines resistance vessels, as opposed to conduit vessels. We believed this to be more clinically significant.

In conclusion, African-American adolescents have impaired endothelial function of the resistance vessels. Additional study will be needed to determine whether this dysfunction predisposes to future cardiovascular disease and type II diabetes in African-American adults. The impairment may be because of increased insulin secretion.

The authors would like to thank the nurses of the Clinical Research Center for their help with the glucose tolerance tests.

REFERENCES


Cardiac Manifestations in Oxidative Phosphorylation Disorders of Childhood

J. Yapito-Lee, MD, R. Weintraub, MBBS, K. Jansen, BSc(Math), C. W. Chow, MBBS, D. R. Thorburn, PhD, and A. Boneh, MD, PhD

Objective To determine the frequency, type, and severity of cardiac involvement in pediatric patients with oxidative phosphorylation (OXPHOS) disorders.

Study design Retrospective review of clinical and laboratory records of all patients with definitive OXPHOS disorders diagnosed and treated at the Royal Children's Hospital in Melbourne between 1984 and 2005.

Results Of a total of 89 patients (male:female ratio 1.5:1) 29 (33%) had cardiac involvement: 9 as presenting symptoms, 9 developing on follow-up, and 11 with subclinical cardiac findings. Leigh or Leigh-like syndrome and complex I and combined complex I, III, and IV deficiencies were the most common clinical and laboratory diagnoses, respectively. Clinically symptomatic patients had hypertrophic cardiomyopathy (5 patients), dilated cardiomyopathy (4 patients), combined ventricular hypertrophy and systolic dysfunction (3 patients), and left ventricular noncompaction (3 patients) at first assessment. A change in the type of cardiomyopathy was noted on follow-up in 2 patients. Conduction and rhythm abnormalities were present in 7 symptomatic patients.

Conclusions Cardiac assessment in children with OXPHOS disorders may reveal subclinical abnormalities of cardiac function. Patients who present with primary cardiac features have a poor prognosis. OXPHOS disorders should be considered in the differential diagnosis of children presenting with otherwise unexplained cardiomyopathy. (J Pediatr 2007;150:407-11)

Mitochondria generate energy for cellular function through the oxidative phosphorylation (OXPHOS) system, which includes the mitochondrial respiratory chain (MRC) comprising complexes I to IV and adenosinetriphosphatase (complex V). Disorders in the mitochondrial OXPHOS system may occur in any organ, at any age, and with any mode of inheritance. The heart, being highly energy dependent, is particularly vulnerable to defects in the OXPHOS system.

There is a wide variability in the reported frequency of cardiac involvement in patients with OXPHOS disorders. Moreover, there is a range of cardiac findings in these patients. Holmgren et al reported a frequency of 17% in their 101 patients with OXPHOS defects. By contrast, Scaglia et al reported a frequency of 40% in 113 patients with confirmed MRC disorders, including those with subclinical findings. Cardiac involvement was usually part of the multisystemic manifestations of OXPHOS disorders. However, decreased specific MRC enzyme activities and mitochondrial DNA point mutations or depletion have also been found in patients with isolated primary cardiomyopathy who presented with severe cardiac failure. The presence of cardiac involvement was associated with a higher mortality rate in patients with OXPHOS defects.

Different types of cardiomyopathy were observed in these studies. Guenthard et al reviewed 22 patients with OXPHOS disorders confirmed by enzyme analysis in a 20-year period and found that all had hypertrophic cardiomyopathy without outflow tract obstruction. Likewise, all 17 patients reported by Holmgren et al had hypertrophic cardiomyopathy, non-obstructive type. By contrast, Scaglia et al reported hypertrophic cardiomyopathy in 58%, dilated cardiomyopathy in 29%, and left ventricular noncompaction in 13% of their 113 patients. Cardiac conduction disturbances have been reported in Kearns-Sayre syndrome with large mitochondrial DNA deletions. Lev et al noted 6 of...
14 of their patients had conduction and rhythm problems, and Scaglia et al\textsuperscript{5} reported these abnormalities in 11.5% of their patients.

In this study we examined the frequency, type, and severity of cardiac involvement in a cohort of pediatric patients with OXPHOS disorders diagnosed in a reference laboratory and treated in a tertiary referral medical center, serving a population of approximately 5 million people.

METHODS

We conducted a retrospective review of clinical and laboratory records of all patients with definitive OXPHOS disorders diagnosed between 1984 and 2005 in the Mitochondrial Research Laboratory at the Murdoch Children Research Institute and treated at the Royal Children’s Hospital, Melbourne. The material used included biopsies taken for the investigation of a possible metabolic disease, explanted hearts at transplantation, and material collected at autopsy. The diagnosis of an OXPHOS disorder was based on our previously published diagnostic criteria.\textsuperscript{9} Mitochondrial enzyme studies in one or more of skeletal muscle, liver, heart, or cultured fibroblasts were available in 71 of 89 (80%) patients.

Cardiac involvement was based on the presence of: (1) clinical symptoms of congestive heart failure; (2) abnormal cardiac findings on two-dimensional (2D) echocardiography or electrocardiography (ECG), or chest x-rays; and (3) abnormal cardiac findings at autopsy. A single pediatric cardiologist classified cardiomypathy types at the time of presentation according to accepted World Health Organization criteria.\textsuperscript{10} Autopsy examination of the heart was undertaken in 24 patients, and the findings were reviewed by a single pediatric pathologist.

Basic demographic, clinical, enzymatic, molecular, and cardiac outcomes were summarized for the entire cohort and for the subgroup that experienced cardiac involvement (cardiac group). T tests were performed to assess differences between the cardiac and noncardiac groups in terms of age at diagnosis and presentation. As the data for these variables were right-skewed, the log transformation was applied. Survival analysis was performed where follow-up time was from date of birth to date of death or date of last visit. Kaplan-Meier plots were constructed for each group and a log-rank test was performed to assess the equality of the two survivor functions.

RESULTS

The clinical, enzymatic, and molecular findings of the whole cohort of patients are detailed in Table I. There were 89 patients, including 54 males and 35 females (male:female ratio 1.5:1). The median age at presentation of the 89 patients was 6 months (range: intrauterine to 15 years). The majority of patients had Leigh- or Leigh-like disease (27/89) and mitochondrial cytopathy (25/89), defined as a multisystemic disorder (affecting the muscular system\textsuperscript{2}, central nervous system\textsuperscript{7}, visual and auditory systems, and possibly kidney and liver).

An OXPHOS enzyme complex defect was found in 71 patients (80%). Complex I deficiency (35/89) and combined deficiencies involving complexes I, III, and IV (23/89) were the most common findings. Complex IV deficiency was found in 10 of 89 and 1 patient had complex III deficiency. Two patients had normal enzyme activity. One was later found to have a high mutant load of the mitochondrial T8993G mutation. The other had cytochrome oxidase-negative fibers on enzyme histochemistry in skeletal muscle.

Cardiac involvement was found in 29 patients (33%) (Table I). Of these, 9 had cardiac manifestations as the

| Table I. Clinical, enzymatic and molecular findings |
|---------------------------------|-------|-------|
| **Presentation and diagnosis** | Cardiac | Noncardiac |
| Male/Female (ratio) | 15/14 (1.07) | 39/21 (1.86) |
| Median age at presentation (range) | 4 mo (Intrauterine-10 y) | 8 mo (0-15 y) |
| Median age at diagnosis (range) | 26.5 mo (0.02-15.2 y) | 29.5 mo (0.02-17.9 y) |
| **Clinical diagnoses** | | |
| Leigh’s disease (27) | 6 | 21 |
| mt cytopathy (25) | 9 | 16 |
| Alpers’ disease (9) | 1 | 8 |
| Barth syndrome (4) | 4 | 0 |
| KSS (3) | 2 | 1 |
| mt cardiomyopathy (6) | 6 | 0 |
| Others (15) | 1 | 14 |
| **Enzymatic diagnoses** | |
| (n = 71) | |
| Complex I deficiency | 14 | 21 |
| Complex I, III, and IV deficiency | 4 | 19 |
| Complex IV deficiency | 2 | 8 |
| Complex III deficiency | 0 | 1 |
| Normal* | 2 | 0 |
| **Molecular diagnoses** | |
| (n = 46) | |
| MtDNA deletions/point mutations | 8 | 15 |
| TAZ mutations | 3 | 0 |
| SURF1 mutations | 1 | 3 |
| NDUFS1 mutations | 0 | 2 |
| NDUFS6 mutation | 0 | 1 |
| POLG mutations | 1 | 12 |
| **Outcome** | |
| Death (or heart transplantation) | 19 (66%) | 37 (62%) |
| Median age at death (range) | 25.8 mo (0 – 20.2 y) | 24.4 mo (0.02-30.6 y) |

Mt, mitochondrial; KSS, Kearns-Sayre syndrome.

*One patient was found to have a high T8993G mutant load. One patient was found to have cytochrome oxidase-negative fibers on enzyme histochemistry in skeletal muscle.
primary presenting symptoms, 9 had noncardiac manifestations as presenting symptoms with cardiac problems evolving later, and 11 had subclinical cardiac involvement with no clinical cardiac symptoms but significant findings on ECG or in postmortem examinations. The median age at onset of cardiac symptoms in the 18 of 29 symptomatic patients was 5 months (intrauterine to 12 years). The median age at presentation of the 9 patients with primary cardiac involvement was 10 days (0 to 9.2 years). Intrauterine onset was observed in 1 patient who had an episode of fetal tachyarrhythmia documented on fetal monitoring and who presented with supraventricular tachycardia (Wolf Parkinson White) on day 1 of life. There were 2 patients who had fetal hydrops because of in utero cardiomyopathy, one of whom was suspected of having Barth syndrome in view of an older affected sibling. The other was born at term by cesarean section and presented with cardiorespiratory distress at birth, requiring ventilatory support. There were 2 older patients who did not have systemic manifestations suggestive of a mitochondrial disease.

Clinical diagnoses in the cardiac group of patients included mitochondrial cytopathy (9), isolated mitochondrial cardiomyopathy (6), Leigh or Leigh-like disease (6), Barth syndrome (4), Kearns-Sayre syndrome (2), Alpers’ disease (1), and cardiovascular collapse (1). Biochemical diagnoses (enzymatic activity of the MRC) were obtained in 22 of 29 patients and included complex I deficiency (14), combined deficiencies (4), and complex IV deficiency (2). The biochemical defect was demonstrated in cardiac biopsies from 5 patients. Molecular diagnoses were obtained in 13 of 29 cardiac patients: 3 with mutations in the X chromosomal TAZ gene (the fourth was a male sibling of one of the patients with a mutation in the TAZ gene, who presented with hydrops-fetalis, as mentioned above), 8 with mitochondrial DNA mutations [deletions (2), T8993G (2), T12706C (1), C3303T (1), and A8344G (1), and rearrangement (1)], and 2 with autosomal mutations [SURF1 (1) and POLG (1)].

Clinical cardiac manifestations included congestive heart failure (12/29), syncope (1/29), exercise intolerance or shortness of breath (6/29), and symptomatic arrhythmia (sudden arrest in 2/29).

The types of cardiomyopathy at presentation included hypertrophic (5), dilated (4), combined ventricular hypertrophy with systolic dysfunction (3), and left ventricular noncompaction (3) (Table II). In 1 patient with the mitochondrial C3303T mutation there was a progression from hypertrophic cardiomyopathy to dilated cardiomyopathy within 1 year, as determined by 2D-echocardiographic examination. Another patient with complex I deficiency, who presented with congestive heart failure at 4 months of age and who had hypertrophic cardiomyopathy at the time of initial evaluation, had a normal repeat 2D echogram at 6 years of age. All patients with dilated cardiomyopathy had congestive heart failure at the time 2D echocardiography was done (3 of these patients presented very early in life [intrauterine, day 10 and <1 day old] and the other 2 patients presented at 4 months and 9.9 years of age). Three of the patients with Barth syndrome had left ventricular noncompaction. One of the patients with Barth syndrome had an unclassified cardiomyopathy diagnosed prenatally.

Table II shows the results of cardiac investigations. One patient with Kearns-Sayre syndrome had subclinical right bundle branch block. There were 2 patients who had both cardiomyopathy and conduction/rhythm defects such as right bundle branch block, supraventricular tachycardia, Wolf-Parkinson White syndrome, and premature ventricular complexes.

**Table II. Cardiac findings**

<table>
<thead>
<tr>
<th>Cardiac Findings</th>
<th>Cardiac</th>
<th>Noncardiac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chest x-ray</td>
<td>23</td>
<td>35</td>
</tr>
<tr>
<td>Cardiomegaly</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>ECG</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>AV Block/Incomplete RBB</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>ST &amp; T wave changes</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>RV/LV hypertrophy</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Arrhythmia (SVT/WPPW)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Left axis deviation/RA enlarged</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>2D Echogram</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>5*</td>
<td>0</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>4*</td>
<td>0</td>
</tr>
<tr>
<td>Combined cardiomyopathy</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Left ventricular noncompaction</td>
<td>3 (+1f)</td>
<td>0</td>
</tr>
<tr>
<td>Structural heart defect</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Postmortem cardiac findings</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Increase in heart weight</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Hypertrophy and endocardial fibroelastosis</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Extensive foci of necrosis</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Fibrosis/myocarditis</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Focal myocarditis</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Normal</td>
<td>3</td>
<td>13</td>
</tr>
</tbody>
</table>

2D, 2 dimensional; RV, right ventricle; LV, left ventricle; AV, atrioventricular; RBB, right bundle branch; SVT, supra ventricular tachycardia; WPP, Wof Parkinson White; RA, right atrium.

*One patient with hypertrophic cardiomyopathy had evidence of dilated cardiomyopathy in a later 2D-echocardiographic examination.
Autopsy examination of the heart was done in 10 of 29 patients of the cardiac group (Table II). One patient who presented with sudden cardiovascular collapse and died 6 days later had extensive foci of necrosis in the heart, probably as a result of the anoxic/ischaemic episode. Of the 3 patients who had normal postmortem cardiac findings, 2 had abnormal ECG findings only. Electron microscopy of the myocardium showed prominent changes in 4 patients [with left ventricular noncompaction (1), dilated cardiomyopathy (2), and cardiovascular collapse (1)] with increased number and enlargement of mitochondria and prominent densely packed cristae, which were sometimes arranged as wavy or concentric stocks.

There were 11 of 29 patients in the cardiac group who had subclinical cardiac findings. Chest radiography was done on 7 of 11 patients and was normal. Subclinical ECG findings were present in 8 of the 11 patients: ST and T wave changes (7) and incomplete right bundle branch block (1). A 2D echocardiogram was done in 6 of 11 patients. One patient had ventricular septal defect and 5 had normal examination. The 3 other patients with subclinical findings had mildly hypertrophied heart at autopsy.

There was little difference between the cardiac and noncardiac groups in terms of age at presentation (log-age means 1.81 vs 2.26; 95% confidence interval 1.29-2.33 vs 1.88-2.64, respectively; \( P = .17 \)) and age at diagnosis (log-age means 2.68 vs 3.17; 95% confidence interval 1.81-3.54 vs 2.75-3.59, respectively; \( P = .24 \)). The survival rate of the cardiac group, as a whole, was not different from that of the noncardiac group. However, the 9 patients in the cardiac group who had primary cardiac manifestations had progressive deterioration from within less than a day and up to 2 months from onset of symptoms. Seven patients died (median age at death: 1.1 months; range: birth to 9.2 years) and the other 2 (one with Barth syndrome and one with mitochondrial cardiomyopathy) survived following heart transplantation (at 15 years, 4 months and 10 years, 8 months, respectively).

DISCUSSION

Cardiac involvement in patients with OXPHOS defects has been reported in several series, with varying prevalence, type, and severity. There is some difficulty in comparing the results of the cited studies\(^2,3,7,8,11,12\) because of the different diagnostic criteria used and possible ascertainment biases as a result of different inclusion criteria. Hence, the true prevalence of cardiac involvement in OXPHOS disorders is unclear. We believe that our systematic review of the clinical, enzymatic, molecular, and postmortem data on a large cohort of patients from a well-defined population, diagnosed according to previously published diagnostic criteria,\(^5\) enabled us to draw solid epidemiological and clinical conclusions regarding cardiac involvement in OXPHOS disorders. In the largest previous review,\(^3\) in which our published diagnostic criteria were used,\(^9\) the median age of presentation was strikingly different from our series (40 months compared with 6 months). This presumably reflects a referral pattern that differs from our population-based cohort.

The frequency of cardiac involvement in our cohort of patients was 33%, which is comparable to the reported frequency range of 17% to 40%,\(^2,3,12\) We included patients with subclinical cardiac involvement in this group, as in the group reported by Scaglia et al.\(^3\) Whether or not patients with subclinical cardiac involvement were included in the “cardiac group” in other studies is not clear. The prevalence of mitochondrial OXPHOS disorders in our population was estimated at 1 in 7634.\(^13\) It follows that the prevalence of OXPHOS-related cardiomyopathy in our population would be at least 1 in 22,500.\(^2\) However, the frequency of subclinical cardiac involvement in our cohort could be an underestimate because not all patients (particularly those dating back several years) had a formal cardiac evaluation such as ECG or 2D echocardiogram unless they were clinically symptomatic. Thus the prevalence of OXPHOS-related cardiomyopathy in our population could be higher. The ratio of males to females in our whole group of patients with OXPHOS defects was comparable to that in previous reports (~1.5:1) but, interestingly, the ratio of males to females in the cardiac group was ~1:1. Larger series of patients would be needed to elucidate the significance of this finding.

An apparent correlation between specific defects in particular MRC complexes and cardiac involvement is not possible, even if we combine our results with those of Scaglia et al.\(^3\) In our study, as in previous studies,\(^7,11\) the most common MRC enzyme defects in the cardiac group were complex I and combined deficiencies. We found few patients with complex IV deficiency, in agreement with a study by von Kleist-Retzow et al,\(^11\) but in contrast to other studies.\(^2,7,14\) It is possible that larger series of patients and cardiac assessment of asymptomatic patients will eventually enable a correlation between specific OXPHOS defects and cardiomyopathy.

Several mitochondrial point mutations, deletions, or mitochondrial DNA rearrangements have been associated with cardiomyopathy\(^7\) and have been reviewed.\(^15,16\) For example, the mitochondrial mutations detected in some patients in our cardiac group (C3303T, T8993G, A8344G) had been previously associated with cardiac involvement.\(^17-19\) There is an increasing awareness of cardiomyopathy in other mitochondrial mutations such as A3243G (causing mitochondrial encephalopathy, lactic acidosis and stroke like episodes).\(^15,20\) Our patients with MELAS had no clinical cardiac symptomatology and did not have a specific cardiac assessment, hence we cannot exclude cardiac involvement in these patients. Mutations in nuclear DNA have also been associated with mitochondrial dysfunction and cardiomyopathy,\(^16\) for example mutations in the \(TAZ\) gene, leading to Barth syndrome (as documented in 3 of 4 of our patients). Complex IV deficiency as a result of mutations in the \(SURF1\) gene has not been associated with cardiomyopathy. Our observation of the postmortem finding of cardiac involvement in 1 patient with a mutation in this gene raises the question of possibly more
patients with this defect, who might have subclinical cardiac involvement.

Hypertrophic cardiomyopathy is the most commonly reported type of cardiomyopathy associated with MRC disorders.2,3,7,8,12 Cardiac hypertrophy in these disorders is thought to result from an increased oxidative stress,20 and its pathogenetic mechanism is thought to involve a cross-talk mechanism between several cellular signalling pathways as well as several transcription factors. The calcium–calcineurin signalling pathway is thought to have a pivotal role in this process.21–23 Sayen et al found decreased complex I and IV activities, decreased amounts of protein content of some subunits of these complexes, both encoded by mitochondrial DNA and genomic DNA, and increased superoxide production in cardiac mitochondria of transgenic mice with cardiomyopathy.21 Thus, overexpression of calcineurin may lead to down-regulation of nuclear and mitochondrial encoded MRC subunits, with loss of electron transfer capacity and increased production of superoxide. In the present study dilated cardiomyopathy was especially common as a hypertrophic cardiomyopathy and a change in pattern (an “undulating cardiomyopathy type”) was observed on follow-up in 2 patients. Dilated cardiomyopathy is thought to result from an acute oxidative stress as a result of a high surge in reactive oxygen species, inducing damage to the mitochondrial DNA, as shown in a mouse model.3,20 It is conceivable that severe oxidative stress in utero could cause early development of dilated cardiomyopathy, as seen in some patients (3 in our group). Such a process is suggested by previously documented case reports8,24 and in a mouse model of an MRC disorder (reviewed in reference 20). In our cohort, 1 patient had evidence of hypertrophic cardiomyopathy in the first 2D-echocardiographic examination and dilated cardiomyopathy in a repeat examination 12 months later, indicating that the timing of the cardiac assessments, particularly that of 2D echocardiography, could be a factor in the classification of cardiomyopathy. Thus, one limitation in our study, as well as in other retrospective studies, is the lack of systematic documentation of possible dynamics in the type of cardiomyopathy. Prospective studies with regular assessments of cardiac structure and function would be more informative for elucidation of the evolution of cardiomyopathy in patients with OXPHOS defects and would add further insights into the proportion of patients who manifest with cardiomyopathy alone.

Repeat cardiac assessment as part of monitoring disease progression is warranted in patients with confirmed OXPHOS disorders, even in the absence of clinical cardiac symptoms. Patients with OXPHOS defects who present with primary cardiac manifestations have a poorer outcome. Our observation that cardiac manifestations alone, particularly in the neonatal period, can be the first manifestations of OXPHOS defects suggests that these defects should be considered in neonates with isolated cardiomyopathy or older patients with conduction defects if the remainder of the diagnostic workup for a cause is unproductive.

We thank Denise Kirby, Simone Walsh, and Erin Oldaker for assistance with the mitochondrial enzyme assays.

REFERENCES

Evaluation of Discharge Management in the Prediction of Hyperbilirubinemia: The Jerusalem Experience

MICHAEL KAPLAN, MB, CHB, RUBEN BROMIKER, MD, MICHAEL S. SCHIMMEL, MD, NURIT ALGUR, MSc, AND CATHY HAMMERMAN, MD

Objective  We evaluated our program for prediction and follow-up of hyperbilirubinemia in preventing plasma total bilirubin (PTB) $\geq 25$ mg/dL and in limiting readmission for hyperbilirubinemia.

Study design  Term and near-term neonates were screened before discharge for risk factors for hyperbilirubinemia. A PTB test was performed when visible jaundice was apparent. Formal postdischarge follow-up was integrated with a possibly unique religious/cultural support system complemented by ritual circumciser (mohel) home visits and a high rate of jaundice awareness in the community.

Results  During 2001-2002, 18,079 term and near-term healthy neonates were cared for in our well baby nurseries. Three hundred forty-two (1.9%) were treated with phototherapy, and 4 with exchange transfusion. Seventy-four (21.6%) of these (0.41% of total) were readmitted for hyperbilirubinemia. Forty-two percent of those readmitted had not been regarded as sufficiently jaundiced to warrant a predischarge bilirubin determination. In only 1 neonate did the PTB exceed $\geq 25.0$ mg/dL (0.006%). No infant had signs of bilirubin encephalopathy.

Conclusions  Our practice was successful in keeping the number of readmitted neonates low and limiting those with extreme hyperbilirubinemia to the minimum. Local customs, rituals, and practices may be successfully adapted as adjuncts in the detection and prevention of hyperbilirubinemia. (J Pediatr 2007;150:412-7)

Current neonatal practice frequently results in discharge of healthy term and near-term neonates at or around 48 hours of life. Because the serum total bilirubin has not yet peaked by this time, there is the potential for postdischarge hyperbilirubinemia.1 In extreme cases, the rare sequel of bilirubin encephalopathy may occur.2,3 The Subcommittee on Hyperbilirubinemia of the American Academy of Pediatrics (AAP) recommends predischarge assessment of every newborn for the risk of development of severe hyperbilirubinemia. Two options for risk assessment are offered, to be used individually or in combination: (1) predischarge measurement of bilirubin level, with either serum/plasma or transcutaneous techniques, and the risk assessed by plotting the bilirubin level on the hour of life specific nomogram1 or (2) assessment of clinical risk factors: the more factors present, the greater the chance of hyperbilirubinemia.4 In any event, postdischarge follow-up is necessary. The AAP Subcommittee regards a total serum bilirubin $>25$ mg/dL at anytime as a medical emergency mandating immediate hospitalization and treatment.

At the Shaare Zedek Medical Center in Jerusalem, Israel, predischarge neonatal assessment for the risk of hyperbilirubinemia is performed by assessing major risk factors in all neonates and performing a plasma total bilirubin (PTB) test in those neonates with clinically apparent jaundice. The PTB results are plotted on the hour of life–specific bilirubin nomogram.1 Jaundiced newborns are followed up as outpatients, supported, as an adjunct, by a possibly unique awareness of jaundice in the community (see Methods section). The primary objective of this analysis was to determine whether our strategies, in term and near–term neonates discharged as healthy from birth hospitalization, were successful in keeping PTB values $<25$ mg/dL and limiting the rate of readmission for hyperbilirubinemia to the minimum. In addition, we compared the etiologic categories for hyperbilirubinemia between those receiving phototherapy during birth hospitalization and those rehospitalized for phototherapy.

METHODS

Consecutive, otherwise healthy term and near–term infants ($\geq 35$ weeks gestation) who were treated for hyperbilirubinemia with phototherapy or exchange transfusion in the well baby nurseries of the Shaare Zedek Medical Center for the years 2001–2002 were included in the data base. Neonates who were ill with conditions such as sepsis, respiratory distress, hypoglycemia, with major congenital anomalies, or requiring neonatal intensive care for any

<table>
<thead>
<tr>
<th>AAP</th>
<th>American Academy of Pediatrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAT</td>
<td>Direct antibody titer</td>
</tr>
<tr>
<td>PTB</td>
<td>Plasma total bilirubin</td>
</tr>
<tr>
<td>G-6-PD</td>
<td>Glucose-6-phosphate dehydrogenase</td>
</tr>
</tbody>
</table>

From the Department of Neonatology (M.K., R.B., M.S.S., C.H.) and Clinical Biochemistry Laboratory (N.A.), Shaare Zedek Medical Center; Faculty of Medicine of the Hebrew University, Jerusalem (M.K.); Faculty of Health Sciences, Ben Gurion University of the Negev, Be’er Sheva, Israel (R.B., M.S.S., C.H.).

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condition other than exchange transfusion, were excluded. Cephalhematoma or mild bruising was not regarded as exclusion criteria. The survey was performed with the permission of the Institutional Review Board, which waived the need for signed, informed consent. The population delivering at this hospital is divided approximately equally between Ashkenazi and Sephardic Jews, and up to 10% Israeli Arabs or Palestinians.

Routine medical care for healthy neonates in our nurseries includes visual observation for development of jaundice at least once per nursing shift. Indications for PTB testing are liberal, and capillary blood samples may be drawn for this purpose at the nurses’ discretion. Breast feeding is encouraged. Routine investigations include screening for glucose-6-phosphate dehydrogenase (G-6-PD) deficiency in high-risk population subsets of the Sephardic Jewish population, and blood group determination and direct antibody titer (DAT) testing for babies born to Rh-negative or O blood group mothers. Neonates are routinely discharged around 48 hours after birth, and those of mothers who had undergone cesarean section at 4 days.

Before discharge, all babies are evaluated for the presence of identifiable risk factors that might increase the chance of subsequent hyperbilirubinemia. These factors include breast feeding, gestational age <38 weeks, previous history of an infant treated with phototherapy or exchange transfusion in the family, G-6-PD deficiency and blood group incompatibility with positive DAT. In neonates with visible jaundice extending to the lower abdomen or legs, or in those who had a previous bilirubin test >40th percentile on the bilirubin nomogram, a PTB is obtained. In those newborns who have had a PTB test, discharge instructions are planned according to the bilirubin percentile, according to the following guidelines: PTB <40th percentile for hour of life—discharge from hospital clinic follow up, but community well baby clinic visit within 2 to 4 days; 40th to 75th percentile—reevaluate in 48 hours, PTB as clinically warranted; 75th to 95th percentile—PTB within 24 hours; >95th percentile—consider delaying discharge, repeat PTB within 8-12 hours, unless meets criteria for phototherapy (below). In the presence of risk factors, or at the discretion of the discharging physician, PTB determinations may be ordered earlier. For the purpose of discharge planning, those neonates who are not regarded as sufficiently clinically jaundiced to warrant a PTB test are grouped among those <40th percentile.

All parents are shown how to check the baby to detect the appearance of clinical jaundice and instructed to return for a bilirubin test in the event they were concerned that the baby was becoming jaundiced. Follow-up is performed daily on an outpatient basis by our medical staff, until either stabilization or decrease of the bilirubin values, or rehospitalization for commencement of phototherapy. In addition to the above, at the time of discharge we recommend that all babies be seen within 2 to 4 days at either the well baby clinics or by their pediatrician for the assessment of jaundice and the success of breastfeeding. Furthermore, many of the Jewish mother-infant dyads spend from a few days to 1 week after delivery either of 2 postnatal convalescent homes set up by the Haredi-orthodox Jewish community. Bilirubin testing and pediatric supervision is available at these institutions, although phototherapy is not performed and neonates are referred to the birth hospital when necessary. Less formal bilirubin evaluation, in the case of males, includes a visit to the home by the ritual circumciser (mohel). This is frequently a source of referral for hyperbilirubinemia because, according to Jewish ritual law, a baby should not be jaundiced at the time of ritual circumcision on the eighth day of life. This injunction serves to increase the awareness of neonatal jaundice in our population and extends to parents of baby girls as well. As a result, our population is aware of neonatal jaundice, and parental compliance for performing testing is excellent.

Neonates requiring phototherapy during the first 10 to 14 days after delivery are usually readmitted to the birth hospital nursery. Home phototherapy is not practiced. For standardization, any neonate who is referred for jaundice from any laboratory other than of our institution has a confirmatory PTB performed in our laboratory before readmission and commencement of phototherapy.

Criteria for phototherapy were based on, but somewhat more stringent than, the 1994 AAP Practice Parameter\(^6\) as follows: <24 hours: PTB 10.0 mg/dL; 24 to 48 hours: 12.0 mg/dL; 49 to 72 hours: 15.0 mg/dL; >72 hours: 17.0 to 18.0 mg/dL. Criteria for readmission for primary treatment of hyperbilirubinemia included PTB concentrations ≥18.0 to 20.0 mg/dL. In infants with risk factors, phototherapy was started at PTB concentrations 1 to 2 mg lower than described above, at the physicians’ discretion.

Phototherapy is provided with babies lying supine in open bassinets, with 1 or 2 overhead blue 4-lamp fluorescent units (Medela Phototherapy Lamp; Medela Medical Technology, Baar, Switzerland) placed 25 cm above the baby. In many instances a phototherapy mattress is used as well (BiliBed; Medela Medical Technology, Baar, Switzerland). Light intensity, measured at the level of the skin of the babies’ abdomen, was 17 ± 3 \(\mu W/cm^2/nm\) (Minolta Airshields Fluoro-lite meter 451, Hatboro, PA). Breast feeding is generally encouraged and is discontinued only in selected infants in whom the PTB continued to rise >20.0 mg/dL despite phototherapy. During phototherapy PTB levels are determined at least twice daily, and more frequently if deemed necessary by clinical judgment. Phototherapy is continued until the PTB concentration decreases to below 12.0 to 13.0 mg/dL. In those cases of early hyperbilirubinemia in whom PTB concentrations did not exceed 12.0 mg/dL, treatment is discontinued when PTB concentrations stabilize and decrease below the 75th percentile on the hour of life specific bilirubin nomogram.\(^1\)

Nursery protocol includes a post-PTB determination 12 to 24 hours after discontinuation of phototherapy but does not require babies to remain hospitalized. In cases of significant rebound, phototherapy is recommenced at the discretion of the attending neonatologist, but not usually at PTB values <15.0 mg/dL and frequently higher. The decision to reinstitute phototherapy may be influenced by the presence of risk factors for hyperbilirubinemia.
Table I. Demographic data of neonates and data related to phototherapy

<table>
<thead>
<tr>
<th></th>
<th>Birth hospitalization</th>
<th>Rehospitalization</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>264</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>3184 ± 453</td>
<td>3294 ± 470</td>
<td>0.07</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>38.9 ± 1.8</td>
<td>38.8 ± 1.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Male:Female</td>
<td>149:115 (56:44%)</td>
<td>53:21 (72:28%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Vaginal delivery (n)</td>
<td>236 (89%)</td>
<td>74 (100%)</td>
<td>0.0007</td>
</tr>
<tr>
<td>PTB (mg/dL) at onset of phototherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>14.7 ± 3.0</td>
<td>18.5 ± 1.7</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Age (hr) at onset or readmission</td>
<td>56 ± 30</td>
<td>123 ± 39</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Age at discontinuation</td>
<td>98 ± 28</td>
<td>153 ± 42</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Duration of phototherapy (hr)</td>
<td>44 ± 26</td>
<td>30 ± 10</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Rate of PTB decrease (mg/hr therapy)</td>
<td>0.12 ± 0.43</td>
<td>0.28 ± 0.11</td>
<td>p = 0.005</td>
</tr>
<tr>
<td>Exchange transfusion (n)</td>
<td>3</td>
<td>1</td>
<td>p = 0.6</td>
</tr>
<tr>
<td>Retreatment (n)</td>
<td>31 (11.7%)</td>
<td>1 (1.4%)</td>
<td>p = 0.01</td>
</tr>
</tbody>
</table>

PTB, Plasma total bilirubin.

Data Analysis

Neonates requiring phototherapy during birth hospitalization and those readmitted for primary phototherapy were compared. Neonates returning for phototherapy because of rebound after an initial round of treatment were not regarded, for the purpose of the study, as new cases of phototherapy. The role of predischarge bilirubin screening, where applicable, in the prediction of subsequent hyperbilirubinemia was examined. Continuous variables were compared by use of Student’s t test, and categorical variables were compared by use of χ² analysis. Significance was defined as a P value < .05.

Laboratory Methods

PTB was measured routinely in the hospital’s clinical biochemistry laboratory by a direct spectrophotometric method with spun capillary tube samples (Wako Bilirubin Tester; Wako Pure Chemical Industries, Ltd., Osaka, Japan). Direct Coombs testing and G-6-PD screening were performed by routine laboratory methods.

RESULTS

During the period 2001-2002 there were 19,437 live births; 1358 were admitted to the neonatal intensive care unit and were not included in the current calculations. The remaining 18,079 term and near-term neonates were cared for in the well baby nurseries; 342 of these (1.9%) were treated with phototherapy, including 4 who required exchange transfusion. Seventy-four of these were readmitted for hyperbilirubinemia, comprising 0.41% of total cohort, and 21.6% of those requiring phototherapy. Demographic details and information related to phototherapy are summarized in Table I, and the number of neonates in each etiologic category appears in Table II. Of the 4 neonates who received exchange transfusion, 3 were performed during the birth hospitalization (for Coombs’ positive ABO isoimmunization, anti-E isoimmunization, and G-6-PD deficiency, respectively), and one was readmitted (G-6-PD deficiency).

In 22 neonates (0.12% of the cohort), a PTB concentration ≥20.0 mg/dL was documented. However, in only 1 of these was the highest noted bilirubin >25.0 mg/dL, and in no neonate was a PTB ≥30.0 mg/dL documented. Of these 22 newborns, the PTB exceeded 20.0 mg/dL during birth hospitalization in 5, and 14 were readmitted for primary phototherapy. An additional 3 had been treated for lower levels of PTB during the birth hospitalization but were readmitted for rebound hyperbilirubinemia. Diagnostic subgroup characteristics of the 22 neonates with the highest PTB values include 2 with G-6-PD deficiency, 4 DAT positive ABO incompatible newborns, 3 of whom were readmitted for repeat phototherapy, 1 with maternal diabetes and 1 ≤37 weeks’ gestation. In 14 of the babies with PTB concentrations ≥20.0 mg/dL, breast feeding was the only identifiable cause for hyperbilirubinemia.

Forty-three (58.1%) of the readmitted neonates had been sufficiently jaundiced, by clinical assessment during the birth hospitalization, to warrant a predischarge PTB determination: of these, 25 (33.8%) were between the 75th and 95th percentile on the hour of life–specific bilirubin nomogram (high intermediate risk zone) and 5 (6.8%) ≥95th percentile (high risk zone). Ten were in the low intermediate-risk zone (40th–75th percentile), and 2 were <40th percentile (low risk zone). Of the latter 2 newborns, one was G-6-PD deficient, although in the second no risk factor other than breast feeding was identified. Thirty-one (42%) of the readmitted neonates had not been regarded sufficiently jaundiced at the time of discharge to warrant bilirubin testing and had not been invited for outpatient follow-up. Mean age at the time of readmission for those neonates who had a predischarge PTB taken (118 ± 36 hours) contrasted with that of those neonates who had not had a predischarge PTB (137 ± 39 hours, P = .03).
Mean weight change from birth to readmission was $-3.5\% \pm 1.7\%$, and weight loss exceeded 10% in only 3 infants. Among those treated during the birth hospitalization, most had a hemolytic cause for their jaundice, and in only 16.3% was breast feeding the only evident cause. The converse was apparent in the readmitted neonates: the minority had hemolytic conditions, whereas in 52% breast feeding was the only identifiable risk factor (Table II). None had signs of bilirubin encephalopathy, and all were neurologically normal at the time of both first and second (where applicable) hospital discharges.

**DISCUSSION**

Despite publication of authoritative guidelines and alerts severe hyperbilirubinemia and kernicterus continue to be encountered. Because neonates frequently leave the hospital environment before their bilirubin level peaks, the development of postdischarge hyperbilirubinemia may not be recognized at home by the parents. The Subcommittee on Hyperbilirubinemia of the AAP has published guidelines for discharge planning and the timing of postdischarge follow-up evaluations.

We assessed the efficacy of our in-hospital prediction, management and discharge program, in conjunction with community services available in Jerusalem. We did not perform routine bilirubin follow-up on every neonate in the cohort. Rather, we integrated our formal outpatient follow-up with available community services, religious authorities, and unique population awareness of jaundice to monitor and refer neonates with hyperbilirubinemia. We are confident that we were aware of the vast majority of those with development of severe hyperbilirubinemia. During the study period we were successful in preventing cases of PTB $\geq 30$ mg/dL and limiting those with PTB levels between 20 mg/dL and 30 mg/dL to the minimum. There was no clinically apparent bilirubin morbidity.

Even when neonates display no visible jaundice, or when predischarge bilirubin values are in the low-intermediate or low-risk zones, postdischarge hyperbilirubinemia requiring readmission may nevertheless be necessary. In fact, before birth hospitalization discharge, 42% of the neonates readmitted for phototherapy had not been recognized as jaundiced and bilirubin was not determined. Low predischarge bilirubin values, or absence of clinically apparent jaundice, should not be regarded as synonymous with very low risk for development of postdischarge hyperbilirubinemia. It is of interest that, of our readmitted neonates, those who had not had a predischarge PTB performed were readmitted significantly later than those who did have a predischarge PTB. It is possible that the latter group was more vigorously followed up because jaundice had already been identified. However, it is also possible that, among those requiring readmission, 2 subgroups of neonates may exist: on the one hand, those in whom jaundice first becomes apparent early, and on the other, those in whom the jaundice becomes evident later.

Our experience does differ from the report of Bhutani et al. In that survey, no infant with a predischarge bilirubin $<40$th percentile for hour of life had subsequent hyperbilirubinemia, although 1 neonate in whom the bilirubin value at age 18 hours was exactly on the 40th percentile did. Reasons for this discrepancy may include genetic or cultural differences between the populations compared or differences in follow-up practice. However, the method in the study by Bhutani et al was dissimilar to ours. For example, case closure occurred earlier in the study by Bhutani et al. Their cohort comprised 22% of the total eligible births during that study period, and, although we did not attempt to perform a bilirubin test on each and every baby, the entire 2-year cohort was subject to the same follow-up facilities.

Readmission to hospital is economically costly, disruptive to the family, and may have adverse effects on the establishment of successful breastfeeding. Hyperbilirubinemia is usually the most common reason for readmission to hospital during the neonatal period. Surveillance of the rate of hospital readmission for hyperbilirubinemia has been suggested as an index of the potential for severe postdischarge hyperbilirubinemia. In this study the rate of readmission for hyperbilirubinemia was 0.38% of live births, at the low end of the reported range (0.17% to 3.2%) for readmission for hyperbilirubinemia. With one exception, maximum recorded PTB concentrations at the time of readmission were below 25 mg/dL. This level of bilirubin is the upper level of the AAP Subcommittee’s recommendations for exchange transfusion, and has been categorized by Bhutani and Johnson as extreme hyperbilirubinemia. However, lower bilirubin levels should not be regarded with complacency because presence of hemolytic conditions or gestational age $<37$ weeks may lower the threshold for bilirubin-induced neurologic damage.

### Table II. Etiology of hyperbilirubinemia in birth hospital phototherapy versus readmission for primary phototherapy

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Birth hospitalization</th>
<th>Rehospitalization</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of neonates</td>
<td>264 (77%)</td>
<td>74 (23%)</td>
<td>$p &lt; 0.0001$</td>
</tr>
<tr>
<td>ABO incompatibility, direct Coombs’ positive</td>
<td>128 (48.5%)</td>
<td>6 (8.1%)</td>
<td></td>
</tr>
<tr>
<td>Other Coombs’ positive</td>
<td>5 (1.9%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ABO incompatibility, direct Coombs’ negative</td>
<td>43 (16.3%)</td>
<td>16 (21.6%)</td>
<td>$p = 0.3$</td>
</tr>
<tr>
<td>$\leq 37$ weeks gestation</td>
<td>64 (24.2%)</td>
<td>11 (14.9%)</td>
<td>$p = 0.1$</td>
</tr>
<tr>
<td>G-6-PD Deficiency</td>
<td>25 (9.5%)</td>
<td>8 (10.8%)</td>
<td></td>
</tr>
<tr>
<td>Breast feeding only identifiable cause</td>
<td>43 (16.3%)</td>
<td>38 (52.1%)</td>
<td>$p &lt; 0.0001$</td>
</tr>
</tbody>
</table>

In some cases there was overlap between certain etiologic categories.
There is no single, all-encompassing reason for the development of postdischarge hyperbilirubinemia. Breast feeding is the most common etiologic factor.\textsuperscript{2,17,22,27-29} Indeed, among our patients, no infant who was exclusively formula fed and who did not have any additional risk factor was readmitted for hyperbilirubinemia. Early discharge has been implicated in some series,\textsuperscript{15,21,22,30,31} although not consistently.\textsuperscript{18,20,32} Borderline prematurity and male sex are frequently cited as a risk factors.\textsuperscript{3,4} Importantly, in many instances no predictive factor other than breast feeding can be identified, an observation that emphasizes the need for routine postdischarge follow-up in all neonates.

Few studies have compared neonates receiving phototherapy during birth hospitalization with those readmitted.\textsuperscript{28} The contrasting cause for hyperbilirubinemia between birth hospitalization and readmitted neonates was striking. In the former group, most had a hemolytic basis for hyperbilirubinemia, although, of those readmitted, breast feeding was the only identifiable risk factor in more than 50%. Similar polarization of etiologic factors has been demonstrated by Maisels and Kring.\textsuperscript{28}

The number of neonates encountered during the 2 years of our study with PTB concentrations \(\geq 20.0\) mg/dL (0.12%), with only 1 neonate \(\geq 25.0\) mg/dL (0.006%) and none \(\geq 30.0\) mg/dL, compares favorably with other surveys\textsuperscript{26,33} and reflects on the efficacy of our surveillance and treatment program. Bhutani et al\textsuperscript{1} estimated the incidence of PTB values \(>20.0\) mg/dL to be 1 in 70 (1.4%), \(\geq 25.0\) mg/dL 1 in 700 (0.14%), and 1 in 10,000 for values \(\geq 30.0\) mg/dL (0.01%). Breakdown of the 22 neonates in our series with the highest bilirubin levels (\(\geq 20.0\) mg/dL) led to surprising conclusions. We had been expecting those neonates with the greatest risk factors, such as DAT-positive ABO incompatibility, G-6-PD deficiency, and borderline prematurity, to comprise the majority. Surprisingly, 14 of the 22 had no identifiable risk factor other than breast feeding. Possibly, those with identified hemolytic conditions had been more vigilantly monitored and treated earlier than those without recognized hemolysis.

Dehydration or unsuccessful breast feeding are sometimes implied in the cause of postdischarge hyperbilirubinemia.\textsuperscript{25,29,30} None of the neonates readmitted in this series had clinical signs of dehydration or appeared ill, lethargic or septic. Weight change from birth to rehospitalization averaged \(-3.5\)% and exceeded 10% in only 3 neonates. It therefore appears unlikely that dehydration was a major etiologic factor in the pathogenesis of late-onset hyperbilirubinemia in our infants.

G-6-PD deficiency is frequently associated with severe hyperbilirubinemia and bilirubin encephalopathy.\textsuperscript{2,3} It is therefore not surprising that the readmitted neonate with the highest PTB concentration was G-6-PD deficient. Severe and unpredictable hemolytic episodes associated with G-6-PD deficiency may be the one reason that kernicterus may not be completely preventable.\textsuperscript{34} G-6-PD screening should be considered in population groups with a high incidence of the enzyme deficiency.\textsuperscript{35}

The onus of the detection of hyperbilirubinemia is in the process of change, from being a virtually totally in-hospital program to one now shared between in-hospital and community health care workers. Our study confirms the potential for significant postdischarge hyperbilirubinemia. However, our predischarge risk assessment program, in combination with a formal follow-up service complemented by a possibly unique community and religious support system, was successful in keeping the number of readmitted neonates low, without forgoing ongoing surveillance for severe postdischarge hyperbilirubinemia. In any community local customs, rituals and practices that may potentially exacerbate hyperbilirubinemia (eg, herbal teas, henna applications, or maternal fava ingestion) should be sought. Our experience shows that other rites or conventions may be successfully adapted as adjuncts to the formal, AAP guideline-based program for the detection and prevention of hyperbilirubinemia.

REFERENCES

In 1957, Tevetoglu described the treatment of childhood constipation with dioctyl sodium sulfosuccinate (also known as docusate sodium), a recently introduced stool softener. In the study, Tevetoglu enrolled 50 children between the ages of 5 weeks and 14 years who had either “persistent chronic constipation” or “acute or subacute... constipation.” The dosages of dioctyl sodium sulfosuccinate used in the study ranged from 10 mg per day in infants to 120 mg per day in older children. The medicine was given once or twice a day and continued for at least 15 days. After the initial 15 days of therapy, the medicine was used on an as-needed basis. Dioctyl sodium sulfosuccinate was found to be very effective in the treatment of childhood constipation, with effects noted as early as 2 days after initiating therapy. Interestingly, several patients enrolled in the study had migraine headaches that were felt to be associated with “faulty elimination.” These headaches disappeared with correction of the constipation.

Constipation is a common childhood problem, with a prevalence reported to be as high as 28%. As noted by Tevetoglu in 1957, the cause of constipation in most young children is “purely functional,” and treatment should include use of an agent that enables passage of soft, painless stools. Dioctyl sodium sulfosuccinate accomplishes this goal by acting as a detergent to draw water into the colon and soften stool, but its use can be limited by the medicine’s bitter taste. Physicians now have tasteless medicines, such as polyethylene glycol without electrolytes, which accomplish the same goal as a detergent to draw water into the colon and soften stool, but its use can be limited by the medicine’s bitter taste. Medical treatments for initial disimpaction and subsequent maintenance therapy are important in treating any child with constipation. In addition to the various medications available for childhood constipation, physicians now recognize that behavioral modification (regular toilet sitting, sticker charts, etc.) and parental education are also important adjuncts of therapy. Many of these medication, educational, and behavioral recommendations have been incorporated into clinical practice guidelines to assist primary care providers in optimizing their management of pediatric constipation.

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REFERENCES


PEDIATRIC USE OF DIOCTYL SODIUM SULFOSUCCINATE
Tevetoglu FJ. J Pediatr 1957;50:304-7

50 Years Ago in The Journal of Pediatrics
Pre-ductal and Post-ductal $O_2$ Saturation in Healthy Term Neonates after Birth

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Objective To determine the pre- and post-ductal oxygen saturation (SpO$_2$) levels during the first minutes after birth in healthy term infants.

Study design In a prospective cohort study, sensors were placed on the right hand and on 1 foot of the neonate. Pre- and post-ductal SpO$_2$ levels were recorded during the first 15 minutes after birth. Exclusion criteria were gestational age <37 weeks, presence of risk factors for asphyxia, emergency cesarean delivery (C/D), congenital anomalies, and multiple pregnancies. Infants who were treated with O$_2$ or positive pressure ventilation were also excluded from the study.

Results The mean (SD) gestational age of the 110 infants was 39 weeks (1.1), and the mean birth weight was 3340 grams (359). At 5 minutes, the mean pre-ductal SpO$_2$ level was 89% (7), and the mean post-ductal SpO$_2$ level was 81% (10). Pre- and post-ductal SpO$_2$ levels were significantly different during the first 15 minutes after birth. The SpO$_2$ level was lower in babies delivered by C/D in comparison to babies born by vaginal delivery.

Conclusions In healthy newly born infants, oxygen saturation rises slowly and does not usually reach 90% in the first 5 minutes of life. A gradient between pre- and post-ductal SpO$_2$ levels remains significant for the first 15 minutes of life. (J Pediatr 2007;150:418-21)

Ever year worldwide, between 5% and 10% of newly born infants require some form of resuscitation. There is currently insufficient evidence to specify the concentration of O$_2$ to be used at the initiation of resuscitation. Oxygen supplementation may be harmful because O$_2$ free radicals may be involved in the pathogenesis of many neonatal diseases. This has lead to questioning about the recommendation to use pure oxygen in neonatal resuscitation. Some studies have shown that starting neonatal resuscitation with air is at least as effective as starting it with 100% oxygen, and some studies even suggest this results in potential better outcomes. A meta-analysis has shown a reduction in mortality in the group of neonates who started resuscitation with room air, and follow-up of survivors at 2 years old found no differences in neurological sequelae in the 2 groups.

Strategies for optimal resuscitation are periodically revised by the International Liaison Committee on Resuscitation (ILCOR) on the basis of the best available evidence. The ILCOR neonatal delegation has recently published new recommendations, acknowledging how difficult it was to reach a consensus on the use of oxygen. For babies born at term, the 2005 NRP guidelines still recommend the use of 100% supplemental O$_2$ when a baby is cyanotic or when positive pressure ventilation is required during neonatal resuscitation. However, the optimal way to assess the response to resuscitative maneuvers needs to be determined better. Traditionally, this has been based on respiratory effort, heart rate, and color. Pulse oximetry is widely used in pediatric and neonatal intensive care, and some pediatricians consider it to be a new vital sign. It has been advocated that O$_2$ supplementation be adjusted after pulse oximetry measurements. Before current standards on oxygen supplementation during resuscitation can be changed, data on the normal levels of oxygen saturation in healthy newly born infants are necessary. Some studies have looked at this issue. However, data on simultaneous determinations of pre- and post-ductal oxygen saturation (SpO$_2$) levels with new technology monitors in healthy term neonates is still insufficient. Newer oximeters with signal extraction technology (Masimo) have improved signal detection and elimination of false input even in adverse situations, which allows measurements of SpO$_2$ levels...
in newly born infants in a more reliable way. Determinations of saturation levels during the first minutes after birth provide information that could be of help in the rational use of oxygen in the newly born infant.

We assessed the physiological changes in both pre- and post-ductal SpO2 levels during the first minutes after birth in healthy term neonates. We also evaluated the pre-ductal SpO2 level that correlated to the clinical perception of pink color.

METHODS

This was a prospective cohort study. The population consisted of healthy term newly born infants born by vaginal delivery or cesarean delivery (C/D). Exclusion criteria are shown in Table I.

For the purpose of the study, 2 investigators and a resident attended every delivery. A second- or third-year resident received the baby and gave all the initial care, and 2 additional physicians (neonatology fellows or attendings) took care of the sensors, monitors, chronometer, and data recording (from visual inspection of the display). Two pulse oximeters (Masimo Radical, UNIC Company) were used. This technology was chosen because there have been problems with the clinical use of traditional oximeters in neonates, particularly with motion artifact, noise, and low perfusion. The new generation pulse oximetry software and Signal Extraction Technology (SET) has improved the threshold of measurement during low perfusion by 10-fold. Signal extraction technology is a set of algorithms, hardware designs, sensor, and patient cable designs that together allow for more accurate arterial SpO2 and pulse rate monitoring during motion and low perfusion. The normal sensitivity mode was used.

As soon as possible after the neonate’s cord had been clamped, sensors were placed on the right hand and on 1 foot of the neonate to record pre- and post-ductal SpO2 levels. Posey wraps were used to secure the sensors. No specific method of sensor application was followed. Times were measured with a chronometer that was started at birth. The time (in seconds) elapsed from birth until the first reliable reading of SpO2 level and heart rate was determined (combining visual appreciation of both the plethysmograph waveform and the signal IQ with direct auscultation when there was uncertainty). Pre- and post-ductal SpO2 levels and heart rate were recorded at 2, 3, 4, 5, 10, and 15 minutes after birth, or until the pre-ductal saturation level was higher than 90%. The investigators who placed the sensors did not intervene in the care of the baby. The resident who attended each delivery was unaware of the SpO2 levels. Routine care was provided as usual, including delayed cord clamping (1 minute), followed by mother-to-child contact. The Neonatal Resuscitation Program (AAP/AHA) was followed in case of need. The resident was asked to state when she or he considered the baby to be pink (central color). The pre-ductal SpO2 level at that time was recorded.

The sample size was calculated to determine the mean level of transcutaneous SpO2 at 5 minutes after birth. For this calculation, we assumed a SD of 10. The sample size calculated to estimate the mean value of oxygen saturation ±2 with a 95% CI was at least 96 neonates. Results were analyzed with the Student t test for parametric data and with the Mann-Whitney U rank sum test for nonparametric data. A P value < .05 was considered to be statistically significant. The protocol was approved by the Hospital Committee of Ethics on Research Protocols, and parental written informed consent was required for inclusion in the study.

RESULTS

Between Oct 10 and Nov 28 2005, 214 babies were born at our institution. Of these births, 104 infants were excluded from the study, for these reasons: 23 were preterm (<37 weeks), 21 had risk factors for asphyxia, 3 were born by emergency C/D, 2 had congenital anomalies, 4 were twin gestations, 8 needed resuscitation, 3 had parents who denied consent, 22 had parents whom the investigators were unable to approach to obtain informed consent, and 18 were excluded for other reasons (simultaneous deliveries, insufficient personnel, insufficient material, etc). Some of these infants had >1 exclusion criteria. A total of 110 neonates were included in the study. The median (5-95 percentiles) gestational age of these neonates was 39 weeks (37-41), and the median birth weight was 3380 g (2745-3865). All mothers received anesthesia during labor, 79% epidural and 21% spinal. Demographic characteristics are shown in Table II.

Median (interquartile range [IQR]) time to get accurate

<table>
<thead>
<tr>
<th>Table I. Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gestational age &lt;37 weeks</strong></td>
</tr>
<tr>
<td><strong>Presence of risk factors for asphyxia</strong></td>
</tr>
<tr>
<td>- Maternal diseases</td>
</tr>
<tr>
<td>- Pregnancy hypertension states</td>
</tr>
<tr>
<td>- Severe oligohydramnios</td>
</tr>
<tr>
<td>- Maternal fever</td>
</tr>
<tr>
<td>- Vagal bleeding</td>
</tr>
<tr>
<td>- Rupture of membranes &gt;18 hours</td>
</tr>
<tr>
<td>- Prolapsed cord</td>
</tr>
<tr>
<td>- Meconium-stained amniotic fluid</td>
</tr>
<tr>
<td>- C/D for fetal health compromise</td>
</tr>
<tr>
<td><strong>Emergency C/D</strong></td>
</tr>
<tr>
<td><strong>Multiple pregnancies</strong></td>
</tr>
<tr>
<td><strong>Congenital anomalies</strong></td>
</tr>
<tr>
<td><strong>Need of resuscitation maneuvers including O2 supplementation, ventilation, and/or medications</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table II. Demographic characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristic</strong></td>
</tr>
<tr>
<td>Maternal age</td>
</tr>
<tr>
<td>Vaginal birth</td>
</tr>
<tr>
<td>Maternal face mask O2</td>
</tr>
<tr>
<td>Time of cord clamping (seconds)</td>
</tr>
</tbody>
</table>
readings was 3 minutes (2.4-4.1). The number of infants contributing to the saturation ranges at 2, 3, 4, 5, 10, and 15 minutes were 20, 62, 92, 106, 109, and 110, respectively. Both pre- and post-ductal O₂ saturations levels increased gradually after birth (Figure). At 5 minutes, the median (IQR) pre-ductal SpO₂ level was 90% (84-94), whereas the median post-ductal SpO₂ level at that moment was 82% (76-89). Pre- and post-ductal SpO₂ levels were significantly different during the first 15 minutes after birth. The mean (SD) time for perceiving the baby pink was 4.4 minutes (2), and the mean pre-ductal SpO₂ at that moment was 86% (7.6). The mean time to reach a pre-ductal SpO₂ level of 90% was 5.5 minutes (2.2).

Pre- and post-ductal SpO₂ levels were lower in babies born by C/D in comparison with babies born by vaginal delivery (Table III). The mean time taken to have a pre-ductal SpO₂ level of 90% was 5.2 minutes (1.5) in vaginal deliveries and 6.3 minutes (2.4) after C/D (P < .05).

All infants included in the study went from the delivery room to room-in with their mothers, and none required admission to the neonatal intensive care unit.

**DISCUSSION**

Our study demonstrates that, in healthy newborn infants immediately after birth, there is a significant difference between pre-ductal and post-ductal SpO₂ levels during the first 15 minutes of life. Most likely this is caused by high pulmonary artery pressure and right-to-left shunt through the ducts arteriosus. Both pre- and post-ductal SpO₂ levels rise gradually and do not usually reach 90% in the first 5 minutes of life. The use of monitors with signal extraction technology makes the measurements of SpO₂ levels immediately after birth feasible and more reliable.¹²¹ ²²

Our results are consistent with those of previous studies. Harris et al studied 76 newly born infants and compared pre- and post-ductal SpO₂ levels in 32 neonates born by vaginal delivery and 44 born by C/D.¹⁶ In babies born by C/D, SpO₂ levels were lower during the first 5 minutes. We had similar findings, including differences in pre-ductal SpO₂ levels. Infants born by C/D took longer to reach a SpO₂ level higher than 90%, a finding consistent with recent reports.¹⁷,¹⁸ Toth et al studied 50 healthy neonates born by vaginal delivery.¹⁹ Using oximeters without new technology, they established pre- and post-ductal saturations until 20 minutes after birth. Their results were similar to those of this study, although they found lower SpO₂ levels throughout the study. The use of a different pulse oximeter technology could explain the difference. Köpotic and Lindner used new generation pulse oximeters to assess SpO₂ levels and heart rate in preterm neonates in the delivery room and compared outcomes with a control group in which monitors were not used.²⁰ They measured post-ductal SpO₂ levels and showed that immediate postnatal SpO₂ monitoring was feasible and may help to make decisions about the type of care babies need. Kamlin et al used pulse oximeters with Masimo technology and determined pre-ductal SpO₂ levels in healthy neonates.¹⁷ They included 54 preterm infants and 121 term infants. For the whole group, the mean (SD) time to reach an SpO₂ level >90% was 5.8 minutes (3.2). However, being preterm and born by C/D were factors independently associated with longer duration of pre-ductal SpO₂ levels <90%. Rabi et al reported similar results in their study of pre-ductal SpO₂ levels using monitors with new technology.¹⁸ They assessed 115 term and near term infants for the first 10 minutes after birth. The median time to reach an SpO₂ level of 90% was 8 minutes. The SpO₂ level was higher in neonates born by vaginal delivery than in those born by C/D.

This study combines the strengths of an adequate sample size, a homogeneous healthy full term population, measurements of pre- and post-ductal SpO₂ levels, and the use of new generation oximeters with Masimo technology, which improves the reliability of the SpO₂ readings.
One of the current controversies in neonatal resuscitation is the optimal use of supplementary O₂. The Neonatal Resuscitation Program recommends making decisions about O₂ administration on the basis of the 3 classical variables of respiratory effort, heart rate, and color. Clinical assessment of color varies widely. We found that the clinical determination of central pink color in healthy term newly born infants correlates with a mean pre-ductal SpO₂ of 86%, and that this is usually reached after 4 minutes in normal conditions. The absence of respiratory effort is certainly a correct variable to provide positive pressure ventilation, but not to decide the optimal oxygen concentration to be given. Resuscitation with 100% O₂ delays the initiation of spontaneous breathings. Heart rate seems to be the most useful sign for adequate response to resuscitation. Considering the limitations of clinical assessment of oxygen requirements and the increasing concern about potential risks of high O₂ exposure to newly born infants, some physicians have suggested an approach using a variable oxygen supply tempered by pulse oximetry measurements to avoid hyperoxia. The results of our study may help to set the reference range of pre- and post- ductal SpO₂ levels in the newly born infant. More studies are needed to understand the physiologic adaptation to extra uterine environment and to be more rational in the use of supplementary O₂.

It was difficult to place the sensors and have accurate readings for the first 2 to 3 minutes. Some factors could explain this observation. First, no intervention was performed until the cord had been clamped. At our institution, a policy of delayed cord clamping is followed, on the basis of several studies showing the safety and beneficial effects of this method of sensor application was followed. It has been recognized earlier drafts of the manuscript and gave us pertinent and valuable suggestions.

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25. van Rheenen P, Brabin BJ. Late umbilical cord-clamping as an intervention for reducing iron deficiency anaemia in term infants in developing and industrialised countries: a systematic review. Ann Trop Pediatr 2004;24:3-16.
Continuing Anemia Prevention Strategies Are Needed Throughout Early Childhood in Low-income Preschool Children

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Objective To assess anemia incidence and persistence in low-income preschool children in the United States.

Study design Using 2000 to 2004 data from Center for Disease Control and Prevention’s Pediatric Nutrition Surveillance System, we constructed 4 cohorts. Children in each cohort had a baseline hemoglobin measurement at either age 12 ± 2 months (n = 583,149), 18 ± 2 months (n = 399,223), 24 ± 2 months (n = 382,605), or 36 ± 2 months (n = 300,817) and a follow-up hemoglobin measurement 12 ± 2 months later, when they were approximately 24, 30, 36, or 48 months old. Defining anemia as a hemoglobin level <11.0 g/dL (<24 months old) or hemoglobin <11.1 g/dL (≥24 mo), we calculated anemia incidence and persistence in each cohort and used multiple logistic regression to identify associated factors (race, sex, birthweight, height, weight, breastfeeding).

Results Anemia incidence declined with age. Persistence remained approximately 30%. In each cohort, 70% of follow-up anemia cases were incident. Compared with white children, black children had greater odds of incident anemia at each follow-up age (odds ratio [OR], 1.84-2.09), while Native American children had lower odds at 36 and 48 months of age (OR, 0.68, 0.65). Both Asian and black children had greater odds of persistent anemia than white children at each age (OR, 1.73-2.60).

Conclusions Most follow-up anemia in each cohort was incident, underscoring the importance of anemia prevention throughout early childhood in this population. Investigation of the causes of anemia is warranted. (J Pediatr 2007;150:422-8)

The prevalence of iron deficiency, iron deficiency anemia, and anemia has declined in US preschool children in the past 2 decades in association with increased iron intake,1-3 but the risk of these conditions remains high in low-income preschool children. Data from the third National Health and Nutrition Examination Survey (NHANES III) (1988-1994) revealed that 12% of 12- to 35-month-old children in the United States who were living below 130% of the poverty threshold were iron-deficient, and 5% had iron-deficiency anemia. This compares to 8% and 1%, respectively, in children living above 130% of the poverty threshold.4 The potential permanent cognitive and developmental consequences of iron deficiency in early childhood underscore the significance of this public health problem.5-7

Because of its low cost and feasibility of use in clinic settings, anemia is a frequently used indicator of iron deficiency, often used to monitor the risk of iron deficiency in low-income children at the state and local levels.8 Although anemia prevalence is most often reported,4,9-13 knowledge of anemia incidence and identification of associated ages and child characteristics could help better target anemia-prevention strategies. The longitudinal design necessary for a study of anemia incidence would also permit evaluation of anemia persistence. In the context of screening, if most anemia cases in older preschool-aged children are persistent, rather than incident or new cases, screening these children becomes less informative. The need for enhanced treatment and follow-up becomes apparent.

Although several studies have provided cross-sectional or prevalence estimates of anemia in low-income preschool children,4,9-13 few have assessed anemia longitudinally. Altucher et al14 evaluated hemoglobin change, but not anemia incidence, from age 1 to 2 years in children enrolled in the Massachusetts Special Supplemental Nutrition Program for Women, Infants, and Children (WIC). Kahn et al15 did report anemia incidence and persistence in young children aged 6 to 59 months attending 3 Chicago WIC clinics, but

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The findings and conclusions in this report are those of the authors and do not necessarily represent the CDC.

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the follow-up period for each child was not uniform, making accurate estimates of the ages of greatest anemia risk difficult.

We assessed 1-year anemia incidence and persistence in low-income preschool children in the United States at approximately 24, 30, 36, and 48 months of age in 4 cohorts of children from 32 states, 4 tribes, and 2 territories who had data submitted to the Centers for Disease Control and Prevention’s (CDC) Pediatric Nutrition Surveillance System (PedNSS). In each cohort, we also assessed associated anemia risk factors, including birth weight, sex, race/ethnicity, breastfeeding status, and baseline height and weight.

METHODS

The CDC established PedNSS in 1973 to monitor the health and nutritional status of low-income children throughout the United States. US states, territories, and tribal agencies annually submit health data collected from children as old as 60 months who are enrolled in federally funded programs to the CDC for compilation, data analysis, and report generation. CDC evaluates the completeness and quality of all data submitted to PedNSS and provides each contributing state with a data quality report detailing missing and miscoded data and biologically implausible values.

More than 90% of the data for this study came from children attending WIC clinics.

Data Set Compilation

Thirty-two states, 6 tribes, Puerto Rico, and Washington, DC, submitted data to PedNSS continually for each year between 2000 and 2004 and were eligible for inclusion in this study. A data-processing contractor aggregated the master files containing all PedNSS clinic visits from 2000 to 2004 from each state and then concatenated the state data sets to form 1 data set. The contractor then matched visits for each child by state, sex, race/ethnicity, date of birth, and state-assigned identifier before replacing this identifier with a sequential child-specific number (1 through the total number of children in the multiple-state data set). Because original identifiers were stripped from the data set before transmission to the researchers, this study was determined to be exempt from human subjects review at CDC.

Description of Cohorts

From the multiple-state data set, we excluded visits with missing or invalid hemoglobin measurements (hemoglobin level <8 g/dL or >17 g/dL) and visits with missing altitude information. We then constructed 4 non-mutually exclusive cohorts of children by selecting children who had a baseline clinic visit at a designated age (12, 18, 24, or 36 ± 2 months) who also had a follow-up visit 12 ± 2 months after the baseline visit, at approximately 24, 30, 36, or 48 months old, respectively. We selected these baseline ages because they corresponded with peaks in clinic visit frequency, and we chose the 12-month follow-up period to capture the most return clinic visits by accounting for the WIC rule that stipulates that children with negative results on anemia tests at a given WIC certification screening need only be re-screened 12 months later. To be eligible to be in a given cohort, each child had to have the opportunity for a complete follow-up period by the end of 2004.

For each cohort, we began with all children who had a baseline hemoglobin measurement in the designated age range (12 ± 2 months: 2,056,407; 18 ± 2 months: 1,349,061; 24 ± 2 months: 1,134,527; 36 ± 2 months: 853,280). We then excluded any child who had >1 visit during the 4-month baseline range (<2% of excluded children for each cohort), any child who had no follow-up visit after baseline (46%-59% of the excluded children for each cohort), any child who did not have a follow-up visit 12 ± 2 months after baseline (38%-52% of the excluded children for each cohort), and any child who had >1 visit 12 ± 2 months after baseline (<2% of excluded children for each cohort). After these exclusions, the final cohort sample sizes were: 12 ± 2 months: n = 583,149; 18 ± 2 months: n = 399,223; 24 ± 2 months: n = 382,605; 36 ± 2 months: n = 300,817. Although the cohorts were not mutually exclusive, approximately 60% of children in the 12-month-, 18-month-, and 36-month-baseline age cohorts were only in 1 cohort, but 35% of children in the 24-month-baseline age cohort were only in that cohort. This lower percentage in the 24-month cohort is perhaps caused by this cohort having the potential to include both children being re-certified after a 12-month visit and children who would be certified again at 36 months old.

In general, excluded children were similar to included children in sex, age, and birth weight. However, in each cohort, a greater percentage of children who were excluded for having a late follow-up visit (ie, after the 12 ± 2-month interval) were Hispanic (31.9%-39.4%) compared with children included in the final cohorts (23.4%-25.9%). Most children (68.2%-79.4%) who were excluded for not having a follow-up visit in the 12 ± 2-month interval had a visit before this interval. These children with early follow-up visits had a higher prevalence of anemia and a lower mean hemoglobin level at baseline (12 months: 18.2%, 11.9 g/dL; 18 months: 19.2%, 11.9 g/dL; 24 months: 26.2%, 11.8 g/dL; 36 months: 23.8%, 11.9 g/dL) than children in the final cohorts, whose mean hemoglobin level and prevalence of anemia were similar to those of children excluded for having a late follow-up visit or for having no follow-up visit at all (data not shown).

Anemia

The HemoCue hemoglobinometer (HemoCue AB, Angelhom, Sweden) was used to measure hemoglobin concentration, with a standardized protocol. Although >80% of states measured hemoglobin values in the health clinic, some clinics in some states referred children to a physician’s office or another laboratory for hemoglobin value measurement. Because hemoglobin value measurements sent in by
these clinics may have been measured with a different instrument or at a slightly different age than the date of visit to the original health clinic, we calculated anemia incidence and persistence both including these states and also excluding these states. Hemoglobin data collected at the state level was checked at CDC for miscodes and biologically implausible values. We defined anemia according to CDC guidelines: <24 months old: hemoglobin concentration <11.0 g/dL; ≥24 months old: hemoglobin <11.1 g/dL. Because our sample included children from different geographic areas, we adjusted hemoglobin values for altitude, according to CDC guidelines.

Additional Assessments

Recumbent length (children <24 months old) or standing height (children ≥24 months old) was measured to the nearest 0.1 cm or 1/8 inch with a standardized protocol. Weight was measured to the nearest 0.1 kg or 1/4 pound with a pediatric scale or other beam balance scale. We calculated height-for-age and weight-for-height values with the CDC SAS software program, which calculates anthropometric measurements and applies the 2000 CDC growth charts to obtain percentile values. Clinic staff obtained birth weight data from maternal or hospital report and breastfeeding information from maternal report. All records were entered onto a standardized paper form or into an automated computer system in the clinics. Once the records were computerized at the state level, they were transferred to the CDC for inclusion in the PedNSS database.

Statistical Analysis

We stratified our analysis first by cohort and then by baseline anemia status. A follow-up anemia case in children who were not anemic at baseline represented an incident anemia case, and a follow-up anemia case in children who were anemic at baseline represented a persistent anemia case. We then divided the total number of follow-up anemia cases (incident cases + persistent cases) in each cohort by the total number of children in that cohort to calculate the prevalence of anemia at follow-up.

For each cohort, we evaluated unadjusted anemia incidence and persistence by anemia risk factor category, including: sex (male, female), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Native American, Asian/Pacific Islander, other [including Southeast Asian refugees]), birth weight category (<2500 g, 2500–4000 g, >4000 g), baseline height and weight (<5th percentile, 5th–94th percentile, ≥95th percentile for height-for-age and weight-for-height), and breastfeeding history (ever, never). WIC requires birth weight data to be collected only for children aged 12 months and younger. Accordingly, we evaluated this anemia risk factor only in the youngest cohort. Because our very large sample sizes rendered P values meaningless, we compared the magnitude of the difference in incidence and persistence rates in anemia risk-factor categories.

We then used multiple logistic regression to identify factors associated with incident and persistent anemia at each follow-up age. For these models, we restricted analysis to include only those children with complete race, height, weight, birth weight (for the youngest cohort), and breastfeeding data. We modeled breastfeeding history both dichotomously (ever, never) and by duration category (0 months, <3 months, 3–5 months, ≥6 months), but found the ever/never variable to have a stronger association with both incident and persistent anemia. Again, because of our very large sample size, we evaluated relative, rather than statistical, significance and considered an odds ratio (OR) <0.80 or >1.25 to represent an important association. SAS software version 9.1 (Cary, NC) was used for all analyses.

RESULTS

The prevalence of anemia at baseline declined with age (Table I). Each cohort consisted primarily of minority children, with the racial distribution being approximately 45% white, 25% black, and 25% Hispanic. Exclusion of the states with some clinics that measured hemoglobin with an instrument other than HemoCue did not change our incidence or persistence rates. Accordingly, we included all states in the final analysis.

Anemia incidence decreased with age, from 13% at 24 months to 7% at 48 months (Table II). In children with incident anemia, the mean hemoglobin concentration de-

<table>
<thead>
<tr>
<th>Table I. Baseline characteristics by cohort</th>
<th>Age at baseline*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 months</td>
</tr>
<tr>
<td>n</td>
<td>583,149</td>
</tr>
<tr>
<td>Age, months†</td>
<td>12.3 (0.7)</td>
</tr>
<tr>
<td>Hb, g/dL‡</td>
<td>11.9 (1.0)</td>
</tr>
<tr>
<td>Anemic, %†</td>
<td>15.7</td>
</tr>
<tr>
<td>Sex, %‡</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>50.6</td>
</tr>
<tr>
<td>Female</td>
<td>49.4</td>
</tr>
<tr>
<td>Race, %‡</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>44.4</td>
</tr>
<tr>
<td>Black</td>
<td>24.0</td>
</tr>
<tr>
<td>Hispanic</td>
<td>25.4</td>
</tr>
<tr>
<td>Native American</td>
<td>1.9</td>
</tr>
<tr>
<td>Asian</td>
<td>2.6</td>
</tr>
<tr>
<td>Other</td>
<td>1.6</td>
</tr>
<tr>
<td>Birth weight, %‡</td>
<td></td>
</tr>
<tr>
<td>&lt;2500 g</td>
<td>10.0</td>
</tr>
<tr>
<td>2500–4000 g</td>
<td>82.3</td>
</tr>
<tr>
<td>&gt;4000 g</td>
<td>7.8</td>
</tr>
</tbody>
</table>

*Age ± 2 months.
†Mean value (SD).
‡Less than 1% of children in each cohort had missing race data.
§A total of 12.8% of children had missing birth weight data.
increased approximately 1.5 g/dL from baseline to follow-up in each cohort, compared with a mean 0.1 g/dL increase in each cohort in children who were not anemic at baseline or follow-up (data not shown). Anemia persistence remained approximately 30% at each follow-up age (Table II). In children with persistent anemia, the mean change in hemoglobin concentration from baseline to follow-up was +0.1 g/dL, compared to a +1.7 g/dL mean change in children whose baseline anemia was resolved at follow-up (data not shown). Again, the hemoglobin concentration differences between baseline and follow-up were consistent across cohorts. Anemia prevalence at follow-up decreased with age, but approximately 70% of total follow-up anemia cases (incident plus persistent cases) in each cohort were incident. Within each cohort, anemia prevalence at follow-up was approximately equal to that at baseline (Tables I and II).

Unadjusted anemia incidence varied by ≤2 percentage points by sex, birth weight category (in children in the first cohort), height-for-age and weight-for-height percentile category, and breastfeeding history for each cohort (Table III; available at www.jpeds.com). However, anemia incidence was consistently highest in black children. Black children had an 8 percentage point greater anemia incidence than white children at 24 months. This discrepancy declined with age to 5.8 percentage points at 48 months. Asian/Pacific Islander children also had an elevated anemia incidence, particularly at 30 months. In contrast, beginning at 30 months, Native American children had the lowest incidence of anemia of all races.

These racial differences in anemia incidence remained when we controlled for other anemia risk factors. With regression analysis, we found that black children, Asian children, and children in the “other” race category all had significantly greater odds of incident anemia at 24, 30, 36, and 48 months of age, compared with white children, after adjusting for sex, birth weight (in the youngest cohort), height-for-age percentile, weight-for-height percentile, and breastfeeding history (Table III). However, the odds of incident anemia at 36 and 48 months of age were 32% and 35% lower, respectively, for Native American children than for white children, despite having similar odds of incident anemia at 24 and 30 months of age.

Unadjusted anemia persistence varied by <4 percentage points by sex, birth weight category, height-for-age or weight-for-height percentile category, and breastfeeding history for each cohort (Table IV; available at www.jpeds.com). Like incidence, persistence varied by race, being highest in black children and Asian/Pacific Islander children. Black children had the highest persistence at 24 and at 48 months of age, although Asian/Pacific Islander children had the highest persistence at 30 and 36 months of age.

With multiple logistic regression, we found that race also was the only risk factor significantly associated with anemia persistence. Children of all other races, with the exception of Native American children, had a greater odds of persistent anemia at 24 months than white children, after adjusting for sex, birth weight (in the youngest cohort), height-for-age percentile, weight-for-height percentile, and breastfeeding history. The odds of persistent anemia at 24 months of age were more than twice as great for black children as for white children. Asian children had the greatest odds of persistent anemia at 30, 36, and 48 months of age, although black children and children in the “other” race category continued to have greater odds of persistent anemia than white children.

Table II. Anemia incidence, persistence, and prevalence at follow-up

<table>
<thead>
<tr>
<th>Age at follow-up*</th>
<th>24 months</th>
<th>30 months</th>
<th>36 months</th>
<th>48 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children not anemic at baseline, n</td>
<td>491,721</td>
<td>347,235</td>
<td>333,908</td>
<td>270,940</td>
</tr>
<tr>
<td>Incident anemia cases, n</td>
<td>63,633</td>
<td>41,249</td>
<td>32,295</td>
<td>18,762</td>
</tr>
<tr>
<td>Anemia incidence, % †</td>
<td>12.9</td>
<td>11.9</td>
<td>9.7</td>
<td>6.9</td>
</tr>
<tr>
<td>Children anemic at baseline, n</td>
<td>91,428</td>
<td>51,988</td>
<td>48,697</td>
<td>29,877</td>
</tr>
<tr>
<td>Persistent anemia cases, n</td>
<td>26,886</td>
<td>16,710</td>
<td>14,213</td>
<td>7,475</td>
</tr>
<tr>
<td>Anemia persistence, % ‡</td>
<td>29.4</td>
<td>32.1</td>
<td>29.2</td>
<td>25.0</td>
</tr>
<tr>
<td>Total children in cohort, n</td>
<td>583,149</td>
<td>399,223</td>
<td>382,605</td>
<td>300,817</td>
</tr>
<tr>
<td>Total follow-up anemia cases, n§</td>
<td>90,519</td>
<td>57,959</td>
<td>46,508</td>
<td>26,237</td>
</tr>
<tr>
<td>Anemia prevalence at follow-up, %</td>
<td></td>
<td></td>
<td>15.5</td>
<td>14.5</td>
</tr>
</tbody>
</table>

*Age ≥ 4 months.
†Incident anemia cases/children not anemic at baseline times 100.
‡Persistent anemia cases/children anemic at baseline times 100.
§Incident plus persistent anemia cases.
||Total follow-up anemia cases/total children in cohort times 100.
were incident, or new, highlighting the importance of anemia prevention in this high-risk population.

Kahn et al. similarly reported a high incidence and persistence of anemia in 3 Chicago-area WIC clinics. In children 6 to 23 months old who were not anemic at their first clinic visit, 11.5% became anemic by their second visit, and an additional 7.5% became anemic by their third visit. More than 30% of the children who were anemic at their first visit were also anemic at their second visit, and of these, >37% remained anemic at their third visit. However, this study had much broader baseline age ranges than our study and did not have a uniform follow-up period, making comparability difficult. Nevertheless, this study, like ours, revealed that anemia remains problematic in low-income preschool children in the United States and that current prevention and treatment practices have not been fully successful.

In contrast with Kahn et al, we found that non-Hispanic black and Asian races were significant risk factors for both incident and persistent anemia. Although Kahn et al did not include Asian children in their analysis, a possible explanation for our discrepant results for black race and anemia is Kahn et al’s use of lower hemoglobin cutoff levels for anemia in black children (<24 months: <10.6 g/dL; ≥24 months: <10.7 g/dL). Although the hemoglobin distribution of the non-Hispanic black population is lower than that of the white population, the CDC does not recommend lowering the hemoglobin cutoff level for anemia in black children because the reason for the observed difference in distributions is unknown. Accordingly, we did not use a separate hemoglobin cutoff level for anemia in black children in our study.

Our finding that Asian/Pacific Islander children had elevated odds of follow-up anemia, in particular persistent anemia, was somewhat unexpected and perhaps points to the need for enhanced anemia treatment and follow-up in children in this race/ethnicity group. Additionally, a recent study reported that Asian children currently account for more than half the North American thalassemia cases in children younger than 10 years, perhaps explaining a portion of the higher anemia persistence in Asian/Pacific Islander preschool children that we observed in our study.

Our finding that Native American children had significantly lower odds of incident anemia than white children was also surprising because the United States Preventive Services Task Force included Native American children as “high-risk” for iron deficiency in its 1996 anemia screening guidelines. Although a recent study found that the prevalence of anemia in preschool-aged Alaska native children was more than twice the US national average, Alaska was not one of the states in our analysis, and consequently, no or very few Alaska native children would have been included in any of the cohorts. Concerted, ongoing efforts by Indian Tribal Organization WIC programs are perhaps responsible for the reduced incidence of anemia in Native American children that we observed. The 2004 PedNSS report similarly demonstrated improved anemia status in Native American children, finding that the prevalence of anemia in this group was the lowest of all race/ethnicity groups except white children.

Aside from race, no other previously identified anemia risk factor was meaningfully associated with anemia incidence or persistence in our study. However, it is possible that our lack of dietary intake or supplement use data could have obscured relationships between anemia risk factors and anemia incidence or persistence. For example, because iron supplementation, either through fortified formula or iron supplements, is recommended for low-birth weight babies, these children may have been more likely to have received iron supplements than children of normal birth weight, potentially attenuating an association between low birth weight and anemia incidence or persistence at 24 months of age.

An additional limitation is the selection bias inherent in studying a population in which >90% receive WIC benefits and are thus, by eligibility specifications, nutritionally at risk. Approximately 4 million children aged 1 to 5 years received WIC benefits each month of fiscal year 2004, and enrollment in WIC decreases with age. Thus, the longer a child remains on the WIC program, the less representative they are of the US population. We chose our design of 4 cohorts with specified baseline ages and follow-up periods to minimize this bias and enable calculation of age-specific estimates of anemia incidence and persistence. Although creating a cohort of children with 3 or 4 sequential visits would have avoided the partial overlapping of our cohorts, such a cohort itself would have increased selection bias by preferentially selecting those children whose continued enrollment in WIC perhaps reflected greater nutritional risk.

The children in each of our final cohorts had similar demographic and hematological characteristics to those children excluded for having no follow-up or for having a follow-up visit after the designated 12 ± 2-month window, which suggests that our final cohorts were representative of low-income children in the United States who were enrolled in federally funded health programs. In contrast, the lower baseline hemoglobin concentration and higher prevalence of anemia in children excluded for having a visit before the 12-month recertification period perhaps indicates that these children are at a particularly high nutritional risk.

Despite the partial overlapping of cohorts, our approach still permitted an accurate calculation of anemia incidence. Although incidence is traditionally used to indicate the proportion of new cases of a disease that occur during a specified period, the term also encompasses the frequency of any new health- or disease-related event. Certain health events, such as anemia, diarrhea, or fever, can occur repeatedly. Calculation of incidence for these events, then, relies on a clear definition of time. For each of our cohorts, we specified a baseline age and 12 ± 2-month follow-up age. Thus, a child who was not anemic at 24 months of age, but became anemic at 36 months of age represents an incident anemia case for that 12-month interval, regardless of past or future anemia status. Anemia persistence can be considered analogously.
Without additional measures of iron status, however, it is impossible to conclude that the anemia we detected reflected iron-deficiency anemia. Anemia, or low hemoglobin concentration, is not entirely sensitive or specific for iron deficiency, even in a high-risk population such as ours.4,11,12 Because anemia represents the final stage of iron deficiency, many children may be iron-deficient but not anemic.4,11,12,31-33 Conversely, children who are anemic may not be iron-deficient because anemia has numerous causes, including infection, other nutritional deficiencies, such as those of B vitamins and vitamin A, and hemoglobinopathies.34-36

Nevertheless, the positive predictive value of low hemoglobin concentration in detecting iron deficiency is maximized in populations in which iron deficiency is prevalent,9 and several recent studies suggest that iron deficiency and iron-deficiency anemia persist as significant public health problems in low-income children.10,31 Further, the extra cost, time, and blood required for additional iron status indicators, such as serum ferritin, erythrocyte protoporphyrin, or transferrin receptor, makes their routine use impractical for routine screening at this time.

Although the incidence of anemia declined with age, approximately two thirds of all anemia cases at each follow-up age were incident. The cause of this continued high proportion of new anemia cases is unknown and warrants further investigation. Additional investigation of the causes of persistent anemia in low-income preschool children in the United States is also warranted. When a child who is enrolled in the WIC program is identified as anemic, that child is referred to a physician for follow-up and treatment. How many children actually go to these referral visits is unknown, and the number may be limited by financial, transportation, or work constraints. Compliance to iron therapy in children who do attend referral visits is also unknown.

The anemia incidence and persistence that we calculated at each follow-up age were sizeable and surprising. Although the incidence, persistence, and prevalence of anemia declined with age, more than two thirds of follow-up anemia cases at each age were new, highlighting the importance of continuing anemia prevention strategies throughout early childhood in low-income preschool children, particularly in black and Asian children. Further investigation of the causes of anemia in these children is a critical next step in addressing this public health problem.

REFERENCES

Table III. Incidence of anemia by risk factors and age at follow-up

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*Age ± 4 months.
†Unadjusted incidence, %.
‡Adjusted odds ratio (95% CI).
Table IV. Persistence of anemia by risk factors and age at follow-up

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<td>1.02 (0.98-1.06)</td>
<td>31.2</td>
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*Age ± 4 months.
†Unadjusted persistence, %.
‡Adjusted odds ratio (95% CI).
Ethical and Legal Implications of Genetic Testing in Androgen Insensitivity Syndrome

JONATHAN S. BERG, MD, PHD, SHANNON L. FRENCH, MD, LAURENCE B. MCCULLOUGH, PHD, SOLEDAD KLEPPE, MD, VERNON R. SUTTON, MD, SHEILA K. GUNN, MD, AND LEFKOTHEA P. KARAVITI, MD, PHD

In many instances, the establishment of a genetic diagnosis in a patient reveals specific or potential genetic information about biologically related individuals. With the large number of genetic tests now available, all physicians must have a working understanding of ethical considerations involved in genetic testing. Here we report on an extended family consisting of numerous members who are likely affected with X-linked androgen insensitivity syndrome (AIS). Individuals with AIS have a 46,XY chromosome complement but are unresponsive to androgens because of mutations in the gene encoding the androgen receptor (AR), resulting in an undervirilized or completely female external phenotype. The proband in this case was identified at 2 months of age when the patient presented with an inguinal hernia that was found to contain testicular tissue. Family history revealed that several additional women in the extended family likely are affected with AIS. Because of the possible health risks associated with AIS, provision of genetic testing for other at-risk family members could be considered an ethical responsibility of the health care team. However, after careful consideration of the relevant ethical issues, we identified a surprising precedent related to the legal definition of sex, raising concerns about the legal implications of genetic testing in other family members.

CASE REPORT

The proband was a full-term, appropriate-for-gestational-age infant who had an inguinal hernia. The external genitalia were unambiguously female. With ultrasound scanning, bilateral hernias with solid structures having internal blood flow but no follicles or Fallopian structures, consistent with testicular tissue, were shown. On repair of the hernias, pathological examination of the tissue revealed seminiferous tubules with germ cell hyperplasia, no vas deferens, and presence of round ligament tissue. Subsequent genetic testing revealed a 46,XY chromosome complement, raising suspicion for AIS. Follow-up magnetic resonance imaging of the pelvis revealed absence of the uterus and ovaries and a blind vaginal pouch.

At the time of initial evaluation, the proband had a 22-month-old full sister and a 9-year-old maternal half-sister who were both healthy. A 4-generation pedigree found a significant bias toward female offspring in previous generations (Figure 1). One of the most striking features of the pedigree is that 10 of 11 individuals in the great-great-grandmother’s generation are female, 5 of whom are infertile and some of whom are known to have absent ovaries (not from oophorectomy), uteruses, or both, suggesting that they are also affected with AIS.

The diagnosis of AIS in our proband was confirmed by means of identification of a novel hemizygous nonsense mutation that is predicted to result in premature termination of coding in exon 1 of the AR gene. This mutation is downstream of the polyglutamine repeat in exon 1, and, therefore, the nucleotide numbering is dependent on the number of CAG repeats that are present (Q118X based on GenBank Accession #NM_000044). After discussing with the family the inheritance of AIS and weighing the possible health risks associated with it, we recommended testing the proband’s sisters. The 9-year-old half sister was found to have a 46,XX chromosome complement, but the 22-month-old sister was diagnosed with AIS on the basis of the finding of a 46,XY chromosome complement and magnetic resonance imaging that revealed no evidence of uterine or ovarian tissue and no prostate, seminal vesicles, or definite testes.

DISCUSSION

AIS, previously known as testicular feminization, is an X-linked recessive condition in which individuals with a 46,XY chromosome complement inherit an altered version of the AR gene. The range of genital phenotypes associated with mutations of the AR is...
Ethical and Legal Implications of Genetic Testing in Androgen Insensitivity Syndrome

AIS, the external genitalia are unequivocally female, although the vaginal introitus typically ends in a blind vaginal pouch. Depending on severity, reconstructive surgery may be needed to provide the means for normal sexual function. Physiologically, individuals with AIS generate testosterone and respond to hCG with an increase in testosterone production from the testes.8

One area of controversy in patients with intersex conditions is the relative importance of genetic, hormonal, and environmental factors in determining cognitive psychology and gender identity. Although the basis of sexual dimorphism in the brain is poorly understood, there are likely androgen effects and gene-specific effects.9,10 A newborn with PAIS and ambiguous genitalia requires urgent examination by a gender medicine team because the gender identity in these individuals is less uniform.11,12 This issue is beyond the scope of our discussion, and from here we will focus on individuals with CAIS. The available data suggest that virtually complete concordance exists between the fully female external phenotype and female gender identity in individuals with CAIS.12,13 Furthermore, rarely does CAIS pose an urgent concern about the sex of rearing because individuals with this condition may live for many years in the female sex, and diagnosis may not be made until childhood (in the case of an inguinal hernia), adolescence (because of amenorrhea), or adulthood (as part of an infertility evaluation). However, establishing the diagnosis of AIS can have a profound psychological impact because of the importance of sex in our sense of identity.

The known health risks associated with AIS include an increased risk of testicular neoplasms,14 which is reportedly greatest after puberty14 and can occur even in the elderly.15 Prophylactic gonadectomy generally is considered in the treatment of these patients, although no consensus guidelines exist about the most appropriate time for performing this procedure.16 In addition, androgens play an important role in regulating bone mineral density,17 and individuals with CAIS are at risk for osteopenia.18,19

We hypothesize that many of the women in this pedigree are at risk for being previously undiagnosed 46,XY females affected with AIS or 46,XX heterozygote carriers of the familial AR mutation who could have affected children with future pregnancies (Figure 1). Because of the risk of gonadoblastoma and other health concerns in individuals with AIS, our gender medicine team (composed of endocrinologists, geneticists, urologists, psychologists, and an ethicist) discussed whether extensive testing of other family members should be recommended.

Ethical Implications of Testing in AIS

Historically, one of the overriding ethical considerations in AIS has been the disclosure of the diagnosis of AIS to patients. An earlier approach of withholding information was based on a paternalistic assumption that physicians were
better able to determine what was in the patient’s best interest. Clearly, this approach has fallen from favor in medicine, and an ethical perspective on AIS disclosure discussing this issue was published recently. Following the ethics of informed consent, for an adult patient in whom an evaluation for AIS is being contemplated by the physician, there should be discussion of the nature of the testing to be performed and the possibility and implications of the diagnosis and genetic counseling before testing. When results are obtained, there should be immediate disclosure of the chromosome analysis and molecular tests and counseling about the significance of the results. Detailed genetic and psychological counseling provided by professionals with expertise in disorders of sexual development also should be part of the diagnostic and therapeutic process.

For minor children in whom a new diagnosis of AIS is made, discussion should be guided by the ethical concept of pediatric assent. Children should participate in decision-making commensurate with their developmental capacity. The goal is not that of consent, as in what an adult decides about medical care for herself, but to make a reasonable effort to ensure that the child understands the nature of her condition and the alternatives for managing it. Decisions about medical management will be made by the parents or guardians for the child, taking into account her beliefs and preferences.

The decision about when to initiate the assent process should be based on a careful clinical assessment by the physician in conjunction with the parents, focusing on the child’s developmental capacity to deal with ambiguity and uncertainty. When treatment can be postponed, and when the child appears to be developmentally unprepared to deal with diagnostic information, decision-making involving the child should be postponed until the child has achieved appropriate cognitive and affective development, preferably by the time of onset of puberty. To prevent distress and confusion in the absence of social support, the child should be informed of her condition before attending school health classes that include discussion of menstruation, and certainly before engaging in sexual activity, which may occur at a younger age than the parents anticipate. Parents should be encouraged and supported by the physician to accept the main result of the assent process, seriously engaging the child in decisions about her medical condition and treatment. Regardless of other concerns, the diagnosis should be disclosed by the time the child reaches the age of 18, whether or not she has symptoms. The patient also should be informed of the availability of support groups (eg, http://www.medhelp.org/ais).

Determination of carrier status is a somewhat different situation because the information would not change the immediate medical management in a child and provides information that a child may not be capable of fully understanding. This is the case for the 9-year-old half-sister of the 2 affected individuals. Because she has a 46,XX chromosome complement, she has a 50% chance to be a carrier of the AR mutation (Figure 2). Investigation of carrier status generally is delayed until an individual reaches adulthood or reproductive maturity and is then provided in the context of counseling about the risk of having an affected child (25% chance with each pregnancy for carriers of AR mutations) (Figure 2).

Another ethical issue that arises is the extent to which a physician is responsible for informing other members of the family that they may be at risk for a given condition or at risk for transmitting the condition to their offspring. In these situations, a conflict sometimes arises between the duty to maintain confidentiality and the duty to protect others from harm that could occur in the absence of their knowledge of clinically relevant information about themselves. The physician should ensure that the parents are well informed about relevant genetic and clinical information and should support them in the process of alerting extended family members of the need to seek medical evaluation. As a general principle, the harm to the child and family should be weighed against the potential harm to the extended family members remaining in ignorance. This ethical problem is best resolved by asking the parents to disclose their child’s condition to other members of the family and to ask those members to contact a physician. Most often, appropriate counseling of affected individuals or the parents of affected individuals can lead to
dispersal of information among other at-risk family members. However, in the event that patients or their parents refuse to divulge information to other family members (or no longer have contact with them), the caregiver should carefully document the discussions held with the family and continue to approach them about divulging this information to others at risk.

Legal Ramifications of Testing in AIS

As we consider whether to offer genetic testing to other at-risk family members in this pedigree, it is important to discuss the legal ramifications of making the diagnosis of AIS, central to which is the legal definition of “sex.” This question has received attention recently as legislatures and courts weigh in on the issue of marriage, and it is a subject of discussion among those in the medical and legal fields. In a 1999 decision potentially relevant to our patients (Littleton versus Prange), the Texas Fourth Court of Appeals nullified a transsexual woman’s marriage to her deceased husband and declared that she did not have legal grounds to sue as a widow in a wrongful death trial, after the defense argued that the marriage was invalid because she was born with a 46,XY chromosome complement. Despite having a female phenotypic appearance and living as a heterosexual female throughout her life, it was determined that because her chromosomes and original birth certificate were “male,” her marriage was invalid. Justice Karen Angelini wrote a concurring opinion pointing out the difficulty posed by intersex individuals about the definition of male and female sex because sex is determined by multiple “biological factors such as chromosomes, gonads, and genitalia” and that “real difficulties will occur if these three criteria are not congruent.” However, the court ruled in favor of a definition of sex based on chromosome complement alone. Although our diagnosis of AIS in the proband and her sister (and testing of others in the pedigree) could substantially benefit their health and well being by preventing medical complications and helping them to understand their condition, it also exposes them to unanticipated legal risks, potentially restricting their civil rights.

Littleton versus Prange and other recent cases that we have not discussed here raise concerns about the way in which the legal system defines sex. Although to our knowledge there has not yet been a case such as this involving an individual with an intersex condition, a strict interpretation of the law in which sex is at issue (eg, discrimination, choice in marriage, participation in sports, housing in higher education and the penal system), prompting an urgent need for legislatures to clarify the definition of sex to include the many aspects of sex, not simply chromosome complement or the initial birth certificate. Unlike race—in which a large number of potential variations exist and individuals are free to identify with the ethnic background(s) of their own choosing—sex identity is a binary concept, and most individuals associate with one of the two sexes and are perceived by others in the same way. However, because an individual’s sexual identity is ultimately personal, perhaps the simplest solution to the problem of defining sex would be to permit each adult individual to select his/her legal sex.

CONCLUSIONS

Our gender medicine team discussed androgen insensitivity extensively with the parents. We emphasized that individuals with CAIS are expected to have external anatomy, self-identities, and sex roles that are fully female. We answered questions about child rearing, the rationale for gonadectomy to prevent testicular malignancy, future hormone replacement and infertility issues, and the potential legal complications that might arise. We assessed the family’s understanding of the medical diagnoses and their comfort with female sex assignment. They were made aware of the availability of psychologists for support and assistance in discussing the diagnosis with the affected girls at an appropriate age. We also strongly encouraged the parents of the proband to disclose the information about AIS to extended family members so that they can seek genetic counseling and testing if desired. In the elderly relatives, we assume that the risk of gonadoblastoma is decreased (though not zero), and, therefore, the medical benefit of reaching a diagnosis of AIS is less of a factor. However, they certainly deserve an explanation for their own infertility if other physicians have not yet raised the possibility of AIS. Younger women in the pedigree at risk to be either affected or carriers should have this information in advance of making decisions about childbearing so they can receive adequate genetic counseling or obtain insight into possible infertility.

The larger societal implications posed by the existence of individuals with intersex conditions are much more complex and difficult to solve. Despite court rulings such as Littleton versus Prange, the true definition of “sex” is clearly more than a simple chromosomal determination, because there are complex molecular and hormonal pathways that ultimately guide the development of the internal and external genitalia and the person’s cognitive psychology and gender identity. This basic biological fact renders legal definitions of sex on the basis of chromosome complement (or, for that matter, any single measure of sex) problematic when incongruity can exist among chromosomal, genetic, hormonal, gonadal, genital, and psychological measures of sex. It concerns us greatly that by providing the standard of medical care, we might inadvertently jeopardize the civil rights of these individuals because of a shortsighted system of law. Therefore, it
is obvious that legislation protecting the rights of intersex individuals, in whom basic biological incongruities exist among various measures of sex, is essential. Pediatricians should be aware of this dilemma and be prepared to advocate on the behalf of these individuals—perhaps in the form of an amicus curiae from the American Academy of Pediatrics—just as we would for any other population with special needs.

REFERENCES

25. Littleton v Prange, 9 SW 3d 223 (4th Cir 1999).

Hearing Loss in Biotinidase Deficiency: Genotype-Phenotype Correlation

Children with symptoms of profound biotinidase deficiency with null mutations are more likely to have hearing loss develop than those with missense mutations, even if not treated for a period of time. Hearing loss appears to be preventable in children with null mutations if treatment is initiated soon after birth. (J Pediatr 2007;150:439-42)

Biotinidase deficiency is an autosomal, recessively inherited metabolic disorder in which both the biotinyl-hydrolase and transferase activities are deficient. Untreated individuals with profound enzyme deficiency (<10% of mean normal serum enzyme activity) usually exhibit neurologic and cutaneous manifestations, including seizures, hypotonia, skin rash or alopecia, developmental delay, conjunctivitis, and visual problems such as optic atrophy and hearing loss, accompanied by ketolactic acidosis and organic academia. Sensorineural hearing loss in biotinidase deficiency occurs in as many as 76% of individuals with symptoms. Although most of the symptoms improve with administration of pharmacologic doses of biotin (5-20 mg daily), hearing loss, visual abnormalities, and developmental delay are usually not reversible. Neonatal screening for biotinidase deficiency is conducted in many countries, but not in most parts of Turkey. Therefore children with the disorder in Turkey are usually ascertained after they have exhibited symptoms.

In this study, we report the clinical features, enzyme activities, mutation analyses, and hearing status of 20 children with profound biotinidase deficiency. The results of this preliminary study indicate the first genotype-phenotype correlation of the type of mutation with hearing loss.

METHODS

This study was performed in children ascertained at Hacettepe University Faculty of Medicine from 1996 to 2005. A total of 20 children with profound biotinidase deficiency participated and were tested in the Audiology Unit. The study was approved by the Ethical Committee of the University (no. HEK 05/94-5). All children were being treated with biotin at the time of study. After a routine otolaryngologic examination, audiologic (pure tone air conduction threshold, speech awareness threshold, and speech reception threshold), impedancemetric, auditory brainstem response, and otoacoustic emissions measurements were performed on all subjects.

Serum biotinidase activities were determined as described. Mutation analyses were performed on isolated DNA samples as described previously. The samples were obtained with signed consent.

RESULTS

Clinical characteristics, enzyme activities, mutation results, and hearing status of the 20 children with profound biotinidase deficiency are summarized in Table I. With the exception of 1 child, all subjects were the product of first-degree consanguineous marriages. The nutritional data obtained from these families did not indicate any differences in diet or the amount of foods containing biotin. Seventeen children were diagnosed because they exhibited symptoms, such as convulsions, dermatitis, and alopecia with lactic acidosis, whereas the remaining 3 children were diagnosed because an older sibling was diagnosed with the disorder. Patient 20 first exhibited symptoms, including respiratory
Table I. Demographic, biochemical, molecular, and auditory status of children with profound biotinidase deficiency

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Consanguinity</th>
<th>Biotinidase activity (U/L)*</th>
<th>cDNA (NM_000060) base change†</th>
<th>Protein effect</th>
<th>Age of diagnosis (mo)</th>
<th>Time from the onset of symptoms to diagnosis (mos)</th>
<th>Hearing status</th>
<th>Clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>F</td>
<td>Yes</td>
<td>0.5</td>
<td>98-104Del7ins3</td>
<td>Frame shift</td>
<td>2</td>
<td>1</td>
<td>Profound hearing loss</td>
<td>Alive</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>Yes</td>
<td>0</td>
<td>171T&gt; G</td>
<td>57Y&gt;stop</td>
<td>3.5</td>
<td>2</td>
<td>Profound hearing loss</td>
<td>Alive</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>Yes</td>
<td>0</td>
<td>171T&gt; G</td>
<td>57Y&gt;stop</td>
<td>3</td>
<td>2</td>
<td>Profound hearing loss</td>
<td>Alive</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>Yes</td>
<td>0.24</td>
<td>171T&gt; G</td>
<td>57Y&gt;stop</td>
<td>4</td>
<td>3</td>
<td>Profound hearing loss</td>
<td>Alive</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>Yes</td>
<td>0</td>
<td>1612C&gt; T</td>
<td>538R&gt; C</td>
<td>180</td>
<td>120</td>
<td>Profound hearing loss</td>
<td>Alive</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>Yes</td>
<td>1.5</td>
<td>98-104Del7ins3</td>
<td>Frame shift</td>
<td>30</td>
<td>26</td>
<td>Profound hearing loss</td>
<td>Alive</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>Yes</td>
<td>0</td>
<td>171 T&gt; G</td>
<td>57Y&gt;stop</td>
<td>3</td>
<td>1</td>
<td>Severe hearing loss</td>
<td>Alive</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>Yes</td>
<td>0</td>
<td>194ins4</td>
<td>Frame shift</td>
<td>4</td>
<td>2</td>
<td>Severe hearing loss</td>
<td>Alive</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>Yes</td>
<td>0</td>
<td>98-104del7ins3</td>
<td>Frame shift</td>
<td>2</td>
<td>1</td>
<td>Moderate hearing loss</td>
<td>Alive</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>Yes</td>
<td>0</td>
<td>98-104del7ins3</td>
<td>Frame shift</td>
<td>3</td>
<td>1</td>
<td>Mild hearing loss</td>
<td>Alive</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>Yes</td>
<td>0</td>
<td>1493ins T</td>
<td>Frame shift</td>
<td>2</td>
<td>1</td>
<td>Mild hearing loss</td>
<td>Alive</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>Yes</td>
<td>0.08</td>
<td>235C&gt; T</td>
<td>79R&gt; C</td>
<td>11</td>
<td>1</td>
<td>Normal hearing</td>
<td>Alive</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>Yes</td>
<td>0</td>
<td>235C&gt; T</td>
<td>79R&gt; C</td>
<td>6</td>
<td>5</td>
<td>Normal hearing</td>
<td>Alive</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>Yes</td>
<td>0</td>
<td>643C&gt; T</td>
<td>215L&gt; F</td>
<td>54</td>
<td>6</td>
<td>Normal hearing</td>
<td>Alive</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>Yes</td>
<td>0</td>
<td>235C&gt; T</td>
<td>79R&gt; C</td>
<td>30</td>
<td>6</td>
<td>Normal hearing</td>
<td>Alive</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>No</td>
<td>0</td>
<td>587C&gt; G</td>
<td>196T&gt; R</td>
<td>30</td>
<td>20</td>
<td>Normal hearing</td>
<td>Alive</td>
</tr>
<tr>
<td>5‡</td>
<td>F</td>
<td>Yes</td>
<td>0</td>
<td>235C&gt; T</td>
<td>79R&gt; C</td>
<td>7</td>
<td>?symptoms</td>
<td>Normal hearing</td>
<td>Died</td>
</tr>
<tr>
<td>1‡</td>
<td>F</td>
<td>Yes</td>
<td>0.5</td>
<td>98-104Del7ins3</td>
<td>Frame shift</td>
<td>0.1</td>
<td>Nosymptoms</td>
<td>Normal hearing</td>
<td>Alive</td>
</tr>
<tr>
<td>2‡</td>
<td>M</td>
<td>Yes</td>
<td>0.48</td>
<td>98-104Del7ins3</td>
<td>Frame shift</td>
<td>0.1</td>
<td>Nosymptoms</td>
<td>Normal hearing</td>
<td>Alive</td>
</tr>
<tr>
<td>3‡</td>
<td>M</td>
<td>Yes</td>
<td>0</td>
<td>1493ins T</td>
<td>Frame shift</td>
<td>0.5</td>
<td>Nosymptoms</td>
<td>Normal hearing</td>
<td>Alive</td>
</tr>
</tbody>
</table>

*Mean biotinidase activity in serum is 7.1 nmol of p-aminobenzoate formed/min/mL (normal range 4.4 to 11).
†All individuals are homozygous for the designated mutation.
‡Treated immediately after birth because they had an older affected sibling.
difficulty, dysarthria, and hearing loss, at 15 years of age. Patient 5 was diagnosed because her older sibling was affected. This child did not have any clinical symptoms and had normal hearing.

Unfortunately, she died at age 13 months of juvenile myelomonocytic leukemia, but until her death, she was doing well with therapy. Clinical outcomes were excellent in the 3 children diagnosed soon after birth, whereas the children with symptoms exhibited psychomotor retardation and permanent neurologic deficits, such as optic atrophy, epilepsy and hearing loss.

All children had normal otolaryngologic findings. Hearing loss was present in 11 of 20 children (55%). In this group, 5 children had profound hearing loss, 3 had severe hearing loss, 1 had moderate loss, and 2 had mild hearing loss. Four of these children were already wearing bilateral hearing aids, and 7 were advised to use bilateral hearing aids after testing.

In Table II, the mean age of diagnosis, the mean age of onset of symptoms, and the mean time from the onset of symptoms to time of diagnosis were compared in children with hearing loss and those with normal hearing. No statistically significant differences were found between children with hearing loss and those with normal hearing with respect to any of these variables.

The most striking result was that all children with symptoms and with hearing loss were homozygous for mutations that resulted in the absence of enzyme protein (null mutations), whereas the children with symptoms and with normal hearing were all homozygous for missense mutations that were expected to result in a defective but altered enzyme protein. Patient 20 is considered as having a null mutation. Moreover, the 2 children with null mutations all had normal hearing. Nonetheless, the children were homozygous for null mutations. On the basis of the genotype similarity with the children with symptoms and with null mutations, we would have expected them to have hearing loss. Children with normal hearing and with hearing loss were followed up for 1 to 5 years after the initial studies. Subsequent testing did not reveal any changes in the auditory thresholds in either group.

### DISCUSSION

Although some of the individuals with null mutations had detectable enzyme activity, this was most likely due to the variability in measuring enzyme activity. It is also possible that the missense mutations result in a defective enzyme with some slight residual biotinyl-hydrolase or transferase activity.

Because of the high frequency of consanguinity in Turkey, almost all children were the products of consanguineous mating, and all of the children were homozygous for specific mutations known to cause profound biotinidase deficiency. Homozygosity in our population afforded us the unique and excellent opportunity to unambiguously evaluate the correlation between the type of mutation and hearing loss. This is unlike other studies in which the children with hearing loss or normal hearing are usually compound heterozygotes for various null and missense mutations, thereby complicating the interpretation of the relationship of specific mutations with clinical features.

The 11 children with symptoms who were homozygous for null mutations all had hearing loss of varying severity, whereas the 6 children with symptoms who were homozygous for missense mutations all had normal hearing. Moreover, the 3 children with null mutations who were treated soon after birth had normal hearing.

A previous study of 33 children with symptoms of profound biotinidase deficiency in the United States revealed that about two thirds had some degree of hearing loss. In the group of 26 children with hearing loss, only 4 were homozygous for null mutations, and 12 were compound heterozygous for 2 null mutations. Although most of the remainder of the children with hearing loss had at least 1 null mutation, some had missense mutations, and several made enzyme protein as determined by the presence of cross-reacting material to antibody to normal biotinidase. Of the 8 children with normal

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**Table II. Comparison of age of onset, age of diagnosis and the time between onset of symptoms to the time of diagnosis of children with profound biotinidase deficiency with hearing loss and with normal hearing**

<table>
<thead>
<tr>
<th></th>
<th>Onset of symptoms (mo) mean (range)</th>
<th>Age of diagnosis (mo) mean (range)</th>
<th>Time from onset of symptoms to time of diagnosis (mo) mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children with hearing loss</td>
<td>6.9 (1-60)</td>
<td>21.5 (2-180)</td>
<td>14.5 (1-120)</td>
</tr>
<tr>
<td>Children with normal hearing</td>
<td>18.6 (1-48)</td>
<td>15.4 (0.1-54)</td>
<td>7.6 (1-120)</td>
</tr>
</tbody>
</table>

No significant differences ($P < .05$) were found between the children with hearing loss and those with normal hearing with respect to each of the above variables using the Mann-Whitney test.

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Hearing Loss in Biotinidase Deficiency: Genotype-Phenotype Correlation

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hearing, only 1 was homozygous for a null mutation, and 6 had at least 1 null mutation. Three of the children in this group had no detectable cross-reacting material. Considering the results of both studies, homozygosity or compound heterozygotes for 2 null mutations appears to increase the risk that a patient with symptoms will have hearing loss. Conversely, children with symptoms and with a single null mutation and a missense mutation and those with 2 missense mutations may be more likely to have normal hearing but could still have hearing loss develop. Other factors that may contribute to the development of hearing loss still remain to be determined.

Our study shows the power of interpreting genotype-phenotype correlations in populations where the individuals are homozygous for specific mutations. These preliminary results indicate that children with symptoms and with 2 null mutations are more likely to have hearing loss develop than those with missense mutations. Moreover, those with missense mutations who have symptoms develop may be spared hearing loss even if they are not diagnosed and treated for a period of time. Once hearing loss occurs, it seems to be irreversible despite biotin treatment, although treatment appears to prevent progression of the hearing loss. In addition, biotin treatment immediately after birth appears to prevent hearing loss in children with null mutations. Further studies of children with biotinidase deficiency are needed to confirm these relationships. The study further indicates that biotinidase deficiency should be considered in all children with sensorineural hearing loss, and all newborns should be screened for this readily treatable disease.

REFERENCES

Pyruvate Kinase (PK) Deficiency in Newborns: The Pitfalls of Diagnosis

SERGE PISSARD, MD, PHD, MARIANE DE MONTALEMBERT, MD, DORA BACHIR, MD, ISABELLE MAX-AUDIT, MD, PHD, MICHEL GOossENS, MD, HENRI WACHMAN, MD, PHD, and BRIGITTE BADER-MEUNIER, MD

Pyruvate kinase (PK) deficiency is asymptomatic in heterozygotes, but it can lead in homozygous neonates to a severe neonatal hemolysis, sometimes life-threatening. We report five cases, with a 1- to 17-month delayed diagnosis, highlighting the need to measure PK activity in neonates and parents in case of an hemolysis at birth. (J Pediatr 2007;150:443-5)

Postnatal adaptation results in rapid change in hemoglobin (Hb) composition and amount. Normally, Hb falls from a high value in term newborns (15.0-22.5 g/dL) to 11.0 g/dL at 2 months of age and normalizes to 13.0 g/dL after 6 years of age because of increased erythropoietic activity. When anemia (Hb <13.0 g/dL in a term baby) is observed at birth, four major etiologies have to be considered: acute hemolysis because of immune incompatibility (ABO or RH group), hereditary spherocytosis, and α-thalassemia and red blood cell (RBC) enzymatic deficiencies, mainly glucose-6-phosphate-dehydrogenase (G6PD) and pyruvate kinase (PK-R).1

PK-R deficiency (EC: 2.7.1.40 and OMIM: 266200) is the most frequent enzyme abnormality of the Embden-Meyerhof pathway.2 In neonates, homozygous or compound heterozygous genotypes may cause clinical patterns, ranging from extremely severe anemia to moderate jaundice. We report five cases of neonatal anemia caused by severe PK deficiency in Caucasian families. Diagnosis was delayed from 1 to 17 months with sometimes unnecessary investigations. These cases illustrate the wide heterogeneity of clinical presentation and the need to include the measurement of PK activity in the evaluation of anemic newborns.

METHODS

Sampling for studies was done after that informed consent was obtained.

The RBC indices were measured by routine procedures. Enzymatic activities for PK and G6PD were measured.3 DNA was prepared from blood using Nucleon BACC3® (Ref. 8512; Amersham Biosciences, Uppsala, Sweden). The first step of our strategy is a restriction fragment length polymorphism analysis for the two most frequent mutations, both located in exon 11 (R510Q and R486W). In case of a negative result, the full sequence is scanned by denaturating high-performance liquid chromatography, and the abnormal region is sequenced.5

RESULTS

In all parents of the probands, except for the mother of patient 4, PK activity was about half of normal, compatible with a heterozygous deficiency (Table). Because of transfusion, PK activity was not measured in patients 1 and 4. In patients 2, 3, and 5, a severe PK deficiency was assessed (<2 UI/g Hb). G6PD activity was in the normal range for all samples.

Case 1

Monozygotic twins, born prematurely to healthy parents, presented with severe anemia, hepatosplenomegaly, and marked jaundice. One infant died a few minutes after birth. The other infant received 60 mL packed RBC because of an Hb of 3.4 g/dL. There was no fetomaternal immunization against erythrocyte antigens, the direct antiglobulin test was negative, and no spherocytes were observed. The total bilirubin level was increased (134 mg/L). At 6 weeks of age, anemia (Hb 6.4 g/dL) was associated with mild
jaundice and splenomegaly, and the reticulocyte count was 82 × 10^9/L. Enzyme activities could not be studied because of transfusions. Bone marrow examination showed an increased proportion of erythroblasts (50.8%) with nonspecific dyserythropoiesis. One year later, enzyme activities assayed in the parents revealed that both carried a PK-R deficiency trait transmitted to the child, who was compound heterozygote (Table). The boy's anemia remained severe (Hb 5.0-6.5 g/dL), requiring 22 packed RBC transfusion units over a 3-year period, and hemochromatosis developed. The child is now 5 years of age with normal growth but needs a blood transfusion every 5 weeks. Iron overload is controlled by chelation.

Case 2
A 17-month-old girl, born of healthy unrelated parents, was referred because of unexplained persistent anemia. Her history revealed neonatal jaundice and two RBC transfusions, at 1 month of age (Hb 6.1 g/dL, reticulocyte count 110 × 10^9/L) and 3 months of age (Hb 6 g/dL, reticulocytes count 80 × 10^9/L). From 3 to 17 months of age, Hb was between 7 and 9 g/dL and the reticulocyte count was between 40 and 110 × 10^9/L. Finally, the erythrocyte enzyme activities were assayed revealing a PK deficiency, the girl being homozygous for the R510Q mutation. After the neonatal period, the patient became transfusion independent (Hb values between 9 and 11 g/dL). She is now 4 years of age with normal growth.

<table>
<thead>
<tr>
<th>Case 1</th>
<th>PK value IU/gHb</th>
<th>PKLR mutations*</th>
<th>Amino acid changes or splice changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband</td>
<td>Transfused</td>
<td>c.1436(G&gt;A) / c.1078(G&gt;C)</td>
<td>R479H / C360Y</td>
</tr>
<tr>
<td>Mother</td>
<td>2.9</td>
<td>c.1436(G&gt;A) / wt</td>
<td>R479H / nl protein</td>
</tr>
<tr>
<td>Father</td>
<td>3.4</td>
<td>c.1078(G&gt;C) / wt</td>
<td>C360Y / nl protein</td>
</tr>
<tr>
<td>Case 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proband</td>
<td>1.0</td>
<td>c.1529(G&gt;A) / c.1529(G&gt;A)</td>
<td>R510Q / R510Q</td>
</tr>
<tr>
<td>Mother</td>
<td>3.6</td>
<td>c.1529(G&gt;A) / wt</td>
<td>R510Q / nl protein</td>
</tr>
<tr>
<td>Father</td>
<td>3.8</td>
<td>c.1529(G&gt;A) / wt</td>
<td>R510Q / nl protein</td>
</tr>
<tr>
<td>Case 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proband</td>
<td>1.8</td>
<td>c.1456(C&gt;T) / c.1618+1(G&gt;C)</td>
<td>R486W / splice IVS-11 +1(G&gt;C)</td>
</tr>
<tr>
<td>Mother</td>
<td>3.4</td>
<td>c.1618+1(G&gt;C) / wt</td>
<td>splice IVS-11 +1(G&gt;C) / nl protein</td>
</tr>
<tr>
<td>Father</td>
<td>4.6</td>
<td>c.1456(C&gt;T) / wt</td>
<td>R486W / nl protein</td>
</tr>
<tr>
<td>Case 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proband</td>
<td>Transfused</td>
<td>c.1456(C&gt;T) / c.1178(A&gt;G) + c.375+10(G&gt;T)</td>
<td>R486W / N393S + splice IVS 4 +10(G&gt;T)</td>
</tr>
<tr>
<td>Mother</td>
<td>5.7</td>
<td>c.1456(C&gt;T) / wt</td>
<td>R486W / nl protein</td>
</tr>
<tr>
<td>Father</td>
<td>4.6</td>
<td>c.1178(A&gt;G) + c.374+10(G&gt;T) / wt</td>
<td>N393S + splice IVS 4 + 10(G&gt;T) / nl protein</td>
</tr>
<tr>
<td>Case 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proband</td>
<td>1.2</td>
<td>c.1529(G&gt;A) / c.1618+1(G&gt;C)</td>
<td>R510Q / splice IVS-11 +1(G&gt;C)</td>
</tr>
<tr>
<td>Mother</td>
<td>3.9</td>
<td>c.1529(G&gt;A) / wt</td>
<td>R510Q / nl protein</td>
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<tr>
<td>Father</td>
<td>2.9</td>
<td>c.1618+1(G&gt;C) / wt</td>
<td>splice IVS-11 -1(G&gt;C) / nl protein</td>
</tr>
</tbody>
</table>

*Numbering is per the system used in Zanella and Bianchi2; nucleotide numbering starts at position 40 of the reference cDNA sequence GenBank D13026, which is the “A” of the translation initiation codon.

Case 3
A 1-month-old girl was referred because of unexplained persistent and moderate anemia. She was born of healthy parents. Her history revealed a neonatal jaundice associated with moderate anemia at 4 days of age (Hb 12.7 g/dL, reticulocyte count 136 × 10^9/L). From 1 to 2 months of age, her Hb varied from 9.2 to 10.8 g/dL and her reticulocyte count from 117 to 150 × 10^9/L. The direct antiglobulin test was negative, and peripheral blood and bone marrow examinations were normal. At 4 months of age, enzyme assays revealed a PK deficiency in the proband and her parents (Table). Molecular analysis showed that she was compound heterozygote (Table). Now, at 2 years of age, she has chronic jaundice and a Hb of about 11 g/dL without transfusion.

Case 4
The third child of healthy parents was referred just after birth because of anemia (Hb 9.0 g/dL) and jaundice (total bilirubin level: 140 µmol/L at Hb). The reticulocyte count was 560 × 10^9/L. The direct antiglobulin test and erythrocyte membrane study (ektacytometry) were normal. The patient’s Hb levels remained stable for 10 days, then fell to 6.4 g/dL and led to the first transfusion. She was then transfused twice more, at 6 weeks (Hb 6.8 g/dL) and 4 months (Hb 6.4 g/dL, reticulocyte count 445 × 10^9/L). Between 4 and 9 months of age, she was pale without hepatosplenomegaly, and her Hb remained stable between 6.5 and 7.5 g/dL. Six months later, PK activity was assayed in the parents and child, revealing
Case 5

The first child of healthy parents was referred for neonatal distress, pallor, marked jaundice, and hepatosplenomegaly. The child had anemia that, despite reticulocytosis (699 × 10^9/L), worsened from an Hb level of 9.9 to 8.2 g/dL with jaundice (total bilirubin level: 145 μmol/L at 12 hours of age), and the child was transfused on day 3. Etiologic investigations were negative except for enzymatic measurements in the child and parents that revealed a PK deficiency. The child was transfused again on days 25 and 50. Molecular investigations revealed that he was a compound heterozygote (Table). At 1 year of age, the child's anemia increased, and he needed RBC transfusion every 2 months to maintain Hb values >7 g/dL.

DISCUSSION

We report five cases of PK deficiency with a neonatal presentation varying from extreme severity (cases 1 and 5) to intermediate severity (case 4) and mild severity (cases 2 and 3). Diagnosis was delayed for up to 17 months. Only cases 4 and 5 had high reticulocyte count (>500 × 10^9/L). Two children remain transfusion-dependent, the others tolerate an anemia between 8 and 11 g/dL. None required a splenectomy. These cases emphasize the heterogeneous presentation of PK deficiency in neonates. The degree of anemia varies widely from very mild or compensated to life-threatening with jaundice and needing exchange transfusion. The hematological features are common to other hereditary nonspherocytic hemolytic diseases. A rapidly declining Hb with a low reticulocyte count, related to the inability of these infants to have an appropriate erythropoietic response to anemia, is noted. Shortly after birth, erythropoiesis enters a hypoplastic phase because of a reduction in erythropoietin secretion, related to both the switch from hepatic to renal erythropoietin secretion and to the marked elevation in oxygen tension. Reticulocytosis is inconstant, and, when noted, it can indicate a large proportion of older improperly matured reticulocytes. On the basis of the biochemical and molecular consequences of the six different PKLR mutations found (Table), we could not predict the disease severity.

In summary, PK deficiency may lead to an anemia with various patterns in the first months of life. It should be investigated in newborns with unexplained persistent anemia and/or jaundice, even in absence of reticulocytosis. Because enzymatic activities in newborns may be difficult to interpret because of reticulocytosis, transfusion, or difficulties in sampling, study of the parents should be performed in the initial screening tests.

We are highly indebted to the physicians who referred the families to the laboratory, namely Dr D. Subtil, Dr F. Blanc, Dr A. Le Querrec, Dr J. J. Benichou, Dr L. Razafimanantsoa, and Dr D. Bachir, and to Marianne Carver, who improved greatly the English language of this article.

REFERENCES

Hospitalizations with Primary versus Secondary Discharge Diagnoses of Asthma: Implications for Pediatric Asthma Surveillance

DAVID G. BUNDY, MD, MPH

Asthma-related hospitalizations are sentinel events in pediatric asthma surveillance. Little is known about hospitalizations that are assigned a secondary discharge diagnosis of asthma (SDDA). The National Hospital Discharge Survey (NHDS) was analyzed to compare hospitalizations with primary versus secondary discharge diagnoses of asthma. Most hospitalizations with SDDA had primary diagnoses of respiratory origin. Surveillance systems tracking the hospitalization burden of pediatric asthma should consider including selected hospitalizations with a secondary diagnosis of asthma. (J Pediatr 2007;150:446-9)

Asthma is one of the most prevalent chronic diseases of childhood and a common cause of pediatric hospitalizations.1 Asthma-related hospitalizations are sentinel events because they represent high morbidity episodes for children and high cost episodes for payers. Surveillance of asthma-related hospitalizations is essential to assess secular trends in morbidity, quantify the burden of disease, and estimate the impact of improvement efforts on outcomes. Using existing data to track the burden of asthma and improve care depends on using case definitions that capture potentially preventable, asthma-related hospitalizations.

Most surveillance programs define asthma-related hospitalizations as those for which a primary discharge diagnosis of asthma (PDDA) was assigned.2 Excluding hospitalizations in which asthma was a secondary diagnosis increases the specificity, at the expense of sensitivity, of such asthma surveillance programs. Little is known about pediatric hospitalizations with secondary discharge diagnoses of asthma (SDDA). Hospitalizations with SDDA may be ones in which asthma complicates the primary diagnosis (eg, primary diagnosis pneumonia) or represents the “true” underlying diagnosis (eg, primary diagnosis respiratory failure). Further, it is unclear whether inverse diagnostic pairs (eg, PDDA/secondary diagnosis pneumonia vs primary diagnosis pneumonia/SDDA) represent distinct disease processes or merely variations in coding.

The purposes of this study were to determine: (1) the prevalence of pediatric hospitalizations with PDDA versus SDDA; (2) the most common primary diagnoses among children with SDDA; and (3) predictors of PDDA versus SDDA among children with co-occurring pneumonia diagnoses.

METHODS

Sample

Data from the National Hospital Discharge Survey (NHDS) 2003 were analyzed. The NHDS collects hospital discharge data from a national probability sample of nonfederal, non-institutional, short-stay hospitals in all 50 states and the District of Columbia; the 2003 sample consisted of 319,530 discharges from 426 hospitals. All discharges of persons >18 years of age (n = 251,706) and discharges identified as newborns (n = 34,094) were excluded yielding the final, unweighted sample of 33,730 pediatric, non-newborn hospital discharges.

Measures

NHDS data include up to seven discharge diagnosis codes. Hospitalizations were analyzed if either the first (primary) or second (secondary) discharge diagnosis was asthma.
The most prevalent nonasthma diagnoses among children with PDDA or SDDA were tabulated using International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) codes (Table I). Descriptive statistics for hospital discharges with PDDA and no secondary diagnosis, PDDA with secondary diagnosis “pneumonia,” and primary diagnosis “pneumonia” with SDDA were calculated. Differences in categorical variables were tested using the Pearson’s $\chi^2$ statistic; continuous variables were compared using simple linear regression in Stata version 8.2 (StataCorp, College Station, Tex).

The publicly available NHDS data set includes sample weights, which were applied to all calculations to generate nationally representative estimates. However, stratum and primary sampling unit identifiers are not included in the publicly available data, making exact calculations of sample errors impossible. NHDS data are completely de-identified and thus exempt from human subjects review.

### RESULTS

After applying sample weights, there were an estimated 306,461 pediatric hospitalizations (8.8% of all non-newborn, pediatric hospitalizations) with PDDA (75%) or SDDA (25%) in the United States in 2003. Among children 0 to 18 years of age with PDDA, the most common secondary discharge diagnoses were "none" (37%), pneumonia (16%), acute upper respiratory infection (6%), and acute respiratory distress/failure (5%) (Table II). Among children with SDDA, pneumonia (50%), acute bronchiolitis (8%), acute respiratory distress/failure (4%), and acute upper respiratory infection (3%) were the most common primary diagnoses. The distribution of associated non-asthma diagnoses varied somewhat by age category, with bronchiolitis, otitis media, and croup more prevalent in the infant and preschool age groups than in older children.

For both PDDA and SDDA, the most common co-occurring diagnosis was pneumonia. Twenty-four percent (n = 74,342) of all hospitalizations with PDDA or SDDA included a primary or secondary diagnosis of pneumonia (Table III; available at www.jpeds.com). Children with PDDA and secondary diagnoses of pneumonia were older, on average, than children with primary diagnoses of pneumonia and SDDA (4.8 vs 3.7 years, $P = .04$). Children with both asthma and pneumonia diagnoses were similar in terms of sex, race, and insurance status, independent of diagnosis order. Children with PDDA and secondary diagnoses of pneumonia were more likely to have documented receipt of nebulizer therapy than children with the reverse diagnoses (13% vs 5%, $P = .003$), though documented prevalence of nebulizer therapy was low overall. Other hospitalization and hospital characteristics varied little between the two asthma/pneumonia groups. In a logistic regression model adjusting for all factors in Table II, pneumonia was more likely to be documented among children with PDDA (odds ratio 1.5, 95% confidence interval 1.1 to 2.0).
III, only older age and documented receipt of nebulizer therapy were independently associated with an increased likelihood of being classified as PDDA/secondary diagnosis of pneumonia compared with primary diagnosis of pneumonia/SDDA (data not shown).

DISCUSSION

Hospitalizations with PDDA or SDDA make up 1 in 12 non-newborn, pediatric hospitalizations in the US. Surveillance systems that track only hospitalizations with PDDA capture approximately 75% of these hospitalizations. Among the 25% of hospitalizations not captured (ie, those with SDDA), the majority coexist with a primary diagnosis of respiratory origin.

There are several possible explanations for the increased likelihood of older children to receive PDDA with secondary diagnoses of pneumonia compared with younger children with asthma and pneumonia diagnoses. First, the diagnosis of asthma can be difficult to make in young children. Nearly 60% of children with lower respiratory tract illnesses associated with wheeze in the first 3 years of life no longer wheeze by 6 years of age. These so-called “transient early wheezers” are no more likely than children who never wheezed in infancy to wheeze at any subsequent time in childhood. Longer-term follow-up suggests that wheezing associated with viral respiratory infections in the first 7 years of life (the “wheezy bronchitis” subgroups in the Melbourne Asthma Study) characterizes a mild asthma phenotype with a high spontaneous remission rate. Given this natural history, physicians may be hesitant to assign a primary diagnosis of asthma to the hospitalization of a young child with a wheezing-associated respiratory illness. Second, clinicians may be more likely to assign PDDA to the hospitalizations of children who have been hospitalized previously for asthma. For children with asthma, their “asthma history” grows as they do; as a result, older children are more likely than younger ones to have previously been hospitalized for asthma and thus be assigned PDDA with subsequent hospitalizations.

Children with PDDA were more likely to have documented receipt of nebulizer therapy than those with SDDA. These data should be interpreted with caution, however. Even among children with PDDA and no secondary diagnosis (ie, “definite” asthma-related hospitalizations), receipt of nebulizer therapy was documented in only 20% of hospitalizations, suggesting significant underreporting may have occurred with this procedure code in NHDS. In addition, children may have received inhaled medications via metered dose inhaler, which would not be captured with NHDS procedure codes. It is unclear, therefore, whether children with PDDA and secondary diagnoses of pneumonia were more likely to receive nebulizer therapy or whether that diagnosis order was associated with a greater likelihood of reporting nebulizer use.

Asthma surveillance systems are designed for a variety of purposes; different case definitions for asthma-related hospitalizations are appropriate depending on the rationale for the surveillance. For example, surveillance systems targeting more definite asthma-related hospitalizations (ie, maximal specificity) could consider excluding hospitalizations with PDDA and secondary diagnoses of pneumonia. Conversely, systems designed to capture all potentially preventable asthma-related hospitalizations should consider including hospitalizations with SDDA and primary diagnoses of respi-

| Table II. Most common discharge diagnoses among children with primary or secondary discharge diagnoses of asthma (weighted counts and percents) |
| Age 0-18 years | Age <1 year | Age 1-5 years | Age >5 years |
| (n = 231,036) | (n = 26,980) | (n = 114,645) | (n = 89,411) |
| *(None)* | 37% | *(None)* | 26% | *(None)* | 38% | *(None)* | 39% |
| Pneumonia | 16% | Pneumonia | 15% | Pneumonia | 18% | Pneumonia | 12% |
| Acute URI | 6% | ARD/ARF | 13% | Acute URI | 5% | Acute URI | 7% |
| ARD/ARF | 5% | Otitis media | 8% | ARD/ARF | 5% | ARD/ARF | 3% |
| Otitis media | 4% | Bronchiolitis | 7% | Otitis media | 4% | Dehydration | 2% |
| Dehydration | 3% | Dehydration | 6% | Dehydration | 2% | Atelectasis | 2% |

| Table III. Most common primary diagnoses among children with secondary diagnoses of asthma |
| Age 0-18 years | Age <1 year | Age 1-5 years | Age >5 years |
| (n = 75,425) | (n = 8,528) | (n = 41,942) | (n = 24,955) |
| Pneumonia | 50% | Pneumonia | 46% | Pneumonia | 63% | Pneumonia | 31% |
| Bronchiolitis | 8% | Bronchiolitis | 35% | Bronchiolitis | 8% | Appendicitis | 6% |
| ARD/ARF | 4% | Croup | 6% | ARD/ARF | 4% | ARD/ARF | 3% |
| Croup | 3% | (No other diagnoses with more than 3 unweighted hospitalizations) | | Influenza | 4% | ARD/ARF | 2% |

ARD/ARF, acute respiratory distress/failure.

| Age 1-5 years | Age >5 years |
| (n = 41,942) | (n = 24,955) |
| Pneumonia | 63% | Pneumonia | 31% |
| Bronchiolitis | 8% | Appendicitis | 6% |
| ARD/ARF | 4% | ARD/ARF | 3% |
| Croup | 6% | ARD/ARF | 2% |
ratory origin, excluding bronchiolitis. For all case definition schemes, specificity will be lower at younger patient ages because of the difficulties in definitively diagnosing asthma in this age group. As a result, a reasonable surveillance strategy in circumstances where high specificity is essential would be to exclude all asthma-related hospitalizations (PDDA and SDDA) among children ≤3 years of age.

NHDS collects a limited number of variables for each hospitalization record; a limitation of this study, therefore, is an inability to characterize asthma-related hospitalizations in greater detail by including information such as discharge medications, chest x-ray results, physical examination findings, past medical history, and family history of asthma or atopy. As a result, it is impossible to know what criteria (eg, physical examination, chest x-ray, laboratory tests, pulmonary function tests) were used to assign diagnoses of asthma or pneumonia. In addition, the cross-sectional nature of the NHDS data precludes drawing conclusions regarding causality or directions of association for related variables. Finally, the absence of primary sampling unit and stratum identifiers in the publicly available NHDS data precludes exact calculation of standard errors. In the absence of these survey design parameters, calculated standard errors are likely to be smaller than design-based ones, increasing the possibility of type I error. Statistically significant differences, therefore, should be interpreted with caution.

Potentially preventable asthma-related hospitalizations are common among children. Hospitalizations with asthma and pneumonia diagnoses, independent of diagnosis order, are similar, suggesting that these hospitalizations may result from the same underlying disease process. Future research should further explore what factors, if any, distinguish hospitalizations with primary versus secondary diagnoses of asthma, and if these hospitalizations indeed result from the same underlying disease processes, what drives the assignment of a primary versus secondary diagnosis of asthma. Until such research is performed, data systems designed to improve the quality of care or for public health surveillance should consider tracking hospitalizations with SDDA and primary diagnoses of pneumonia, acute respiratory distress/failure, or acute upper respiratory infection, in addition to those with PDDA.

REFERENCES

### Table III. Comparison of discharge diagnosis pairs (weighted values)

<table>
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<tr>
<th>Parameter</th>
<th>Primary/Secondary discharge diagnosis</th>
<th>Asthma/(None) (n = 86,135)</th>
<th>Asthma/Pneumonia (n = 36,213)</th>
<th>Pneumonia/Asthma (n = 38,129)</th>
<th>P value*</th>
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<td>Age</td>
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<td>11</td>
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<td>Sex (%)</td>
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<td>Race (%)</td>
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<td>Insurance (%)</td>
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<td>Admitted via emergency room (%)</td>
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<td>49</td>
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<td>Discharge season (%)</td>
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<td>Length of stay</td>
<td>Mean (SE)</td>
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<td>&lt;1 day (%)</td>
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<td>2 days (%)</td>
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<td>3 or more days (%)</td>
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<td>Number of beds (%)</td>
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<td>500 or more</td>
<td>8</td>
<td>7</td>
<td>5</td>
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*P values reflect comparisons between the Asthma/Pneumonia and Pneumonia/Asthma groups. Asthma/(None) data presented for reference only.
Esophageal Stenosis Due to Vascular Ring

A 6-year-old boy was referred for follow-up of a ventricular septal defect, which was first diagnosed when he was a neonate. Cardiac catheterization performed at 7 months of age revealed an aberrant left subclavian artery arising from the right aortic arch. Recurrent respiratory infection that caused stridor and/or wheezing occurred in early infancy, but it was deemed not related to the cardiac defect because the pulmonary-to-systemic flow ratio was 1.6:1 and there was no pulmonary hypertension. With time, his respiratory symptoms remitted, and he was lost to follow-up for several years, while his family worried about his dysphagia. No heart murmur was auscultated, and the second heart sound was split and fixed. Electrocardiography showed incomplete right bundle-branch block. Ultrasonography indicated a small infundibular ventricular septal defect and right ventricular volume overload with intact atrial septum.

Contrast-enhanced 16-row multislice computed tomography was performed, and three-dimensional reconstruction (Figure 1) visualized anomalous connection of the right-upper pulmonary vein to the superior vena cava (A) and the right retroesophageal aortic arch with an aberrant left subclavian artery (B). It also revealed the esophagus breaking off above the aorta and running inferiorly from right to left (C). Esophagography confirmed severe esophageal stenosis (Figure 2; available at www.jpeds.com). The patient underwent surgical division of the ligamentum arteriosum, repair of the anomalous pulmonary venous connection, and direct closure of the ventricular septal defect through a median sternotomy.

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10.1016/j.jpeds.2007.01.053
Figure 2. Esophagogram in left anterior oblique view showing marked posterior indentation (arrow).
Definition of metabolic syndrome

To the Editor:

We read with interest the report by Chi et al comparing and contrasting definitions of metabolic syndrome used in pediatric samples. However, we wish to qualify the characterization of our study in this article. Our study compared and contrasted the prevalence of and demographic disparities in the World Health Organization and National Cholesterol Education Panel (NCEP) Adult Treatment Panel III definitions of metabolic syndrome in adolescents in the Princeton School District (PSD) Study. We did not create a “pediatric”-specific definition of metabolic syndrome. Instead, we extended the adult definitions to the adolescent age group. We felt this was important because there are discontinuities between the adult definitions and the various proposed pediatric definitions. For example, 14- to 18-year-old girls and 18-year-old boys can meet the adult NCEP cutoff point for waist circumference yet miss the 90% “pediatric” cutoff point. In addition, Table II indicates that our study population was NHANES, which is incorrect. Our data were drawn from the PSD Study.

Chi et al specifically mention a “consensus clinical definition.” We urge caution in clinically applying any of the proposed definitions to children of any age. There is continued debate about the very existence of this syndrome. The evidence base does not support the use of metabolic syndrome in “risk profiling” among children or drug treatment in this age group. Among adults with impaired glucose tolerance, metformin had no effect on prevalence of metabolic syndrome, whereas increased physical activity, which would be standard intervention for obesity, produced a significant decline. Both the American Diabetes Association and the American Heart Association agree that, in childhood and adolescence, prevention and treatment of obesity and vigilant attention to the early diagnosis of diabetes mellitus are currently the most evidence-based methods for addressing the clustering of cardiovascular risks metabolic syndrome represents.

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Reply

To the Editor:

We appreciate the comments of Dr Goodman and her colleagues. In response to their first comment, we apologize for the error in Table II and acknowledge that their study population was from the Cincinnati PSD, as cited in Table I. The data presented in their study used modified definitions of the adult metabolic syndrome (MS), specifically the ATP III and WHO criteria, to estimate prevalence of MS among adolescents. Although Goodman et al did not create original pediatric criteria for MS, they used “pediatric”-specific body mass index percentiles for measurement of obesity rather than established adult cutoff points for waist circumference. Several pediatric studies have validated body mass index as a reliable method for predicting body fatness in children. However, as pointed out by Goodman et al, different subgroups of obese children are identified with body mass index versus waist circumference. This disagreement in classification causes concern from an epidemiological and clinical standpoint.

We recognize the limitations in applying a universal definition of MS in children. However, the purpose of choosing particular criteria and their cutoff points is to identify the highest risk subset among obese children who may benefit from both lifestyle intervention and medical therapy. Current studies are now showing that “clustering” of multiple cardiovascular risk factors can accelerate atherosclerosis in young people. The Bogalusa Heart Study, a longitudinal community-based study of cardiovascular risk factors in children, revealed “tracking” of risk factor clustering related to MS from childhood to adulthood, especially among obese subjects. For instance, when Bao et al studied 1176 individuals aged 5 to 17 years during an 8-year period, they found that 61% of subjects who were initially in the highest quintile of their multiple risk index remained there 8 years later. Tracking of the multiple
risk index increased with higher ponderal index (weight/ height3).7

If the clinical end point of treatment of pediatric obesity is prevention of adult cardiovascular disease, then creating a standardized MS definition may help identify the highest risk children who need immediate medical intervention. Conversely, if the clinical end point is prevention of type 2 diabetes mellitus, then following the American Heart Association and American Diabetes Association recommendations for lifestyle intervention may be sufficient.11 Perhaps what is ultimately needed is a paradigm shift from MS to a composite “risk index” score that alerts patients and physicians of all potential health problems related to obesity. The “risk index” score would be composed of both physical and nonphysical attributes that are associated with obesity-related health problems. Risk score cutoff points would not be absolute but “continuous” and dependent on the targeted health problem.

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External validation of a scoring system to predict resistance to intravenous immunoglobulin

To the Editor:

Egami et al1 described a new scoring system that predicts resistance to intravenous immunoglobulin (IVIG) treatment in patients with Kawasaki disease (KD). Because no special equipment is needed, this score can be used in almost any children’s hospitals and pediatric services and should help guide clinicians in decision making regarding primary therapy. Although the scoring has acceptable predictive values in the authors’ database, they did not perform an external validation that involved completely new data to further assess the generalizability of their proposed scoring model. Therefore, we performed external validation using a database made up from clinical records of 750 consecutive KD patients treated with intravenous immunoglobulin (IVIG).2

Of the 750 KD patients, 137 were classified as having resistance to IVIG. We defined IVIG nonresponders as KD patients with persistent fever (≥37.5°C) that lasted more than 24 hours. In our database, the area under the receiver operating characteristics curve was 0.74 (95% confidence interval, 0.69 to 0.79) on Egami et al’s scoring. Using a cutoff point of ≥3 with this prediction score, we could identify IVIG nonresponders with 66% sensitivity and 72% specificity and coronary artery lesions with 70% sensitivity and 68% specificity. We also compared the score of Harada3 with the score of Egami et al. With the score of Harada, the area under the receiver operating characteristics curve was 0.62 (95% confidence interval, 0.56 to 0.67). IVIG nonresponders at a cutoff of ≥4 could be predicted at 69% sensitivity and 47% specificity; coronary artery lesions, at 46% sensitivity and 86% specificity. Although the score of Egami et al was superior to that of Harada, the prediction performance was not as high as the authors reported.

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10.1016/j.jpeds.2006.12.036

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REFERENCES
Reply

To the Editor:

We appreciate the comments regarding our article. However, some specific concerns are listed below.

First, the definitions of resistance to intravenous immunoglobulin (IVIG) are varied and have not been universally established.1–4 In Japan, almost all institutions define fever as $\geq 37.5^\circ\text{C}$, although $38.0^\circ\text{C}$ or $38.3^\circ\text{C}$ is accepted in the United States and other western countries. In addition, we defined patients who are resistant to IVIG as showing persistent fever or the lack of a decrease in C-reactive protein. Therefore our definition of resistance to IVIG is stricter than those used in other articles.

Second, we believe that the prediction of coronary artery lesion and that of IVIG resistance should be discussed separately. The score of Harada was intended to predict patients who would require IVIG to prevent coronary artery lesion, not IVIG resistance.5 In addition, patients who were treated with IVIG within 4 days of illness were rare in the decade since the score of Harada score was proposed.

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Finding a direction for pediatric assent

To the Editor:

A significant point has recently been made by Miller and Nelson in the ongoing debate about how and when to obtain pediatric assent.1 They appropriately note that assent, based on the framework of informed consent, holds children to the unreasonable ethical standard of autonomy rather than upon respect for persons.

There is another compelling reason to reject the reliance on informed consent as the model for pediatric assent. Apart from providing the wrong ethical framework, informed consent provides the wrong legal foundation as well. One only has to consider the legal evolution of informed consent to recognize its limitations.

The law of informed consent developed through a series of fragmented retrospective decisions resulting in the propagation of a series of complicated and poorly executed legal formalities. Where once medical judgment dictated the degree of medical disclosure, now legal requirements developed in the courts have been imposed on the doctor-patient relationship.

The notion of pediatric assent is currently developing as a malleable concept. Federal regulations require assent but fall short of either defining it or providing guidance as to how to obtain it.2 As we have seen from the example of informed consent, where there is a void left by standards and guidelines, courts will step in to fill the gap. The potential exists for a resulting patchwork of laws placing practitioners, researchers, and institutions in legal jeopardy while ultimately failing to uphold the underlying ethical principles.

Miller and Nelson rightly caution against the tendency to conflate assent with informed consent. Pediatric assent must not follow the same trajectory. Rather than waiting for the courts to fill the gaps left by federal regulations, a proactive approach is needed to define and shape pediatric assent in a way that fully embodies the underlying ethical values.

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<td>Stanley H. Inkelis, MD</td>
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<td>St. Louis, MO</td>
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The Journal of Pediatrics • April 2007
The American Board of Pediatrics announces recertifications in Gastroenterology.

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Randall S. Nederhoff, MD, Albuquerque, NM
Valerie Newman, MD, Portland, OR
Jeanne Sabine Nunez, MD, Laurel, MD
Chinwe C. Ofoor, MB, BS, Dix Hills, NY
Oluwemimi Oyetunji Okanlami, MB, BS, Granger, IN
Mary Elizabeth Olguin, MD, Tijeras, NM
Clayton J. Olney, DO, Dallas, TX
Manuel Ortega-Elias, MD, Coral Springs, FL
Jack Darren Owens, MD, Madison, MS
Mariann Pappagallo, MD, Fort Lee, NJ
Saroj J. Parida, MB, BS, Lilitiz, PA
Lance Alan Parton, MD, Setauket, NY
James W. Pellegrini, MD, West Boylston, MA
Manuel Angel Peregrino, MD, Roanoke, VA
Agnes R. Perenyi, MD, New York City, NY
Anand Peters, MB, BS, Nagercoil, Tamilnadu, India
Jean Ann Petershack, MD, Fort Worth, TX
Anthony Joseph Piazza, MD, Atlanta, GA
Maria Rodriguez Pierce, MD, San Antonio, TX
Nicolas F. M. Porta, MD, Chicago, IL
Cynthia Kay Powell, MD, Plano, TX
Mark Peter Preziosi, MD, Lakeland, FL
Marina Eugenia Quevedo-Riley, MD, Dallas, TX
Robyn Lee Rairigh, MD, Denver, CO
Vigna Rajan, MB, BS, Pasadena, CA
Ganapathy P. Rama, MB, BS, Mathews, NC
Nestor Alfonso Ramirez-Lopez, MD, Champaign, IL
Deepa Ranganathan, MD, BS, Whitter, CA
Raghavendra Bangrakuluro, MD, BS, New Brighton, MN
Shantanu Rastogi, MB, BS, Scarsdale, NY
Shobhana Ravikumar, MB, BS, Bolingbrook, IL
Vijaya-Kumar Konda Reddy, MB, BS, Morgantown, NC
Judy Ann J. Rigby, MD, Duluth, MN
Samuel Edward Rogers, MD, Sioux Falls, SD
Marta R. Rognol, MD, Morrisstown, NJ
Jerod M. Rone, MD, Dayton, OH
Dennis Alan Rosenblum, MD, Cedar Rapids, IA
Siamak Safar, MD, Westlake Village, CA
Shashi Kumar Sahdev, MB, BS, Dayton, NJ
Walid A. Salhab, MD, Dallas, TX
Gerardo de Jesus Sanchez-Montemayor, MD, Brownsville, TX
Bikramjit Singh Sangha, MB, BS, Sylmar, CA
Monisha Deodhar Saste, MD, Virginia Beach, VA
Sabitha S. Sehgal, MB, BS, Los Angeles, CA
Darshan S. Shah, MB, BS, Swainsboro, GA
Pradip Kumar Shah, MB, BS, Riverside, CA
Fauzia M. Shakeel, MB, BS, St. Petersburg, FL
Malini Jabeen Shareef, MB, BS, Lombard, IL
Renu Sharma, MB, BS, Jacksonville, FL
Calvin Tsing Shen, MD, Morristown, NJ
Khalid Akhtar Siddiqui, MB, BS, Rockford, IL
Israel David Simchowitz, MB, BCh, Houston, TX
Ronald Stuart Sklar, MD, Portland, OR
Robin S. Smith, MD, Salt Lake City, UT
Terry Muynihan Snidow, MD, Midlothian, VA
Michele Vickers Snyder, MD, Raleigh, NC
Gregory M. Sokol, MD, Indianapolis, IN
Alan Richard Spitzer, MD, Sunrise, FL
Shanthy Sridhar, MB, BS, Stony Brook, NY
Hari Bhaskar Srinivasan, MB, BS, Westmont, IL
Sudhir Sriram, MB, BS, Chicago, IL
Craig Killian Steiner, MD, College Station, TX
Joel David Stenzel, MD, Cumming, IA
Kelly A. C. Stuart, MD, Oilville, VA
Poornima Subramanyam, MB, BS, Regina, Saskatchewan, Canada
Kirin Noelle Suri, MB, BS, Scarsdale, NY
Dan Suskin, MB, BCh, Atlanta, GA
Denise M. Suttner, MD, San Diego, CA
Anthony John Tackman, MD, Cary, NC
Ajay J. Talati, MB, BS, Memphis, TN
Noel D. Tan, MD, Tampa, FL
Rosemarie Cecelia Tan, MD, Coronado, CA
Luciano Tanfulla, MD, Coral Springs, FL
Barron Keith Taylor, MD, Lynchburg, VA
Katarzyna Tesmer, MD, Santa Ana, CA
Dean T. Theophilopoulos, MD, Tarpon Springs, FL
Patti J. Thureen, MD, Denver, CO
Kajori G. Thureen, MB, BS, Clovis, CA
The American Board of Pediatrics announces recertifications in **Nephrology**.

Rajeev Agarwal, MB, BS, Phoenix, AZ
Samhar Ibrahim Al-Akash, MB, BS, Corpus Christi, TX
Emmanuel Sylvestre Lamug Apostol, MD, Indianapolis, IN
Mazen Yousef Arar, MD, San Antonio, TX
Carlton Matthew Bates, MD, Hilliard, OH
Donald L. Batisky, MD, Columbus, OH
Nancy Anne Bishop, MD, Lexington, KY
Richard Thomas Blaszak, MD, Little Rock, AR
 Lavjay Butani, MB, BS, Sacramento, CA
Anselmo E. Cepero-Akselrad, MD, Miami, FL
Aftab Shakir Chisti, MB, BS, Al-Khobar, Saudi Arabia
Katherine MacRae Dell, MD, Cleveland, OH
Vikas Ramnath Dharnidharka, MD, Gainesville, FL
Meiul P. Dixit, MB, BS, Winter Garden, FL
Francisco Xavier Flores, MD, Tampa, FL
Jens W. D. Goebel, MD, Cincinnati, OH
Stuart Leonard Goldstein, MD, Houston, TX
Paul C. Grimm, MD, La Jolla, CA
Robert C. Holfman, Jr., MD, Columbia, SC
Stephanie McGee Jernigan, MD, Atlanta, GA
Farahaba Rajab Lakhdir, MB, BS, Roanoke, VA
James Arthur Listman, MD, Manlius, NY
Tej K. Mattoo, MB, BS, Troy, MI
Kevin E. C. Meyers, MB, BCh, Drexel Hill, PA
Michael L. Moritz, MD, Pittsburgh, PA
Shashi K. Nagaraj, MB, BS, Lewisville, NC
Rulan Parekh, MD, Baltimore, MD
Gerard Guy Prosper, MD, Englewood, NJ
Izhak U. Raafat, MB, BS, Flossmoor, IL
Reem H. Raafat, MB, BS, Virginia Beach, VA
Kathleen Mary Sardegna, MD, West Hartford, CT
Asher Daniel Schachter, MD, Needham, MA
Azra M. Sehic, MD, Kingston, PA
Rita D. Sheth, MB, BS, Houston, TX
Douglas Marc Silverstein, MD, New Orleans, LA
Jordan M. Symons, MD, Seattle, WA
Lynne Pei-Lan Yao, MD, Silver Spring, MD

The American Board of Pediatrics announces recertifications in **Pulmonology**.

Ibrahim Abdulhamid, MB, BS, Farmington Hills, MI
Elizabeth DeFrancis Allen, MD, Columbus, OH
Dora M. Alvarez, MD, Passaic, NJ
Ran Dani Anbar, MD, Fayetteville, NY
Arthur Bernard Atlas, MD, Morristown, NJ
Bruce Alan Barnett, MD, Toledo, OH
Naim S. Bashir, MB, BS, Richmond, VA
Martin L. Bauer, MD, Overland Park, KS
David J. Birnkrantz, MD, Moreland Hills, OH
Dorothy Stein Biskig, MD, South Orange, NJ
Mark Allen Brown, MD, Tucson, AZ
Terence L. Carey, MB, BCh, Tulsa, OK
Barbara Allison Chatfield, MD, Salt Lake City, UT
Barry Alan Cohen, MD, New York, NY
Andrew Robert Amiel Colin, MD, Newton, MA
Karen Lynne Daigle, MD, Hartford, CT
Donald Frederick Davison, Jr., MD, Urbana, IL
Daniel A. Deane, MD, Cleveland, OH
Mary F. A. C. DiMaio, MD, New York, NY
Anthony G. Durnowicz, MD, Gaithersburg, MD
Marie E. Egan, MD, Madison, CT
Jeffrey Mark Ewig, MD, St. Petersberg, FL
William M. Gershon, MD, Mequon, WI
Ronald L. Gibson, Jr., MD, Bellevue, WA
Marilyn Alley Gowen, MD, Norfolk, VA
Louis H. Guerney, Jr., MD, Chester Springs, PA
Margaret F. Guil, MD, Augusta, GA
Marc B. Hershenson, MD, Ann Arbor, MI
Bettina Clarice Hiltman, MD, Papillion, NE
Bonnie Boyer Hudak, MD, Jacksonville, FL
Julie Pamela Katkin, MD, Houston, TX
Kevin Kirchner, MD, Atlanta, GA
Richard Mark Kravitz, MD, Durham, NC
Lucille A. Lester, MD, Chicago, IL
Diana B. Lowenthal, MD, Briarcliff, NY
Peter M. Luckett, MD, Dallas, TX
Joseph Marc Majure, MD, Durham, NC
Carole Lesley Marcus, MB, BCh, Philadelphia, PA
Monique Faricy Margetis, MD, Pasadena, CA
John David Mark, MD, Santa Cruz, CA
Roy C. Maynard, MD, Eden Prairie, MN
The American Board of Pediatrics announces recertifications in Rheumatology.

Leslie S. Abramson, MD, Burlington, VT
Barbara Sadoff Adams, MD, Ann Arbor, MI
Thasawee Arkachaisri, MD, Pittsburgh, PA
Karyl S. Barron, MD, Bethesda, MD
John Francis Bohnsack, MD, Salt Lake City, UT
Michael Steven Borzy, MD, Portland, OR
Suzanne Louise Bowyer, MD
Robert A. Colbert, MD, Cincinnati, OH
Andrew H. Eichenfield, MD, New York, NY
Melissa Ellen Elder, MD, Gainesville, FL
Helen Margaret Emery, MD, Seattle, WA
Christos A. Gabriel, MD, Norfolk, VA
Kerry Teresa Gallagher, MD, Pacific Palisades, CA
Ellen A. Goldmuntz, MD, Washington, DC
Donald P. Goldsmith, MD, Jenkintown, PA
Kathleen Ann Haines, MD, Hackensack, NJ
Gloria C. Higgins, PhD, MD, Columbus, OH
Raphael Hirsch, MD, Pittsburgh, PA
J. Roger Hollister, MD, Denver, CO
Donna M. Sedlak Hummell, MD, Nashville, TN
Norman T. Ilowite, MD, Bronx, NY
Ilidy Margaret Katona, MD, Bethesda, MD
David R. Keim, MD, Green Bay, WI
Yukiko Kimura, MD, Tenafly, NJ
Herbert M. Lazarus, MD, New York, NY
Thomas J. A. Lehman, MD, New York, NY
Carol Jean Betlack Lindsley, MD, Kansas City, KS
Daniel Joseph Lovell, MD, Cincinnati, OH
Deborah K. McCurdy, MD, Los Angeles, CA
Richard John Mier, MD, Lexington, KY
Stephen Ray Mitchell, MD, Alexandria, VA
Mary Denise Moore, MD, Kalamazoo, MI
Barry Lee Myones, MD, Houston, TX
Robert W. Nickeson, Jr., MD, Saint Petersburg, FL
Kathleen M. O’Neil, MD, Oklahoma City, OK
Judyann C. Olson, MD, Milwaukee, WI
Nancy Young Olson, MD, Prairie Village, KS
Murray H. Passo, MD, Cincinnati, OH
Maria De Los Angeles Perez, MD, Houston, TX
Consuelo D. Ega Rabinovich, MD, Durham, NC
Ann M. Reed, MD, Rochester, MN
Christy I. Sandborg, MD, Stanford, CA
Laura Eve Schanberg, MD, Durham, NC
Kenneth N. Schikler, MD, Louisville, KY
Mandel Reid Sher, MD, Largo, FL
David D. Sherry, MD, Philadelphia, PA
Leonard Donald Stein, MD, Chapel Hill, NC
Linda Wagner-Weiner, MD, Chicago, IL
Carol A. Wallace, MD, Seattle, WA
Robert Wells Warren, MD, Houston, TX
Dorothy Woodward Wortmann, MD, Tulsa, OK
Lawrence Stoner Zemel, MD, Hartford, CT

The American Board of Pediatrics announces recertifications in Sports Medicine.

Douglas Catron Cobble, MD, Greenville, TN
Joseph Anthony Congeni, MD, Akron, OH
Michael J. Coraggio, MD, Flemington, NJ
Jorge Emilio Gomez, MD, San Antonio, TX
Bernard A. Griesemer, MD, Springfield, MO
Delphis C. Richardson, MD, Phoenix, AZ

The American Board of Pediatrics announces recertifications in General Pediatrics.

Maria L. de la Morena, MD, Rye, NY
Ileana de la Sota, MD, Parkland, FL
Thomas E. de Brigard, MD, Brandon, FL
Sarah D. de Ferranti, MD, Boston, MA
Deborah Enad de Guzman, MD, Houston, TX
Alla Aminova, MD, Campbell, CA
Sherlita N. Amler, MD, Brewster, NY
Mohammad Samer Anmar, MD, Springfield, IL
Yaw Amaoeteng-Adjepong, MB, ChB, Milford, CT
Amira R. Amonker, MD, Overland Park, KS
Jorge Hernan Amor, MD, Pompton Plain, NJ
Kathy Amoroso, MD, Roanoke, VA
Kwabena K. Ampofo, MB, BS, Salt Lake City, UT
Rick Allan Anaka, MD, Santa Rosa, CA
Meena Chandrasekaran Anant, MB, BS, Centreville, VA
Jintanat Ananworanich, MD, Bangkok, Thailand
Nancy Comeau Anastasi, MD, South Lyon, MI
Carlos Anaya, MD, El Dorado, AR
Joey S. Ancheta, MD, Tampa, FL
Eric A. Andersen, MD, Lake Mills, WI
Angela C. Anderson, MD, Providence, RI
Arne John Anderson, MD, Montgomery Village, MD
Eric E. Anderson, MD, Pearland, TX
Laura Lee Anderson, MD, Pearland, TX
Ramona V. Anderson, MD, Albuquerque, NM
Toren J. Anderson, MD, Birmingham, AL
Joseph Ricardo Andrade, MD, Scarsdale, NY
Gregor Andree, MD, San Diego, CA
Carol Anderson Andrews, MD, Gulf Breeze, FL
Rajesh K. Aneja, MB, BS, Pittsburgh, PA
Jorge G. Ang, MD, Whitesburg, KY
Nadezna Lyn P. Ang, MD, Hilo, HI
Thomas A. Angello, MD, Yonkers, NY
Margarite Angelopoulos, MD, Lecanto, FL
Satish Angra, MB, BS, Potomac, MD
Joelle M. Angsten, MD, Little Rock, AR
Asad Ansari, MB, BS, Sioux Falls, SD
Richard Joseph Antaya, MD, Orange, CT
Gheorghe Antonescu, MD, Pulaski, PA
Donna Lynn Antonucci, MD, Jenkintown, PA
Sima Asadi, MD, Merced, CA
Ogiemwonyi E. Asemota, MB, BS, Fayetteville, NC
Shahab Asgharzadeh, MD, Los Angeles, CA
Nancy L. Ashburn, MD, Westminster, MD
Imreen M. Ashraf, MB, BS, Fremont, CA
Jeremy David Asnes, MD, New Haven, CT
Rosalyn Sarmiento Assef, MD, Houston, TX
Dagnachew Assefa, MD, Morristown, NJ
Ast A. Astraksas, MD, Palos Hills, IL
Joanne Therese Asuncion, MD, Pasadena, CA
Thomas L. Atkins, MD, Oakland, CA
Valerie Ann Atkins, MD, Jaffrey, NH
Mark Peter Atlas, MD, Great Neck, NY
Marilyn Christine Augustyn, MD, Somerville, MA
Jeffery J. Auletta, MD, Strongsville, OH
Jennifer M. Austin, MD, Portland, OR
Kishor Vasasara, MB, BS, Oakland, CA
Jacquelyn Marie Aveta, MD, Philadelphia, PA
Jeffrey R. Avner, MD, Teaneck, NJ
Abraham Joseph Avni-Singer, MD, New Haven, CT
Khalid A. Awad, MD, Papillion, NE
Rebecca Ayala, MD, Presque Isle, ME
Arkady Ayngorn, MD, Lisle, IL
Katherine Frankel Azaro, MD, Chatham, NJ
Azim U. Azhand, MD, Temecula, CA
Michelle Baack, MD, Ft. Pierre, SC
Carmina F. Babao, MD, Dawsonville, GA
Michelle Murphy Babb, MD, Edwardsville, IL
Janine G. Babcock, MD, Kensingtown, MD
Ranjit Terence Baboolal, MB, BCh, Etobicoke, Ontario, Canada
Edgar Bacaers, MD, Liberty, NY
Philippe Ferdinand Backeljauw, MD, Cincinnati, OH
Sylvie M. Backman, MD, Salt Lake City, UT
Donna J. Backus, MD, Canton, OH
Nicki A. Bacon, MD, Wheat Ridge, CO
Rachel L. Bacon, MD, Escondido, CA
Mohamed K. Badawy, MB, ChB, Rochester, NY
Janina Szukuczk Badowkska, MD, Naperville, IL
Robert E. Badwey, MD, Atlanta, GA
Soungwon S. Bae, DO, Tuscon, AZ
Roomika T. Baig, MB, BS, Urbana, MD
Won Hee Balik-Han, MD, Great Neck, NY
Edith Proctor Bailey, MD, Lakeside, AZ
Lynn Ann Bailey, MD, San Diego, CA
Verna Bain, MD, Hendersonville, TN
Romana Y. Bairan, MD, Chiefland, FL
Zarar M. Bajwa, MB, BS, Pottsville, PA
Agoritsa G. Baka, MD, Athens, Greece
Sima Habibi Bakalian, MD, Rockville, MD
David Richard Baker, DO, Lewiston, ME
Karen Furlow Baker, MD, Conway, AR
Marcy Solomon Baker, MD, Tampa, FL
Stephen Scott Baker, MD, Eagle River, AK
Saad Matti Bakhaya, MB, BCh, Lancaster, CA
Corinna Adelaida Balanon-Soriano, MD, Jackson, KY
Anne Estelle Baldwin, MD, Washington, DC
Dariusz A. Balinski, MD, Bay City, MI
Rachel R. Ballard, MD, San Antonio, TX
Linda M. Balogh, MD, Novi, MI
Joseph Raymond Baltrun, MD, Salado, TX
Barbara Jean Bambach, MD, East Amherst, NY
Nigel S. Bamford, MD, Bellevue, WA
Amal Chandra Banerjee, MD, Piscataway, NJ
Noel L. Bansil, MD, Hilldale, NJ
Cynthia Hope Barabas, MD, W. Allenhurst, NJ
Lena Diane Baram, MD, East Setauket, NY
Małgorzata U. Barar-Pressen, MD, Mount Prospect, IL
Susan Amy Barasch, MD, Green Brook, NJ
Cami S. Barger-Jones, MD, Muncie, IN
Patsy Carrasquillo Barker, MD, Wichita, KS
Mark Alan Barnhardt, DO, San Antonio, TX
Rumana A. Barodawalla, MB, BS, Mount Pleasant, MI
Cara Pizzo Barone, MD, Los Altos, CA
Joel D. Barron, MD, Murray, UT
Menard dela Cruz Barruga, MD, Calimesa, CA
David Howe Barry, Jr., MD, Houston, TX
Brenda Anne Barshel, MD, Toledo, OH
Matthew F. Bartels, MD, East Amherst, NY
Tracy M. Basler-Decker, MD, Tucson, AZ
Fathy Z. Basilios, MD, Champaign, IL
Govindasamy Baskar, MB, BS, Avon, OH
Gary Allen Bass, MD, Greenville, SC
Felicia Bassey-Akamune, MB, BS, Olney, MD
Andrea Lynn Bateman, MD, Anchorage, AK
Kathryn Wellington Bates, MD, Richmond, VA
Sandep Batra, MB, BS, Aberdeen, SD
James H. Batson, MD, Cookeville, TN
F. Keith Battan, MD, Westminster, CO
Mislen Stol Bauer, MD, Miami, FL
Rebecca A. Baum, MD, Gahanna, OH
Beverly Helene Baum, MD, Portland, OR
Penny S. Baumeier, DO, South Lyon, MI
Rowena G. Baumgartner, MD, Tulsa, OK
Eduardo R. Bautista, MD, Toms River, NJ
Maria Lourdes de Guzman Bautista, MD, New Hyde Park, NY
Amy L. Baxter, MD, Atlanta, GA
Hulya Bayir, MD, Pittsburgh, PA
Mohamed S. Bayoumy, MB, BCh, Jeddah, Saudi Arabia
Menouchehr Bazyani, MD, Clovis, CA
Pamela H. Beahm, MD, Nashua, NH
Scott Douglas Beane, MD, Easley, SC
Mark E. Beatty, MD, Jacksonville, FL
Marta A. Beaubien, MD, Moses Lake, WA
Annette Marie Beck, MD, Independence, MO
Cynthia M. Beck, MD, Valencia, CA
Ann Nicoloff Becker, MD, Wellesley, MA
Christopher Thomas Becker, MD, Rowlett, TX
David Kenneth Becker, MD, San Francisco, CA
Jeffrey Alan Becker, MD, Silver Spring, MD
Jonathan Wingate Becker, MD, Seattle, WA
Theresa Creneti Becker, MD, Huntingdon Valley, PA
Diane Helene Bedrosian, MD, Carlsbad, CA
Lee Ann Savio Beers, MD, Washington, DC
Razia Begum, MB, BS, Michigan City, IN
Arthur Allen Beisang III, MD, North Oaks, MN
Amy Elizabeth Beiter, MD, Tucson, AZ
Caryn B. Belafsky, MD, Ashland, OR
Cathy K. Bell, MD, Kaneohe, HI
Patricia Louise Bellissimo, MD, Middleton, WI
Gary Andrew Bellus, MD, Carmel, IN
Nancy Launensack Belser, MD, Blue Bell, PA
Robert Allen Belza, MD, Louisville, KY
Eyal Ben-Isaac, MD, Santa Monica, CA
Sandra Benanti, DO, Hillsborough, NJ
Lee Morris Benaroch, MD, Parkland, FL
Paul Christian Benda, MD, Eugene, OR
Brian P. Benfield, MD, Shelby, NC
Jose A. Bengochea, MD, Coral Gables, FL
Bengt Ola S. Bengtsson, MD, Camarillo, CA
Elizabeth Benitez, MD, Jonesville, FL
Sudha Sagar Bennuri, MB, BS, Tuscaloosa, AL
Katherine Hoshiko Bentley, MD, Ashland, OH
Mary E. Benton, MD, Memphis, TN
Rebecca Lynn Benton, MD, Oakton, VA
Arthur E. Benzick, MD, Colleyville, TX
Sona Hornung Berdia, MD, Gaithersburg, MD
Stacey Lynn Berg, MD, Houston, TX
Sven Thomas Berg, MD, Spring, TX
John Torrey Berger III, MD, Washington, DC
Lesly Berger, MD, Ellicott City, MD
Rachel Parde Berger, MD, Pittsburgh, PA
Shaun Steve Berger, MD, Las Vegas, NV
Sara Berhanu, MD, Santa Barbara, CA
Joseph Berk, MD, Owings Mills, MD
Rosario Salgado Bermisa, MD, Chesapeake, VA
Maria Lourdes S. Bernardo, MD, Union Springs, AL
Joshua E. Bernstein, MD, Asheville, NC
Ulla Lindholm Philbert Berringer, MD, Parker, CO
Pablo Antonio Berrios, MD, Hollywood, FL
Marcy L. Berry, MD, Plano, TX
Mark W. Bertagnoli, MD, De Pere, WI
Ernest G. Bertha, MD, Coral Springs, FL
Dawn Bertram-Stewart, MD, Naples, FL
Kelly M. Betha, MD, New Milford, NJ
Heath L. Bettencourt, MD, Terrytown, LA
John T. Beuervlein, MD, Knoxville, TN
Jennifer A. Bevan, MD, Libertyville, IL
Vijay Bhardwaj, MB, BS, Bloomfield, MI
Neena Bhargava, MB, BS, Rochester Hills, MI
Shobha Bhaskar, MB, BS, Osage Beach, MO
Anju R. Bhatia, MB, BS, Milpitas, CA
Parul Sheht Bhatia, MD, Los Angeles, CA
Manisha K. Bhatt, MB, BS, Brea, CA
Anuradha M. Bhimavarapu, MB, BS, Sharon, MA
Donna M. Bhishikul, MD, Lakeland, FL
Swati S. Bhobe, MB, BS, Lisle, IL
Prakash S. Bhoompal, MB, BS, Yorktown, IN
Adnan Tarid Bhutta, MB, BS, Little Rock, AR
Tonu Alan Bianchetta, MD, Bear, DE
Laarni Serquina Bibay, MD, Chesapeake, VA
Anthony A. Biehl, MD, Fort Wayne, IN
James Paul Bien, MD, West Lafayette, IN
Douglas R. Bierma, Jr., MD, Shorewood, IL
Gurur Biliciler Denktas, MD, Houston, TX
Michael P. Binder, MD, League City, TX
Mark Lee Binion, MD, Shelby, NC
Karen M. Binns Loveman, MD, Roanoke, VA
Debra Stein Birdsong, MD, Washington, NC
Bruce J. Birk, MD, Portland, OR
Nancy Anne Bischof, MD, Lexington, KY
Sandra Hagedus Bispo, MD, Vineland, NJ
Jennifer M. Bivens, MD, Ooltewah, TN
Alexander B. Black, MD, San Antonio, TX
Virginia G. Black, MD, Jacksonville, FL
Abigail Walters Blackmon, MD, Powell, TN
Rebecca Smiley Blackwell, MD, Tarzana, CA
Monica T. Botsch, MD, New Port Richey, FL
Salvador Bou, MD, Tampa, FL
Correne L. Boucher, MD, Portland, CT
Douglas A. Boudreau, MD, Jefferson City, MO
Margaret Mary Boudreaux, MD, Cumming, GA
Abdelhamid Bourbia, MD, Charleston, WV
Susan B. Boutilier, MD, Greenville, NC
Joseph D. Bouvier, MD, Gilbert, AZ
David West Bowe, MD, Seattle, WA
Mary Jo A. Bowman, MD, Canal Winchester, OH
Arlene Risk Boykin, MD, South Riding, VA
Kathleen Ann Boyls, MD, Tulsa, OK
Virginia Carle Brack, MD, Norwich, VT
Stephen Robert Braddock, MD, Charlottesville, VA
Melita Melson Bradley, MD, Murphysboro, TN
Brenda A. Bradshaw, MD, Fredericksburg, VA
Pilar A. Bradshaw, MD, Eugene, OR
Kathleen A. Brady, MD, Slingerlands, NY
Lynda Brady, MD, St. Louis, MO
Rebecca Charlene Brady, MD, Cincinnati, OH
Timothy Brian Brady, MD, Indianapolis, IN
Margaret Edith Braile, MD, Gig Harbor, WA
Ada Brainsky, MD, Merion Station, PA
Harry P. Bramley, DO, Elizabethtown, PA
Hilary Jane Branch, MD, Springfield, MA
Greta L. Branford, MD, Plymouth, MI
Deanna Branscom, MD, Rougemont, NC
Gagandeep Thind Brar, MB, BS, Maumee, OH
Sardul S. Brar, MB, BS, Lakeland, FL
Clay B. Brashears, MD, Benton, AR
John Kevin Bratsch, MD, Pinetop, AZ
Adrianna Bravo, MD, Salisbury, CT
Bolling Whitfield Brawley, MD, Newport, TN
Michael Joseph Brazelton, MD, Golden, CO
Lori A. Breaux, MD, Brentwood, TN
Karolina Anne Breikss, MD, Victoria, British Columbia
Canada
Andrea Marie Brescia, MD, Tuckahoe, NY

Joel W. Bonaparte, MB, ChB, Sugar Land, TX
Harry Victor Bond, MD, New York, NY
Teresa Rozon Bondoc, MD, La Mirada, CA
Brian K. Bonham, MD, Hagerstown, MD
Gena M. Bonitatibus, MD, Evans, GA
Robert Louis Bonner, Jr., MD, North Wales, PA
Scott David Bookner, MD, Scarsdale, NY
Karen Lynn Booth, MD, Emerald Hills, CA
Susan E. Borba, MD, Santa Cruz, CA
Camille Arnita Borders, MD, Gary, IN
Annette R. Borger, MD, Berwick, PA
Peter William Borrowdale-Cox, MD, Mount Sterling, KY
Rebecca G. Bosomworth, MD, Lexington, KY
Juan M. Bossano, MD, Lake Charles, LA
Deborah Edith Bostic, MD, Clifton Park, NY
Bruce Alan Boston, MD, Lake Oswego, OR
Carmen Julia Botero, MD, Tarzana, CA
Monica T. Botsch, MD, New Port Richey, FL
Salvador Bou, MD, Tampa, FL
Correne L. Boucher, MD, Portland, CT
Douglas A. Boudreau, MD, Jefferson City, MO
Margaret Mary Boudreaux, MD, Cumming, GA
Abdelhamid Bourbia, MD, Charleston, WV
Susan B. Boutilier, MD, Greenville, NC
Joseph D. Bouvier, MD, Gilbert, AZ
David West Bowe, MD, Seattle, WA
Mary Jo A. Bowman, MD, Canal Winchester, OH
Arlene Risk Boykin, MD, South Riding, VA
Kathleen Ann Boyls, MD, Tulsa, OK
Virginia Carle Brack, MD, Norwich, VT
Stephen Robert Braddock, MD, Charlottesville, VA
Melita Melson Bradley, MD, Murphysboro, TN
Brenda A. Bradshaw, MD, Fredericksburg, VA
Pilar A. Bradshaw, MD, Eugene, OR
Kathleen A. Brady, MD, Slingerlands, NY
Lynda Brady, MD, St. Louis, MO
Rebecca Charlene Brady, MD, Cincinnati, OH
Timothy Brian Brady, MD, Indianapolis, IN
Margaret Edith Braile, MD, Gig Harbor, WA
Ada Brainsky, MD, Merion Station, PA
Harry P. Bramley, DO, Elizabethtown, PA
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