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The impact of chronic disease

Pediatricians celebrate the small successes of our chronically ill patients: the survivals, hospital discharges, school entry, years without unplanned hospitalizations, etc. There are a whole host of potential successes, however, that are outside our usual purview and about which we may not always think: social independence, employment, marriage, parenthood, etc. In the current issue of The Journal, Groothoff and associates examine some of these adult milestones in a large population of Dutch patients with renal failure developing in childhood. Depending upon one’s viewpoint, this is a classic “half full” versus “half empty” situation.

Overall, these 144 adults had about twice the level of unemployment of their age-matched controls, and were much more likely to be unmarried, living with their parents, and having a “low” occupational level. On the other hand, the employment rate of these adults is actually significantly above that of Dutch citizens with renal failure which had its onset in adulthood rather than in childhood. Additionally, there appears to be a trend toward some measures of independence being improved in adults who were part of the more recent generation of children treated for renal failure. It also appears that a long duration of time on dialysis without a functioning renal transplant does not bode well for adult adjustment.

I believe that the glass is half-full. We should tell the parents of children with renal failure that modern treatment has the potential to give them productive, independent adult lives. This should be our goal for any child with a chronic disease.

—Thomas R. Welch, MD

Should adolescents be paid to participate in research?

In order to obtain best evidence for pediatric practice, it is critical that research be performed in pediatric patients. However, this research can be difficult and may present ethical dilemmas. One concern is the issue of financial compensation for participation in research. It is well accepted that adults may be compensated for their time spent in research. For adolescents, concerns have been raised regarding whether such compensation could be coercive.

In this issue of The Journal, Scherer et al report the results of a study to evaluate the impact of financial compensation on decision making about participation in research on pediatric asthma. Their results indicate that financial compensation did not affect decisions about participation. Estimates of fair compensation for participation were lower in adolescents, lower income patients, and potential participants who were naïve about compensation. Fair compensation was judged to be higher than actual compensation for low risk studies, but lower than actual compensation for higher than minimal risk studies.

These results should be helpful to investigators and IRB members as they consider what would be fair compensation in studies of children and adolescents.

—Stephen R. Daniels, MD, PhD

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Adenovirus and ophthalmologic examinations

Faden et al describe an outbreak of adenovirus 30 disease in a neonatal intensive care nursery - remarkable for high morbidity and intermittently recurrent cases over 6 months. The following findings make it likely that virus was transmitted or facilitated by examinations for retinopathy of prematurity: clinical disease in ophthalmologists, multiple examinations of affected infants by ophthalmologists, lack of following adequate sterilization procedures for equipment, and the association of outbreaks of adenovirus conjunctivitis in outpatients with visits to source ophthalmologists' offices reported in the medical literature.

The dire consequences of adenovirus infection in very young term neonates and prematurely-born infants with underlying pulmonary conditions are reinforced in this report. It is imperative that we all re-examine our protocols for safe use of instruments for neonatal eye examinations and reinforce standard rules of “wellness” for all individuals entering the nursery.

—Sarah S. Long, MD

Severe BPD is decreasing

The major associations with the development of BPD in preterm infants are low gestational age at delivery, chorioamnionitis prior to delivery, mechanical ventilation, supplemental oxygen, postnatal sepsis, and patent ductus arteriosus. Clearly BPD is a multifactoral disease. BPD is a significant medical problem because it results in longer hospital stays, increased risks of lung diseases after discharge (severe RSV pneumonia, increased airway reactivity), and severe BPD is associated independently with poor neurodevelopmental outcomes. Despite the widespread use of antenatal corticosteroids and postnatal surfactant, the incidence of BPD has not changed.

Smith et al confirm in this issue of The Journal that there has been no change in the incidence in the gestation-adjusted risk of BPD in a large database over a 9-year period. Nevertheless, severe BPD, defined as the need for oxygen plus respiratory support (mechanical ventilation or CPAP), decreased from 9.7% in 1994 to 3.7% in 2002. This represents real progress as the infants with severe BPD are at highest risk for adverse outcomes. A cautionary note is the validity of the diagnosis of BPD based on an oxygen requirement at 36 weeks corrected age only. Walsh MC et al (Pediatr 2004;114;1305-11) demonstrated that oxygen use varies widely between neonatal units and recommends testing if an infant needs supplemental oxygen at 36 weeks corrected age.

—Alan H. Jobe, MD, PhD
What is the best method to identify cardiovascular risk related to obesity?

Obesity is emerging as an important risk factor for cardiovascular disease in children and adolescents. It has been recommended that body mass index (BMI) is the most useful clinical method to identify obesity. In children, because they are growing, the use of BMI percentiles has been recommended.

In this issue of the Journal, Kahn et al present the results of a study designed to compare the waist to weight ratio versus sex and age specific percentiles of BMI and evaluate which better determines risk of future cardiovascular disease. They found that when children were discordant for the two measures, the waist to height ratio was more closely associated with total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels than BMI. The waist/height ratio was less associated with elevated blood pressure levels.

It is clear that pediatricians need to use anthropometric measures to identify overweight children. BMI percentiles appear to be useful as an overall measure of adiposity; however, they do not identify the distribution of fat. Other measures such as the waist to height ratio may be useful for that purpose.

—Stephen R. Daniels, MD, PhD

Exploring risks for sexually transmitted infections

Ellen et al performed a complex recruitment and interview study of sexually active adolescents as well as their sexual partners to determine whether partners' sexual activity outside of the primary identified association affected risk of sexually transmitted infection. It shouldn't be a surprise that partners' additional partners outside of the identified local network mattered. This work will help guide conceptualizing interventions that are multilayered and consider social and sexual networks in communities.

—Sarah S. Long, MD
For some time, the pediatric community has exhibited a greater appreciation than many of its sister disciplines in the medical world of the broad array of factors that affect health. This perspective is demonstrated in the American Academy of Pediatrics’ Bright Futures initiative and the existence of major textbooks on Ambulatory Pediatrics and Community Pediatrics. Despite these efforts, residency and medical school curricula in Pediatrics are heavily weighted toward the biomedical aspects of training.

A recent report of the Board of Children Youth and Families of the National Academies (Institute of Medicine [IOM] and National Research Council [NRC]) examined the ways by which we define and think about child health. The report, *Children's Health, the Nation's Wealth: Assessing and Improving Child Health*, was commissioned by Congress and was prepared by an interdisciplinary committee including pediatricians, psychiatrists, state and county health workers, obstetricians, an economist, and epidemiologists. It has major implications for refocusing attention on the issues of balance in our training of pediatricians and on the range of research topics in our academic departments.

The charge of the committee was to examine the ways in which child health is defined and currently assessed as well as what is known about risk and protective factors that have been shown to affect child health. The committee was asked to assess the adequacy of current efforts to measure child health, the factors that affect child health, and to recommend efforts for better monitoring in the future. Although the charge did not directly consider the implications for pediatric education, these considerations should lead to a reassessment of current educational and research priorities.

*Children's Health, the Nation's Wealth* includes a comprehensive review of what is known about factors that contribute to health of children. These are called influences in the report because so many of them can either increase risks or be protective, depending on the circumstances. For example, the family can be both protective and a source of risk, depending on the circumstances and nature of their effects.

The report contains a number of important contributions, including a new definition of child health that considers the importance of development and evolving health. Also included is an exciting model of child health that goes beyond the usual linear model to consider the more dynamic interaction among health influences. It calls for a number of short- and long-term initiatives for better acquisition and improved utilization of data, as well as for a higher prioritization of child health on the federal and state agenda. All these are key contributions, which we believe will resonate in the pediatric community.

However, it is the review of influences that is of strategic importance when thinking about our pediatric mission as academicians—as educators and investigators dedicated to improving child health. The review of influences is organized into six key areas: biology, behavior, social environment, physical environment, services, and policy. Strikingly, almost all of the current focus of pediatric education is on the first of these broad areas. Only recently has there been some attention to the issue of behavior, but it is still relegated to a small fraction of the pediatric training program, usually 1 month in most residencies. In residencies, there is minimal attention to the other influences recognized by the report. How often do pediatric residencies even discuss issues pertaining to the built environment (the presence or absence of sidewalls, if given space in an urban area, and so forth) or the effects of highway safety policies on child health? What importance is ascribed to the role of cultural values, or availability of good nutritional options in poor communities that lack healthy shopping options in our teaching of the growing problems of obesity and the epidemic of metabolic syndrome among children and adolescents? Few residents without other training outside the routine have a working knowledge of the frameworks with which to approach such issues as services, physical environment, or policy. They are not routinely trained in child development, sociology, economics, cultural anthropology, epidemiology, or advocacy. They lack an introductory familiarity with many of the concepts embodied in the report.

Thus, in reviewing what we know about the influences that contribute to child health, it becomes clear that only a small fraction of those influences are taken into account in what we
do currently in the clinical venue, whether at the bedside or in the office.

It is critically important to extend our knowledge of the biological determinants of diseases or the biological variation that explains why some people respond to therapies that fail miserably in others. However, even if that is the primary focus of much of the care of ill children, pediatricians need to understand the context of child health within a far broader framework. That broader understanding is a prerequisite to improved capacity to care effectively for all children—even the very sickest infants, children, and adolescents.

Surely it was not just a coincidence that independently and only weeks before the release of the IOM report, the Josiah Macy Jr. Foundation issued a report of the conference Pediatric Education in the 21st Century. This report likewise raised concerns about the neglect of genetic and environmental influences, particularly sociocultural issues, in the education of child health providers—and the grave implications that this deficit has for equity in health care delivery. Unlike Children's Health, The Nation's Wealth, the Macy Report specifically focused on education. It called for significant changes in the education of pediatricians and child health workers—an educational format that recognizes the gene-environmental interaction and the important role of development not only in child health, but throughout life.

Without an understanding of the child’s own behavior, of his social and physical world, and of the public policies and service sectors with which he and his family members interact, care for even the most extreme tertiary problems is inadequate. Reports repeatedly document that adherence to difficult therapeutic regimens, and hence therapeutic success, is determined by nonbiological factors often seen as extrinsic to current pediatric training. That our graduates are inexperienced when faced with these issues was described in the original Future of Pediatrics Report and again in both the FOPE II report and the recent Commonwealth Fund Survey. Most graduates in practice report that they have to learn about these nonbiological factors on their own, to figure them out by trial and error as they go through their pediatric careers. They report that they did not receive adequate training and understanding of the comprehensive context in which pediatric health care services take place. They are particularly weak in behavioral and social issues, and as environmental issues take more center stage, it is likely that they will also become an area of relative deficit.

As emphasis shifts increasingly to chronic health conditions for which services are provided predominantly on an ambulatory basis, these issues become even more pertinent. When care involves brief and intermittent encounters in the office, there is little opportunity to gather sufficient information to understand the broader context, especially if the pediatrician is not comfortable with how to process, integrate, and interpret the information. As pediatricians try to treat asthma and obesity and their complications, issues other than biology become central to therapy.

Addressing complex childhood problems often requires that pediatricians share care responsibilities with colleagues from other disciplines. Such coordination requires that at the very least, pediatricians have a basic understanding of the perspective and expertise of these colleagues to communicate and to work collaboratively to coordinate care. All of the above considerations should direct us to rethink elements of the core curriculum for pediatric training and to make provision for introducing new elements that include some of the areas in the IOM/NRC and Macy reports as key influences on child health.

Similarly, few departments of pediatrics organize research priorities around the challenging interdisciplinary tasks of seeking to understand the interconnections among the various influences of child health. Most departments lack well-developed dry bench research units dedicated to understanding the behavior, social and physical environments, and policies and services that affect child health. Such enterprises require teams that include, among others, skilled social scientists, epidemiologists, biostatisticians, and economists. These teams are needed to work with biomedical teams and to do cutting edge research on the relative importance of different influences and combinations of influences on child health. They are also needed to develop and evaluate theory-based preventive interventions to avoid some of the health concerns that now are reaching epidemic levels among our children. If integrated into faculties of departments, they will also be available to help shape the tenor of discussions and provide expertise in developing some of the additional curriculum discussed above.

As we move into the 21st century, it is time to rethink the content and format of pediatrics and make changes that will more adequately prepare future pediatricians for all the challenges they will face in improving child health.

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ADENOVIRUS IN THE NEONATAL INTENSIVE CARE UNIT: FORMIDABLE, FORGOTTEN FOE

In this issue of The Journal, Faden et al describe an outbreak of adenovirus type 30 in a neonatal intensive care unit. They highlight the importance of hospital epidemiology, infection control measures, and the impact of an uncommon adenovirus on a vulnerable population. In their retrospective observational study, 21 of 333 infants in a neonatal intensive care unit were infected over a 6-month period. Premature discontinuation of effective control measures resulted in additional cases. Adenovirus type 30 was associated with pneumonia, conjunctivitis, upper respiratory tract illness, and asymptomatic cases. Infants with chronic lung disease of prematurity requiring mechanical ventilation and those who received corticosteroids were significantly more likely to have development of pneumonia and die. Ophthalmologists who performed eye examinations on the infants appeared to have played a significant role in the outbreak by working while symptomatic and not adhering to infection control guidelines of the American Academy of Ophthalmology.

There are at least 49 distinct adenovirus serotypes in human beings that are classified into 6 groups, based on hemagglutinating characteristics, oncogenic properties, and DNA homology. Adenoviruses are nonenveloped, icosahedral double-stranded DNA viruses that are highly stable even at room temperature. Adenoviruses are associated with a broad spectrum of clinical conditions in children and adults. Respiratory tract disease is the most common condition associated with adenovirus infection, although other sites of infection, such as the gastrointestinal tract, central nervous system, cardiovascular system, urinary system, and eyes, occur with regularity. The illnesses in healthy children and adults generally are acute and nonfatal. On occasion, however, epidemic outbreaks with serotypes 3, 7, and 21 cause severe lower respiratory tract infections and appreciable mortality rates. Infants, neonates, and preterm infants are susceptible to severe disease, often with extrapulmonary dissemination. It must be kept in mind, however, that the common childhood adenovirus types 1, 2, 3, 4, 5, 6, and 7 that account for approximately 85% of the adenovirus infections in older infants and children are recovered less frequently from term infants younger than 6 months of age. The data suggest that adenovirus serotype-specific antibody acquired transplacentally protects the young infant.

Adenovirus type 30 is rarely identified in infants and children. It belongs to group D adenoviruses that are often associated with conjunctival and gastrointestinal disease. Some of these serotypes such as types 8, 19, and 37 are well known to cause epidemic keratoconjunctivitis in adults and pseudomembranous conjunctivitis in infants. The most common infection of the eye is acute follicular conjunctivitis caused by most of the group D adenovirus serotypes. In the only previously reported case in a preterm infant, adenovirus type 30 was associated with diffuse necrotizing bronchiolitis and pneumonia resulting in death. In the current report by Faden et al, adenovirus type 30 caused conjunctivitis in 8 of 21 infected infants and it was the only clinical finding in 7 of the infants. Five infected infants were asymptomatic and were only discovered because of surveillance cultures. Pneumonia occurred in 8 infants, and in 7 of these infants it was the sole presentation. Six of the infants with pneumonia died. Importantly, the use of corticosteroids was highly associated with death. Corticosteroids are often used in the neonatal intensive care unit setting with intent to hasten the reduction in supplemental respiratory requirements in preterm infants with lung disease. The use of corticosteroids should be reassessed in preterm infants with acute deterioration in their respiratory status or those with conjunctivitis, in particular during an adenovirus outbreak. As shown by this report and others, adenovirus outbreaks in the neonatal intensive care unit are associated with significant morbidity and mortality rates.

Adenoviruses are very stable agents that account for the ease in nosocomial spread from respiratory tract secretion, fecal–oral route, droplets, contaminated surfaces, contaminated hands, equipment, or ophthalmologic solutions. The prolonged replication and release of adenovirus from the gastrointestinal tract and shedding of virus by asymptomatic infants contribute to the difficulty in containing this virus. Rapid and accurate detection of adenovirus and monitoring of an outbreak are important in instituting the correct infection control measures and for insuring “best practices” in complying with effective control measures. Primary human embryonic kidney cells are ideal for isolating adenovirus serotypes 1 to 39, although other cell lines such as human lung carcinoma cells are appropriate. Unfortunately, standard
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DYSPHAGIA IN ADOLESCENCE

The Rome II criteria for functional gastrointestinal disorders, and adopted by the pediatric working group, defines dysphagia as “a feeling of persistent or recurrent pain or discomfort in the upper abdomen,” most often aggravated by meal ingestion.1 When no physical or organic cause for the symptom is identified with conventional testing, the condition is termed “functional dysphagia” (FD). The precise prevalence of FD in children is not known. In a recent community-based study, the prevalence of FD in Italian children aged birth to 12 years was 13%.2 This may be an under-representation, because the study, the prevalence of FD in Italian children aged birth to 12 years was 13%.2 This may be an under-representation, because the study, the prevalence of FD in Italian children aged birth to 12 years was 13%.

The most common symptoms of the dysphagia “symptom complex” in adults include post-prandial fullness and bloating, epigastric pain, early satiety, nausea, and belching.4 Weight loss, traditionally considered to be a “red flag” for more serious disease, has also been reported as a symptom associated with FD in adults.4 The heterogeneity in the presenting symptoms of FD reflects the uncertainty about the pathophysiologic mechanisms underlying this disorder. The 2 most extensively studied mechanisms are altered gastric accommodation and abnormal gastric.
emptying. Other potential contributing factors include visceral hypersensitivity to gastric distension, *Helicobacter pylori* infection, abnormal duodenojejunal motility, response to duodenal lipids or acid, and central nervous system dysfunction.  

The human stomach is a complex neuromuscular organ, both in structure and function. The proximal (fundus) and distal (body and antrum) parts of the stomach have different but coordinated sensory-motor functions. The fundus plays an important role in accommodation, a phenomenon that enables us to eat a large meal without producing symptoms. The stomach is able to handle an increase in volume without a proportional increase in pressure. This is accomplished by 2 related mechanisms of receptive relaxation and adaptive relaxation. The former is induced by the passage of food along the esophagus and is centrally mediated via the vagus nerve. Adaptive relaxation, a locally mediated neuromuscular response to gastric distension, is independent of external (vagal) innervation. A defective accommodation reflex leading to a reduced post-prandial relaxation of the fundus has been suggested as an underlying mechanism for FD in adults. In FD, there is an abnormal intragastric distribution of food, with preferential accumulation in the distal stomach. It is unclear whether the symptoms are generated by distension-induced activation of the mechanoreceptors in the fundus or in the antrum.

In contrast to the “passive” reservoir function of the fundus, the gastric body and antrum have an active contractile function that mixes, grinds, and sorts food and propels it toward the pylorus. Gastric emptying is mediated via peristaltic contractions in the gastric body that are initiated at a specific site along the greater curvature. Histological studies have demonstrated a dense network of pacemaker cells known as Interstitial Cells of Cajal (ICC) at this site. These specialized cells reside within and between the muscle layers. They have the ability to generate and propagate electrical slow-wave activity in the gut. Working in concert with the enteric nervous system and smooth muscle, the ICCs also serve as mediators of gastric motor neurotransmission. Disruption of this complex interaction among the ICC, the enteric nervous system, and the smooth muscle can potentially cause altered gastric emptying. Delayed gastric emptying of solids has been reported in 30% to 40% of adult patients with FD; this also may be true for liquids.

Several techniques have been used in adults to evaluate the sensory-motor function of the stomach in health and disease; these include intragastric barostat, transabdominal ultrasound scanning, magnetic resonance imaging, single photon emission computed tomography, satiation drinking, and water load tests. However, there is a woeful lack of understanding of the normal sensory–motor function of the stomach in children. Adolescence, in particular, is a period of transition, and the biophysical changes occurring during this period could potentially affect gastric function also. It is therefore challenging to interpret the scant data available on the pathophysiologic basis of FD in children. Maes et al have reported a high variability in the gastric emptying in healthy children 4 to 15 years old when compared with that in adults. In addition, they found the emptying of milk to be significantly slower in children than in adults. All subjects were given the same “standardized” test meal despite a wide range of body mass index (BMI), which may account for the variability noted because BMI can affect satiation in patients with FD.

The report by Chitkara et al in this issue of the journal represents an important step toward filling the knowledge void about FD. The authors determined that FD was the most common functional gastrointestinal disorder in adolescents (49%) who were referred to a tertiary care center. They found that bloating was independently associated with both rapid gastric emptying and delayed small bowel transit and that 16% of adolescents with FD had delayed small bowel transit but normal gastric emptying. If this is indeed true, it is possible that the increased small bowel acid or nutrient load is a factor in causing symptoms, as suggested in adult studies. Notwithstanding the limitations in performing clinical research in pediatrics, there are methodological issues in the study by Chitkara et al that need to be addressed to put their interpretations into perspective. First, subjects in the series were retrospectively selected on the basis of the pediatric Rome II criteria that have not yet been validated. In addition, a standardized 300 Kcal, 99technetium-labeled egg meal was given to all subjects irrespective of their weight or BMI. As shown by Maes et al, varying nutrient load could have potentially affected gut transit. Finally, gastric emptying and small bowel transit of solids were investigated with scintigraphy, a method validated in adults at their center, and the results were reported with data derived from their adult studies. Because of the complexity of growth and development during adolescence and the sparse data on normal gastric and small bowel motor function in children, it may not be scientifically valid to interpret the results in the context of adult normative data using methodology not validated in children.

There is growing evidence that, in adults, FD may represent a heterogeneous disorder with different subsets of patients on the basis of clinicopathophysiologic characteristics. In pediatric subjects, there is a dearth of information on the demographic, clinical, and pathophysiologic features of FD. Because of the adult data, it may be naïve to expect a single gastric sensory or motor abnormality to explain all the symptoms in a given child with FD. Also, there may be other, as yet unidentified, central or peripheral mechanisms contributing, singly or in combination, to the generation of the “dyspepsia symptom complex” in children. It is likely that the increasing sophistication of newer, non-invasive, imaging technology that does not involve radiation, combined with computer simulation, mathematical modeling, and mechanics-based analysis, may provide much-needed data in healthy children and reveal novel contributing mechanisms for pediatric FD. This may assist in correlating a particular symptom or combination of symptoms, with a specific underlying gastric dysfunction and help develop therapy targeted to correct the identified abnormality.
Assisted reproductive technologies (ART’s) have made a huge difference for infertile couples. However, in North American culture, perhaps to a lesser degree in Europe and Australia, infertility carries with it embarrassment and even shame, so there is still an aspect of secrecy about the utilization of ART’s. Once babies are conceived by ART’s, the families often do not tell their pediatrician or even their obstetrician that this pregnancy is the product of ART’s. Thus, if you, the pediatrician, do not ask, you may not find out and consequently not be suspicious of possible complications. The article by Yoon et al1 in this issue of The Journal brings to light many of the potential issues.

A recent review of published articles on ART’s, by a combined committee of the American Academy of Pediatrics and the American Society for Reproductive Medicine and sponsored by the Genetics and Public Policy Center at Johns Hopkins University aimed to determine whether there was an increased risk to mother and child(ren) with ART’s. The conclusion of that review was that no studies have been properly done to be actually assess the risk, but if there is an increased risk for congenital anomalies, neurologic abnormalities, learning defects, and so forth, it is primarily related to the increase in multiples births (ie, twins, triplets, and so forth).2 It is well known that twins have more complications than singletons and tend to be small for dates and born prematurely; however, the products of ART’s have even more intrauterine growth restriction for their gestational age.3 There does not appear to be a marked increase in congenital anomalies or handicaps beyond that which might be expected as related to the products of a “multiples” pregnancy. The concern in North America is that more than 1% of conceptions are now the products of a “multiples” pregnancy. The concern in North America is that more than 1% of conceptions are now the products of ARTs, leading to a remarkable increase in multiple births that have a major effect on special care nurseries and on the costs of pregnancies. However, the pediatrician should be aware that there is also likely to be an increase in rare complications, highlighted in the excellent case report by Yoon et al. Single case reports can be extremely important to identify rare pathogenic events. In the case described by Yoon et al,

References
there is a conjunction of the rare complications that can be seen in ARTs.

In addition to the increased number of dizygotic twins expected because of the replacement of multiple fertilized eggs during the procedure of ARTs, there has been an observed increase in monozygotic twinning (MZ) with ARTs. 4 This increase rate of MZ twinning is 3 to 5 times the spontaneous background rate of MZ twinning. It is not clear whether these are true monozygotic twins or whether, as reported in Yoon's case report, it is because they have a monochorionic placenta and therefore are assumed to be MZ twins. Normally, dizygotic twins (DZ) have completely separate placentas. Rarely, there can be implantation of their placentas close enough together to consider them fused. However, it is extremely rare, or at least it has been thought to be extremely rare, for dizygotic twins to have a single chorion.

There are now two reports of DZ twins with a monochorionic placenta, that is, the current report by Yoon et al and the report by Souter et al, 5 both associated with ARTs, and it is rumored that there are at least two more monochorionic DZ twins that were products of ARTs.

The case of DZ twins reported by Souter et al and the case reported by Yoon et al were both the product of intracytoplasmic sperm injection (ICSI). ICSI appears to improve fertilization when there is oligospermia. ICSI is often combined with allowing the zygote to grow to the blastocoele stage before returning the zygote to the uterus for reimplantation. Neither of these reports 1, 5 indicate whether the embryos were placed in the uterus immediately or allowed to grow to blastocoele stage. It may be that placing two zygotes back into the uterus in a particular way or at a particular stage of development influences whether or not they will implant so close to each other that they can have a monochorionic placenta. Spontaneous dizygotic twins are likely to be ovulated and fertilized at slightly different times, whereas ART's twins are replaced within minutes of each other. Unfortunately, the details of the ART's procedures as to medium, culture conditions, difficulty with ICSI, and so forth are rarely reported and may be difficult to obtain because of privacy issues. Nor is the procedure concerning replacing the zygotes and the timing of replacement usually reported (as seen in these two cases).

Most pediatricians are well aware that monochorionic twins are usually MZ. 6 In fact, the presence of a single chorion is traditionally the way to identify MZ twins. 70% to 75% of MZ twins are monochorionic. However, there is less awareness that 25% to 30% of MZ twins have separate placentas and are dichorionic diamniotic, just like DZ twins. Those MZ twins who are monochorionic are thought to undergo formation/separation into twins later (4 to 8 days of embryonic development) than dichorionic MZ twins, who are thought to separate in the first 4 days of embryonic development. 7

Because MZ twins share a single placenta, they have an increased risk for complications that include vascular connections, twin-twin transfusions, and vascular compromise, which can then lead to limb reduction anomalies, gastroschisis, central nervous system destructive lesions, and so on. 8 The pediatrician needs to be aware of the increased risk for MZ monochorionic twins to have these types of vascular problems that predispose to disruptive types of congenital anomalies. It is the monochorionic status that gives this increased risk, and thus the DZ twins who are monochorionic have the same risk.

What is also important about the recognition of DZ monochorionic twins is that because they share vascular connections, their bone marrow can be expected to be chimeric (a mixture of the white blood cells from each twin) or may be taken over completely by one of the twins. Thus, looking at white blood cell DNA to establish dizygosity may not work in monochorionic DZ twins. It may be necessary to do fibroblast or buccal smear DNA to determine whether the twins are MZ or DZ.

There appears to be a small increase in imprinting defects among the products of ARTs. This has not yet been sorted out completely, but several registries of Beckwith-Wiedemann patients indicate a marked increase in cases among the products of ARTs. 9 Also, in the case of Angelman syndrome, there appears to be an increase in a very rare type of imprinting defect. 10 Interestingly, Beckwith-Wiedemann syndrome has been reported for years to be increased in one of MZ twins, usually female twins. This phenomenon appears to be related to the failure to maintain normal imprinting on chromosome 11 in one of the MZ twins. 11 Weksberg has suggested that this failure to maintain imprinting may actually produce the MZ twinning. In Yoon's report, there are DZ twins who are the product of ART's utilizing ICSI, and the source of the imprinting defect leading to Beckwith-Wiedemann syndrome has not been elucidated. Thus, it seems likely that the presence of Beckwith-Wiedemann syndrome in one of these twins is related to ART's rather than to the MZ twinning process and the failure of maintaining imprinting usually seen with discordant MZ Beckwith-Wiedemann twins.

An increase in aneuploidy is seen in the offspring of ARTS. 12 This probably primarily relates to the infertility in the parents that brings them to use ARTS. For instance, men with Klinefelter syndrome have produced children with Klinefelter syndrome, and men with a Y deletion have produced children with Y deletion. In Yoon's report, we would have suspected that the father of this child might have Klinefelter syndrome and that he was the source of the extra chromosome; however, analysis of the child's sex chromosomes have demonstrated that the extra X came from mother.

Because of the cost of chromosome analysis, many couples with infertility do not have a proper workup for the cause of their infertility before using ART's (eg, careful parental chromosome studies to rule out small deletions or translocations). This can then lead to the production of chromosomal abnormalities in their children derived from their own chromosomal abnormalities, which could also be the cause of their infertility. In general, there has not appeared to be an increased occurrence of chromosome abnormalities other than those associated with advanced maternal age or those producing the infertility problems in the parents.

Nevertheless, it has been suggested for some time that when there are dizygotic twins and one twin has aneuploidy,
the pregnancy of the aneuploid twin could be maintained rather than miscarried, by the presence of the normal twin. Half of Down syndrome pregnancies and 99% of Turner syndrome pregnancies miscarry. However, the presence of a normal dizygotic twin appears to maintain discordant twin pregnancies. In Yoon’s case, Klinefelter syndrome is not a situation that would be expected to miscarry, but it does raise the issue of the presence of aneuploidy being increased and maintained by the presence of a normal twin in ARTs when multiples are produced.

The pediatrician needs to be aware of the possibility of rare complications when ARTs is used. Pediatricians are undoubtedly aware of the increased incidence of twinning related to ARTs because of the presence of so many twins, triplets, and quadruplets in the special care nurseries. It appears that the problems related to multiple births increases the true cost of ARTs, that is, the overall cost to the health care system. Because each attempt to attain a pregnancy by using ARTs is expensive, families tend to opt to put more fertilized ovum into the uterus than are necessary. It appears that in young women younger than 35 years of age, it is not necessary to put back multiple ovum to achieve a pregnancy.2 In addition, it is best to work up the infertility of the couple before using ARTs. Interestingly, if only one zygote had been replaced in the case presented by Yoon et al, as was appropriate because the mother was younger 35 years old, we would not have learned about these many complications from this remarkable case report.

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REFERENCES

Objectives  To determine whether the pattern of brain injury in term neonatal encephalopathy is associated with distinct prenatal and perinatal factors and to determine whether the pattern of injury is associated with 30-month neurodevelopmental outcome.

Study design  A total of 173 term newborns with neonatal encephalopathy from 2 centers underwent magnetic resonance imaging (MRI) at a median of 6 days of age (range, 1-24 days). Patterns of injury on MRI were defined on the basis of the predominant site of injury: watershed predominant, basal ganglia/thalamus predominant, and normal.

Results  The watershed pattern of injury was seen in 78 newborns (45%), the basal ganglia/thalamus pattern was seen in 44 newborns (25%), and normal MRI studies were seen in 51 newborns (30%). Antenatal conditions such as maternal substance use, gestational diabetes, premature rupture of membranes, pre-eclampsia, and intra-uterine growth restriction did not differ across patterns. The basal ganglia/thalamus pattern was associated with more severe neonatal signs, including more intensive resuscitation at birth ($P = .001$), more severe encephalopathy ($P = .0001$), and more severe seizures ($P = .0001$). The basal ganglia/thalamus pattern was associated with the most impaired motor and cognitive outcome at 30 months.

Conclusion  The patterns of brain injury in term neonatal encephalopathy are associated with different clinical presentations and neurodevelopmental outcomes. Measured prenatal risk factors did not predict the pattern of brain injury. (J Pediatr 2005;146:453-60)
profund asphyxia produced deep gray nuclei (basal ganglia and thalamus) injury. A comparable regional vulnerability is observed in the term newborn, resulting in 2 major patterns of injury detectable by MRI: a watershed predominant pattern involving the white matter, particularly in the vascular watershed, extending to cortical gray matter when severe; and a basal ganglia/thalamus predominant pattern involving the deep gray nuclei and perirolandic cortex, extending to the total thalamus pattern of injury. It is unclear whether these patterns in the human newborn are associated with different antenatal risk factors or timing of injury. The first aim of this cohort study of term neonatal encephalopathy was to determine the antenatal and perinatal risk factors for the watershed and basal ganglia/thalamus patterns of brain injury. If watershed injury follows relatively prolonged and milder insults and basal nuclei injury follows more abrupt severe insults, we hypothesized that the watershed pattern would be more strongly associated with long-standing antenatal risk factors, whereas the basal ganglia/thalamus pattern of injury would be more strongly associated with acute intrapartum risk factors.

The American College of Obstetricians and Gynecologists task force on neonatal encephalopathy and cerebral palsy concluded that an acute intrapartum event could only result in cerebral palsy of the spastic quadriplegic or dyskinetic type and could not account for cognitive deficits alone. Our clinical experience and data suggest that the outcome of neonatal encephalopathy is not homogeneous and may include cognitive deficits in the absence of cerebral palsy. The second aim of this study was to discriminate the specific deficits associated with each of the patterns of brain injury on neonatal MRI. This is important to target appropriate rehabilitative services to newborns.

**METHODS**

Two cohorts of term newborns were studied at the University of California San Francisco (UCSF) and at Loma Linda University Children’s Hospital (LLUCH). The Committees on Human Research at each site approved the protocol.

The UCSF cohort consists of 121 newborns studied with MRI (1994-2000), all of whom had neonatal encephalopathy or a marker of perinatal depression: umbilical artery pH <7.1, umbilical artery base deficit >10, or a 5-minute Apgar score ≤5. These inclusion criteria were chosen to include the entire range of neonatal encephalopathy and neurodevelopmental outcomes, from normal to severe impairment. Newborns were excluded when their gestational age was <36 weeks or there were suspected or confirmed congenital malformations, inborn errors of metabolism, or congenital infections on the basis of clinical examinations and laboratory studies. Infants with transient metabolic derangements such as hypoglycemia were not excluded from the study. The LLUCH cohort of 52 newborns was derived from newborns examined with MRI during the same period as part of a larger study of neonatal brain injuries. The UCSF inclusion and exclusion criteria were applied at LLUCH.

**MRI**

MRI was acquired comparably at both centers at a median of 6 days of life (range, 1-24 days) when newborns were stable for transport to the MR scanner and imaging time was available. At UCSF, MRI used a circularly polarized head coil on a 1.5Tesla Signa EchoSpeed system (GE Medical Systems) and included 4 mm (1 mm “gap”) sagittal spin-echo (SE; 500/11/2 [TR/TE/excitations]), 4 mm (1 mm “gap”) axial SE (500/11/2) images, and 4 mm (2 mm “gap”) axial SE (3000/60,120/1) images through the entire brain. At LLUCH, MRI used a circularly polarized head coil on a 1.5T Magnetom SP4000 (Siemens Medical Systems) and included 5 mm sagittal SE (550/22/4), 5 mm axial triple SE imaging (3000 ms/22,60,120 ms/1), and 5 mm coronal SE (1800/22,90 and 90/ half Fourier acquisition).

At each institution, a neuroradiologist who was blinded to the subjects’ clinical condition reviewed the MRI scans. With a previously validated system for acute and subacute signal abnormalities, the severity of injury in the watershed region was scored from 0 to 5, and in the basal ganglia/thalamus region, the severity of injury was scored from 0 to 4. Both neuroradiologists independently interpreted 10 MRI studies with a Kappa of 0.85, suggesting good reliability of the scoring at both sites. The intraobserver reliability of these scores was reported previously in this cohort as ranging from a Kappa of 0.85 to 1.0.

Newborns were grouped into 3 patterns of injury on the basis of the predominant site of injury on MRI: normal, watershed predominant, and basal ganglia/thalamus predominant. Newborns had the watershed pattern when the watershed scores were higher than the basal ganglia/thalamus scores. Newborns were classified as basal ganglia/thalamus when the basal ganglia/thalamus scores were as high as or higher than the watershed scores. The basal ganglia/thalamus pattern included newborns with total brain injury (maximum basal ganglia/thalamus score and watershed score), because extensive deep gray nuclei injury was often accompanied by cerebral cortical injury that was not limited to a watershed pattern. In previous work, isolated hyperintensity of the lateral thalamus on T1 weighted images was found to be a normal variant and was classified with the normal pattern.

**Neonatal Condition**

Prenatal, perinatal, and postnatal variables thought a priori to be associated with neonatal brain injury were collected prospectively at UCSF. Two investigators who were blinded to the MRI and outcomes then retrospectively reviewed the obstetric and neonatal charts at LLUCH for these data. Maternal substance use was defined as drug, ethanol, or cigarette use because of the high frequency of concurrent recreational drug and ethanol use in mothers who smoke cigarettes during pregnancy. Furthermore, previous work has demonstrated that, in infants exposed to cocaine in utero, maternal cigarette smoking was predictive of an abnormal neurologic examination, whereas the cocaine exposure itself was not. A maternal inflammatory condition
was considered to be present when a treating physician diagnosed chorioamnionitis or endometritis on the basis of clinical symptoms or when antepartum or peripartum maternal fever or infection were documented. Intra-uterine growth restriction (IUGR) was defined as a birth weight <2 SDs below the mean for the gestational age at birth. Maternal thyroid studies were not systematically collected. Caesarean section was classified as emergent or elective on the basis of the clinical indication. Complicated vaginal delivery was defined as arrest of descent and failed vacuum delivery. Fetal distress included fetal bradycardia and variable or late decelerations documented by the treating physician. Placental/cord insults included abruptio placenta, vasa previa, cord prolapse, nuchal cord, cord rupture, or uterine rupture. The amount of resuscitation at birth was summarized by using a resuscitation score: 1 = no intervention, 2 = blow-by oxygen, 3 = endotracheal suctioning, 4 = bag-mask positive pressure ventilation, 5 = endotracheal intubation with positive pressure ventilation, and 6 = endotracheal intubation with ventilation and medication (sodium bicarbonate with or without epinephrine). The severity of neonatal seizures was graded from [0] no documented seizure to [10] severe seizures by using a previously developed score measuring seizure frequency and onset, electroencephalogram abnormalities, and the number of anticonvulsant medications used.18 The severity of neonatal encephalopathy was graded from 0 (no encephalopathy) to 6 (severe encephalopathy) by using a validated score on the basis of alertness, feeding, tone, respiratory status, reflexes, and seizure activity.19

Developmental Examinations

The UCSF cohort was followed prospectively to 12- and 30-months of age. At both assessments, cognitive development was assessed by the Mental Development Index (MDI) of the Bayley Scales of Infant Development II.20 At each assessment, a pediatric neurologist who was blinded to the neonatal course and imaging results performed a standardized neurological examination. The neurologist scored neuromotor outcome with a validated score: 0 = normal, 1 = abnormal tone or reflexes, 2 = abnormal tone and reflexes, 3 = decreased power in addition to tone or reflex abnormality (functional deficit of power), 4 = cranial nerve involvement with motor abnormality, and 5 = spastic quadraparesis.21 The LLUCH cohort was not prospectively observed for neurodevelopmental outcomes.

Data Analysis

Statistical analysis was performed with Stata software version 8 (Stata Corporation, College Station, Texas). Variables were compared across the 3 patterns of injury with the Kruskal-Wallis tests for continuous variables and the Fisher exact tests for categorical variables. A P value ≤.05 was considered to be significant. Univariate comparisons of antenatal and perinatal variables with the MRI scores were made with linear regression. Variables associated with MRI scores on univariate analysis with a P value <.2 were included in multivariate models. Because of the categorical nature of the outcome variable, we investigated non-normality of the outcome variables using bootstrap modeling (5000 repetitions). Because these variables modeled similarly with and without bootstrap modeling, we present results without the bootstrap methods. The Spearman rank correlation (r) was used to compare MRI scores with scores for resuscitation, seizures, and encephalopathy.

RESULTS

Patterns of Injury

MRI findings consistent with acute or subacute brain injury were common in this cohort, particularly the watershed predominant pattern. At UCSF, 63 newborns had the watershed pattern (52%), 26 had the basal ganglia/thalamus pattern (22%), and 32 had normal MRI results (26%). At LLUCH, 15 newborns had the watershed pattern (29%), 18 had the basal ganglia/thalamus pattern (35%) and 19 had normal MRI results (37%). The predominant region of injury was often accompanied by lesser damage to the other region. Twenty-four newborns with the watershed predominant pattern (31%) had some deep gray nuclei injury. Twenty newborns with the basal ganglia/thalamus predominant pattern (45%) had total brain injury, and another 9 newborns (20%) had some watershed injury.

The day of life for MRI did not differ by the pattern of injury (P = .8). Only 11 newborns underwent their MRI study in the first 2 days of life (6.3%), including 2 newborns who underwent an MRI on their first day of life. Of the 11 newborns who underwent early imaging, 2 had normal scan results, 4 had the watershed predominant pattern, and 5 had the basal ganglia/thalamus predominant pattern. The basal ganglia/thalamus scores were significantly higher in the group studied in the first 2 days (median, 2) relative to the remainder of the cohort (median, 0; P = .02), whereas the watershed scores did not differ significantly (P = .1).

Most antenatal and perinatal variables associated with neonatal encephalopathy were similar across the 3 MRI patterns, except that emergent Caesarian section delivery was most common in the basal ganglia/thalamus predominant pattern (Table I). Although other prenatal and perinatal conditions were common in the cohort, in particular maternal inflammatory condition and fetal distress, these factors were similar in newborns with normal MRI scan results and newborns with brain injury. The antenatal and perinatal variables measured were similar in newborns with total brain injury as compared with the other newborns with the basal ganglia/thalamus predominant pattern (all P >.2).

Newborns with the watershed pattern had lower birthweights, but did not differ in gestational age, head circumference, or body length. The clinical presentation of the normal and watershed patterns was similar, whereas newborns with the basal ganglia/thalamus pattern had more severe clinical signs. Only 2 newborns in the entire cohort did not require resuscitation at birth (1 with normal MRI results and
Each of these newborns had clinical seizures at presentation. Newborns with the basal ganglia/thalamus pattern had at birth the most intensive need for resuscitation and the most severe clinical encephalopathy and seizures. Among newborns with the basal ganglia/thalamus pattern, newborns with total brain injury had more severe clinical seizures, but did not have a significantly different need for resuscitation (P = .7), 5-minute Apgar score (P = 1.0), or severity of encephalopathy (P = .06). The median seizure score in newborns with total brain injury was 5, whereas that of the other newborns with the basal ganglia/thalamus pattern was 3 (P = .01).

**Antenatal and Perinatal Predictors of the Severity of Brain Injury in Each Region**

Antenatal and perinatal predictors of the severity of brain injury in each region were determined. Birthweight was the only measured variable associated with the watershed score on univariate analysis (Table II). Maternal substance use was associated with the basal ganglia/thalamus region score on univariate analysis. In a multivariate model adjusting for maternal substance use, maternal inflammatory state, and prolonged rupture of membranes, newborns of lower birth-weight had higher watershed scores (Table II). Adjusting for these factors, newborns with maternal inflammatory conditions had lower basal ganglia/thalamus scores compared with newborns without a maternal inflammatory condition.

**Severity of Brain Injury in Each Region and the Neurological Syndrome**

The basal ganglia/thalamus score was significantly correlated with the intensity of resuscitation at birth (ρ = 0.32; P < .0001), the severity of encephalopathy (ρ = 0.42; P < .0001), and the severity of seizures (ρ = 0.41; P < .0001). In contrast, the watershed score was less strongly correlated with the intensity of resuscitation at birth (ρ = 0.21; P = .006), the severity of encephalopathy (ρ = 0.32; P < .0001), and the severity of seizures (ρ = 0.29; P = .0001).

**Neurodevelopmental Outcome**

Of the UCSF cohort, 89 infants (74%) were observed to 30 months of age. The basal ganglia/thalamus predominant pattern was associated with more impaired cognitive and

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### Table I. Clinical characteristics by magnetic resonance imaging pattern

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Watershed predominant</th>
<th>Basal ganglia/thalamus predominant</th>
<th>P value</th>
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<tbody>
<tr>
<td>Number</td>
<td>51</td>
<td>78</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>27 (54%)</td>
<td>51 (65%)</td>
<td>24 (56%)</td>
<td></td>
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<tr>
<td>Antenatal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substance use</td>
<td>8 (16%)</td>
<td>11 (14%)</td>
<td>11 (25%)</td>
<td>.3</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>4 (8%)</td>
<td>9 (12%)</td>
<td>6 (14%)</td>
<td>.6</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>2 (4%)</td>
<td>6 (8%)</td>
<td>3 (7%)</td>
<td>.7</td>
</tr>
<tr>
<td>Prolonged rupture of membranes</td>
<td>6 (12%)</td>
<td>16 (21%)</td>
<td>8 (19%)</td>
<td>.4</td>
</tr>
<tr>
<td>Intra-uterine growth restriction</td>
<td>0</td>
<td>4 (5%)</td>
<td>2 (5%)</td>
<td>.3</td>
</tr>
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<td>Maternal inflammatory state</td>
<td>19 (37%)</td>
<td>35 (45%)</td>
<td>12 (28%)</td>
<td>.2</td>
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<tr>
<td>Perinatal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetal distress</td>
<td>33 (66%)</td>
<td>51 (66%)</td>
<td>23 (56%)</td>
<td>.5</td>
</tr>
<tr>
<td>Complicated vaginal delivery</td>
<td>8 (16%)</td>
<td>17 (18%)</td>
<td>10 (23%)</td>
<td>.7</td>
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<td>Caesarian section delivery</td>
<td>24 (47%)</td>
<td>42 (54%)</td>
<td>22 (50%)</td>
<td>.7</td>
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<tr>
<td>Emergent Caesarian section</td>
<td>18 (75%)</td>
<td>29 (69%)</td>
<td>22 (100%)</td>
<td>.006</td>
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<tr>
<td>Placenta/cord insult</td>
<td>16 (31%)</td>
<td>21 (27%)</td>
<td>15 (34%)</td>
<td>.7</td>
</tr>
<tr>
<td>Postnatal</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>40 (36-42)</td>
<td>40 (36-42)</td>
<td>40 (36-42)</td>
<td>.5</td>
</tr>
<tr>
<td>Birthweight (kg)</td>
<td>3.5 (2.2-5.2)</td>
<td>3.2 (2.0-5.4)</td>
<td>3.4 (1.6-4.9)</td>
<td>.01</td>
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<tr>
<td>Head circumference (cm)</td>
<td>35 (32-38)</td>
<td>35 (31-44)</td>
<td>35 (29-39)</td>
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<tr>
<td>Length (cm)</td>
<td>51 (38-56)</td>
<td>51 (41-59)</td>
<td>51 (44-56)</td>
<td>.9</td>
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<td>Resuscitation score (1-6)</td>
<td>4 (1-6)</td>
<td>4 (2-6)</td>
<td>5 (1-6)</td>
<td>.001</td>
</tr>
<tr>
<td>Five-minute Apgar score</td>
<td>6 (1-9)</td>
<td>5 (1-9)</td>
<td>4 (0-9)</td>
<td>.0005</td>
</tr>
<tr>
<td>Meconium aspiration</td>
<td>14 (28%)</td>
<td>17 (22%)</td>
<td>10 (23%)</td>
<td>.8</td>
</tr>
<tr>
<td>Encephalopathy score (0-6)</td>
<td>2 (0-6)</td>
<td>3 (1-6)</td>
<td>5 (1-6)</td>
<td>.0001</td>
</tr>
<tr>
<td>Clinical seizures (yes/no)</td>
<td>14 (28%)</td>
<td>34 (44%)</td>
<td>36 (82%)</td>
<td>.0001</td>
</tr>
<tr>
<td>Seizure score (0-10)</td>
<td>0 (0-6)</td>
<td>0 (0-8)</td>
<td>4.5 (0-9)</td>
<td>.0001</td>
</tr>
</tbody>
</table>

Data is presented as median (range) or number (%). P values refer to comparisons across the 3 groups.
motor outcomes at 30 months of age, with the watershed predominant pattern having an intermediate outcome (Table III; Figure). None of the newborns with normal MRI results had an MDI score <70 (2 SD below the mean) or functional motor deficits (neuromotor score $^3)$. Eight newborns with the basal ganglia/thalamus pattern (50% of survivors) had an MDI score <70, and 9 newborns with the basal ganglia/thalamus (56% of survivors) had functional motor deficits (spastic quadraparesis in all). In newborns with the basal ganglia/thalamus predominant pattern, the median MDI and neuromotor scores were not significantly different in those with isolated deep gray nuclei injury compared with those with deep gray nuclei and watershed injury ($^P$.1). Similarly, the median MDI and neuromotor scores were not significantly different in newborns with total brain injury compared with the remainder of the basal ganglia/thalamus predominant group ($^P$.1). Eight newborns with the watershed predominant pattern (18% of survivors) had an MDI score <70, and 5 newborns with the watershed predominant pattern (11% of survivors) had functional motor deficits (spastic quadraparesis in 3, spastic hemiparesis in 1, and spastic triaparesis in 1). Three of the 5 newborns with a watershed pattern and functional motor deficits had isolated watershed injury without deep gray nuclei abnormalities. Of the 32 newborns lost to follow-up at 30 months of age, 20 were examined at 12 months (5 with normal MRI results [16%), 10 with the watershed predominant pattern [16%), and 5 with the basal ganglia/thalamus predominant pattern [19%]); the outcomes at 12 months of this group were similar to that of the cohort observed to 30 months. In the infants evaluated at both times, the MDI of infants with the watershed pattern was significantly lower at 30 months than at 12 months ($^P$.0007), but did not differ with time in the infants with normal MRI results ($^P$.5) or basal ganglia/thalamus

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Change in watershed score</th>
<th>95% CI</th>
<th>P value</th>
<th>Change in basal ganglia/thalamus score</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birthweight (per 100-gram decrease)</td>
<td>0.06</td>
<td>0.02-0.11</td>
<td>.006</td>
<td>0.02</td>
<td>0.01-0.06</td>
<td>.17</td>
</tr>
<tr>
<td>Maternal substance use</td>
<td>0.54</td>
<td>-0.24-1.32</td>
<td>.18</td>
<td>0.70</td>
<td>0.1-1.3</td>
<td>.02</td>
</tr>
<tr>
<td>Maternal inflammatory state</td>
<td>-0.27</td>
<td>-0.88-0.34</td>
<td>.40</td>
<td>-0.38</td>
<td>-0.85-0.09</td>
<td>.11</td>
</tr>
<tr>
<td>Prolonged rupture of membranes</td>
<td>0.71</td>
<td>-0.07-1.48</td>
<td>.07</td>
<td>0.30</td>
<td>0.30-0.91</td>
<td>.30</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>-0.57</td>
<td>-1.51-0.38</td>
<td>.24</td>
<td>-0.02</td>
<td>-0.76-0.71</td>
<td>.90</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>0.73</td>
<td>-0.48-1.94</td>
<td>.24</td>
<td>0.33</td>
<td>-0.61-1.28</td>
<td>.50</td>
</tr>
<tr>
<td>Fetal distress</td>
<td>0.11</td>
<td>-0.26-0.48</td>
<td>.60</td>
<td>-0.06</td>
<td>-0.35-0.22</td>
<td>.70</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>0.21</td>
<td>-0.8-0.38</td>
<td>.50</td>
<td>-0.01</td>
<td>-0.48-0.48</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Change in watershed score</th>
<th>95% CI</th>
<th>P value</th>
<th>Change in basal ganglia/thalamus score</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birthweight (per 100 gram decrease)</td>
<td>0.06</td>
<td>0.01-0.10</td>
<td>.01</td>
<td>0.02</td>
<td>0.01-0.05</td>
<td>.26</td>
</tr>
<tr>
<td>Maternal substance use</td>
<td>0.30</td>
<td>-0.49-1.08</td>
<td>.45</td>
<td>0.60</td>
<td>-0.01-1.21</td>
<td>.06</td>
</tr>
<tr>
<td>Maternal inflammatory state</td>
<td>-0.42</td>
<td>-1.02-0.18</td>
<td>.17</td>
<td>-0.48</td>
<td>-0.95-0.01</td>
<td>.05</td>
</tr>
<tr>
<td>Prolonged rupture of membranes</td>
<td>0.63</td>
<td>-0.14-1.41</td>
<td>.11</td>
<td>0.25</td>
<td>-0.36-0.87</td>
<td>.42</td>
</tr>
</tbody>
</table>

Table II. Antenatal and perinatal predictors of the severity of brain injury measured by the magnetic resonance imaging scores

Table III. Neurodevelopmental outcome of newborns observed to 30 months of age by magnetic resonance imaging pattern

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Normal</th>
<th>Watershed predominant</th>
<th>Basal ganglia/thalamus predominant</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>20</td>
<td>48</td>
<td>21</td>
<td>.01</td>
</tr>
<tr>
<td>Died *</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>.0007</td>
</tr>
<tr>
<td>30-month MDI</td>
<td>101 (77-121)</td>
<td>84 (50-116)</td>
<td>62.5 (50-104)</td>
<td>.0001</td>
</tr>
<tr>
<td>12-month MDI</td>
<td>93 (53-109)</td>
<td>91.5 (50-120)</td>
<td>58 (50-109)</td>
<td>.0006</td>
</tr>
<tr>
<td>30-month NMS</td>
<td>0 (0-2)</td>
<td>1 (0-5)</td>
<td>5 (0-5)</td>
<td>.0001</td>
</tr>
<tr>
<td>12-month NMS</td>
<td>0 (0-3)</td>
<td>1 (0-5)</td>
<td>5 (0-5)</td>
<td>.0008</td>
</tr>
<tr>
<td>30-month head circumference</td>
<td>50 (47-57)</td>
<td>50 (41-53)</td>
<td>47 (39-51)</td>
<td>.005</td>
</tr>
</tbody>
</table>

Data is presented as median (range) or number (%). P values refer to comparisons across the 3 groups.

NMS=Neuromotor score.

*All infants died before 12 months of age.
patterns ($P = 1.0$). The neuromotor scores did not change significantly from 12 to 30 months in any group (all $P > .3$)

**DISCUSSION**

In this cohort representative of the spectrum of severity of term neonatal encephalopathy, the watershed predominant injury pattern was most common, seen in 45% of the cohort, and was often accompanied by less severe basal ganglia/thalamus injury. The basal ganglia/thalamus predominant pattern was less common, seen in 25% of the cohort, and was frequently accompanied by more diffuse cortical injury. These patterns are consistent with the regional vulnerability of the term neonatal brain.\textsuperscript{1,11-13,22}

In an earlier study of risk factors for neonatal encephalopathy, 69% of cases had antepartum risk factors, 24% had both antepartum and intrapartum risks, and 5% only had intrapartum risks.\textsuperscript{6,7} In our cohort, although Caesarian section delivery was common in all 3 MRI patterns, emergent Caesarian delivery was significantly more common in the basal ganglia/thalamus pattern. Although many risk factors are clearly prenatal,\textsuperscript{7} recent evidence from prospective cohorts of neonatal encephalopathy using MRI demonstrate that the brain injury actually happens close to the time of birth.\textsuperscript{8} The MRI findings in our cohort were also consistent with recent rather than chronic brain injury, and the antenatal conditions measured were remarkably similar in newborns with normal and abnormal MRI scan results. These observations highlight the potential of interventions to ameliorate brain injury in the newborn.

The association of lower birthweight with the severity of watershed injury supports our hypothesis that antenatal risk factors are more strongly associated with this pattern of injury. However, lower birthweight may be a marker for multiple risk factors of neonatal encephalopathy.\textsuperscript{23} We were also unable to attribute more severe brain injury to a maternal inflammatory condition. The failure to identify the specific antenatal risk factors associated with the pattern and severity of brain injury suggests that either we did not measure the relevant antenatal risk factors or that better antenatal markers need to be identified.

The intensity of resuscitation, the severity of encephalopathy, and the severity of seizures were associated more strongly with the basal ganglia/thalamus predominant pattern than with the watershed pattern. This is consistent with previous observations relating deep gray nuclei injury with profound asphyxia and severe encephalopathy.\textsuperscript{13} The surprising observation of more severe seizures in newborns with the basal ganglia/thalamus pattern as compared with the watershed pattern may relate to an overall increased severity of injury, including the cerebral cortex. This is supported by the observation that seizures were most severe in newborns with total brain injury. The dissociation of antenatal risk factors from the severity of the clinical presentation supports the hypothesis that the etiology of brain injury in neonatal encephalopathy is distinct from these antenatal risk factors.

The MRI scoring system applied in this cohort was developed to better evaluate the severity of neonatal brain injury, particularly milder abnormalities, and discriminates abnormal neurodevelopmental outcomes at 12 months of age.\textsuperscript{11} This is consistent with other studies demonstrating that the severity of brain lesions on MRI in the term newborn is predictive of neurodevelopmental outcome.\textsuperscript{24-26} Although it is accepted that the risk of an abnormal neurodevelopmental outcome increases with the severity of the injury, the pattern of injury also conveys important prognostic information. The MRI patterns of injury were associated with impairments in different developmental domains. Similar to previous observations with computed tomography, the basal ganglia/thalamus predominant pattern was associated with severely impaired motor and cognitive outcomes at 30 months of age.\textsuperscript{27} This is also consistent with the seminal observation that abnormal signal intensity in the posterior limb of the internal.
capsule on MRI, a structure involved in the basal ganglia/thalamus predominant pattern, is an accurate predictor of neurodevelopmental impairment in term neonatal encephalopathy.28 Because of the frequent occurrence of cerebral watershed injury with the basal ganglia/thalamus predominant pattern, cognitive deficits cannot be directly attributed to damage to the deep gray nuclei themselves. In contrast, the watershed pattern had predominantly cognitive impairments at 30 months that were not detected at 12 months of age. Cognitive deficits in this group often occurred without functional motor deficits. This highlights that abnormal outcome after neonatal encephalopathy is not limited to cerebral palsy and often requires follow-up beyond 12 months of age to be detected.15

A limitation of this study is that not all newborns underwent imaging at a uniform time after brain injury, so the extent of damage, particularly in the basal nuclei, may have been underestimated in some newborns.29,30 However, the basal ganglia/thalamus scores were significantly higher in the group studied in the first 2 days compared with the remainder of the cohort. Although some injury in this group may have been underestimated, it is less likely that these newborns were inadvertently classified as normal or as watershed injury. Because of our sample size, we a priori included newborns with total brain injury with the basal ganglia/thalamus pattern. This was done because extensive deep gray nuclei injury was often accompanied by cerebral cortical injury not limited to a watershed pattern. We found that, besides more severe clinical seizures, the clinical presentation of newborns with total brain injury was similar to that of other newborns with the basal ganglia/thalamus pattern. Because of the broad inclusion criteria for this cohort, it is not surprising that a substantial number of the newborns had normal MRI study results. Although the cohort was recruited from 2 specialized care centers, the range of normal and abnormal MRI study results suggests that the entire severity spectrum of neonatal encephalopathy is represented. Incomplete follow-up at 30-months may have exaggerated the difference in cognitive outcome between the patterns because cognitive deficits in the watershed groups were most evident at 30 months. However, newborns “lost to follow-up” were evenly distributed across the MRI patterns.

Because newborns with more severe encephalopathy are more likely to be identified for research studies in the intensive care nursery and these newborns are more likely to have the basal ganglia/thalamus injury pattern, it is possible that prospective MRI studies of neonatal encephalopathy will over-represent perinatally acquired injury as compared with population-based epidemiological surveys. Because population-based retrospective studies identify a preponderance of antenatal risk factors and smaller prospective cohort studies identify the perinatal occurrence of brain injury,8 our results indicate the pressing need to establish the mechanistic link between prenatal risk factors and etiology of brain injury. This is critical to the prevention of acquired neonatal brain injury and may be achieved with the development and application of more accurate in-utero measures of brain injury, such as fetal MRI.31 Until MRI is routinely applied to study neonatal encephalopathy, it is likely that discrepancies across study designs will be related to the heterogeneity of the brain injury.

In conclusion, the pattern of brain injury in neonatal encephalopathy can distinguish associated risk factors and clinical presentation and can identify those newborns who are at a higher risk for abnormal outcomes. This is important when considering which newborns should be targeted for emerging strategies to protect the brain after injury. Knowing the pattern of brain injury can also help parents and physicians care for the survivors of neonatal encephalopathy by identifying newborns who may benefit from rehabilitative services, in particular, the developmental domains requiring specific attention.

The authors thank the neonatal nurses of the Pediatric Clinical Research Center at UCSF for their work on this study.

REFERENCES


GROWTH AND DEVELOPMENT OF PRETERM INFANTS FED INFANT FORMULAS CONTAINING DOCOSAHEXAENOIC ACID AND ARACHIDONIC ACID

M. THOMAS CLANDININ, PHD, JOHN E. VAN AERDE, MD, PHD, KIMBERLY L. MERKEL, RPH, CHERYL L. HARRIS, MS, MARY ALICE SPRINGER, BA, JAMES W. HANSEN, MD, PHD, AND DEBORAH A. DIERSEN-SCHADE, PHD

Objectives  To evaluate safety and benefits of feeding preterm infants formulas containing docosahexaenoic acid (DHA) and arachidonic acid (ARA) until 92 weeks postmenstrual age (PMA), with follow-up to 118 weeks PMA.

Study design  This double-blinded study of 361 preterm infants randomized across three formula groups: (1) control, no supplementation; (2) algal-DHA (DHA from algal oil, ARA from fungal oil); and (3) fish-DHA (DHA from fish oil, ARA from fungal oil). Term infants breast-fed ≥4 months (n = 105) were a reference group. Outcomes included growth, tolerance, adverse events, and Bayley development scores.

Results  Weight of the algal-DHA group was significantly greater than the control group from 66 to 118 weeks PMA and the fish-DHA group at 118 weeks PMA but did not differ from term infants at 118 weeks PMA. The algal-DHA group was significantly longer than the control group at 48, 79, and 92 weeks PMA and the fish-DHA group at 57, 79, and 92 weeks PMA but did not differ from term infants from 79 to 118 weeks PMA. Supplemented groups had higher Bayley mental and psychomotor development scores at 118 weeks PMA than did the control group. Supplementation did not increase morbidity or adverse events.

Conclusions  Feeding formulas with DHA and ARA from algal and fungal oils resulted in enhanced growth. Both supplemented formulas provided better developmental outcomes than unsupplemented formulas. (J Pediatr 2005;146:461-8)

Exogenous sources of the long-chain omega-3 and omega-6 polyunsaturated fatty acids docosahexaenoic acid (DHA) and arachidonic acid (ARA), respectively, are important for premature infants. DHA and ARA accumulate rapidly in the developing brain during the last trimester of gestation, and preterm infants are deprived of in utero accretion. Supplementing preterm formula with DHA and ARA at human milk levels results in circulating levels of these fatty acids similar to those seen in preterm infants fed breast milk. DHA supplementation of preterm formula has been associated with accelerated visual maturation and improved mental development. Some early studies of feeding preterm formula with fish oil providing DHA and another omega-3 fatty acid, eicosapentaenoic acid (EPA), but no ARA resulted in reduced growth, which was associated with reduced ARA status of infants in one study. EPA and its metabolites compete with and have other antagonistic effects on ARA and some of its metabolic effects. Several subsequent studies of preterm formulas containing ARA as well as DHA found no consistent differences in growth compared with unsupplemented formulas, suggesting that balanced addition of omega-6 and omega-3 long-chain fatty acids addressed the reduced growth seen in earlier studies. However, one study reported reduced growth at 18 months after term associated with preterm formula containing DHA, EPA, and ARA from egg lipids. A second study found significantly higher weights in the first few months of life with preterm formula with single-cell algal...
and fungal oils providing DHA and ARA but no EPA. Thus, questions remain about whether and how long-chain polyunsaturated fatty acids and specific sources of these fatty acids affect growth.12

Given the possible benefits of providing DHA and ARA to preterm infants and the growth concerns raised by some early trials, we considered it imperative to carefully evaluate clinical performance of formulas containing potential commercial sources of these fatty acids. Therefore, we conducted a large, double-blinded, randomized, controlled trial to assess the safety and efficacy of feeding preterm infants premature, discharge, and term infant formulas supplemented with DHA from algal oil or fish oil and ARA from fungal oil until 92 weeks PMA.

**METHODS**

**Subjects**

Infants were eligible for enrollment in the first phase of this two-phase, multisite study if gestational age was ≤35 weeks PMA and they had received <10 total days of enteral feedings of >30 mL/kg per day (Figure 1). Infants initially fed human milk were not enrolled unless formula was started within 10 days after completing the first day of human milk feeding. Exclusion criteria included congenital abnormalities of the gastrointestinal tract, hepatitis, hepatic or biliary pathology, necrotizing enterocolitis confirmed before enrollment, or history of underlying disease or congenital malformation likely to interfere with evaluation. Given the minimal exclusion criteria, the study included many infants with concomitant medical conditions related to prematurity.

Preterm subjects eligible for inclusion in the second phase met the following criteria: successful completion of the first phase, ≥80% of enteral intake from study formula during hospitalization, and 100% of caloric intake from study formula at completion of the first phase (40 weeks PMA). A protocol amendment implemented shortly after enrollment began excluded preterm infants with birth weight >1500 g to limit inclusion to very and extremely low birth weight infants. Healthy, appropriate-for-gestational-age term infants (38 to 42 weeks gestational age) who were to be exclusively breast-fed for ≥4 months were enrolled as a reference group between birth and 4 weeks of age. Institutional review boards at each site reviewed and approved the protocol and procedures. Parents/guardians of all infants provided written informed consent.

**Design**

Infants enrolled in the first phase of this prospective, randomized, double-blinded, controlled study were stratified by birth weight (<1000 g, 1000 to 1500 g, and >1500 g initially and <1000 g and 1000 to 1500 g after the protocol amendment) and sex. Computer-generated random assignment schedules assigned infants to 1 of 3 study formula groups: (1) control, formulas with no added DHA or ARA; (2) algal-DHA, formulas with 17 mg DHA/100 kcal from algal oil and 34 mg ARA/100 kcal from fungal oil (Martek Biosciences, Columbia, MD); or (3) fish-DHA, formulas with 17 mg DHA/100 kcal from tuna fish oil (Roche Vitamins Inc, Parsippany, NJ) and 34 mg ARA/100 kcal from fungal oil. These levels of DHA and ARA (Table I) are similar to median worldwide amounts reported for mature human milk of approximately 0.3% by weight of fatty acids as DHA and 0.6% as ARA.19,20 Subjects from the first phase who were eligible for enrollment in the second phase at 40 weeks PMA remained in their assigned formula group throughout the study.

Each study group was provided with premature (24 kcal/oz), discharge (22 kcal/oz), and term (20 kcal/oz) ready-to-use formulas, with the only differences being the polyunsaturated fatty acid profiles due to absence of DHA and ARA in control formulas and the sources of DHA in the supplemented formulas. The algal-DHA formulas were similar in ingredient and nutrient composition to Enfamil Premature LIPIL with Iron, EnfaCare LIPIL, and Enfamil LIPIL with Iron (Mead Johnson & Company, Evansville, IN). The protocol recommended feeding premature formula ≥14 days until at or near hospital discharge, discharge formula to 53 weeks PMA (3 months after term), and term formula to 92 weeks PMA (12 months after term). However, investigators were allowed discretion in selecting the formula type to meet the nutritional needs of each infant. Study formulas were to be the sole source of nutrition for preterm subjects until 57 weeks PMA (4 months after term) and the primary source of nutrition until 92 weeks PMA. Information was collected on which type of formula (premature, discharge, or term) each infant was consuming at 40, 44, 48, 53, and 57 weeks PMA. Study formula was stopped at 92 weeks PMA. Subjects in the second...
phase were monitored until 118 weeks PMA (18 months after term).

Growth, Intake, and Tolerance

Weight, length, and head circumference were measured by standardized procedures. All infants were assessed at birth and at 40, 44, 48, 53, 57, 66, 79, 92, and 118 weeks PMA. For preterm infants, growth data were collected weekly before hospital discharge and enteral intake and formula tolerance were recorded daily during hospitalization. Parents provided 24-hour diet and tolerance data at 40, 44, 48, 53, and 57 weeks PMA. Similar data were collected for the breast-fed term reference subjects at 44, 48, 53, and 57 weeks PMA.

Laboratory Measurements

Blood samples were collected from preterm subjects by heel prick or venipuncture at 57 weeks PMA, the age when exclusive formula feeding ended. Analyses included hematology; serum glucose, cholesterol, high-density lipoproteins, triglyceride, mineral, and electrolyte measurements; and liver and kidney function tests.

General Development

Trained testers at each site who were blinded to infants’ study group assignments administered the Bayley Scales of Infant Development II21 Mental Development Index (MDI) and Psychomotor Development Index (PDI) to all infants at 118 weeks PMA (18 months after term).

Morbidity and Adverse Events

Concomitant medical conditions (categorized by ICD-9-CM codes22) and use of concomitant therapies and medications were recorded for preterm infants during hospitalization. Detailed information was collected for specific conditions related to prematurity, including intraventricular hemorrhage, necrotizing enterocolitis (using modified Bell staging criteria), sepsis (confirmed by culture) or suspected sepsis, bronchopulmonary dysplasia (defined as requiring oxygen at 36 weeks PMA with severe or chronic changes to the lungs as seen on chest radiographs), and retinopathy of prematurity. All subjects were monitored for the occurrence of adverse events throughout the study. Investigators documented the occurrence and clinical outcome of each event.

Sample Size and Statistical Methods

The primary outcomes of this study were weights at 57 and 92 weeks PMA. A sample size of 58 infants per group would provide 80% power to detect a 500 g difference in weight among the study formula groups at 57 weeks PMA (SD = 950 g, α = 0.05, 2-tailed); 31 infants per group were required to identify an 870 g difference at 92 weeks PMA (SD = 1200 g, α = 0.05, 2-tailed). Anthropometric measurements at birth and subsequent study visits were analyzed by using analysis of variance (ANOVA). Study site, feeding regimen, and sex were included in the ANOVA models used to evaluate growth parameters. Bayley scores were analyzed by using an ANOVA model, including terms for study site and feeding regimen. The Van Elteren test, blocked for study site, was used to analyze laboratory measurements. Categoric variables were analyzed by using the Fisher exact test. Unadjusted pairwise comparisons were performed if initial tests were significant (P < .05).

RESULTS

Infant Characteristics

Preterm infants (n = 361) were enrolled in the first phase and randomly assigned to study formula groups (119 control, 112 algal-DHA, 130 fish-DHA). The fish-DHA group had significantly lower mean weight and head circumference at birth compared with the control and algal-DHA groups (Table II). The mean gestational ages at birth and at first consumption of study formula for the fish-DHA group also were less than those of the control group. The fish-DHA group had a somewhat lower mean weight and head circumference at birth compared with the control and algal-DHA groups (Table II). The mean gestational ages at birth and at first consumption of study formula for the fish-DHA group also were less than those of the control group. The fish-DHA group had a somewhat (P = .052) higher incidence of multiple (>2) births (27% vs 15% for control and 16% for algal-DHA) and fewer twins (15% vs 22% for control and 27% for algal-DHA). There were no significant differences among groups in distribution by sex, birth weight categories, or racial groups (data not shown). Fifty-six infants (21 control, 17 algal-DHA, 18 fish-DHA) in the first phase discontinued before 40-week PMA. The most common reasons for discontinuation were formula intolerance (n = 15), medical complications unrelated to the study (n = 13), and parental request (n = 11). There were no differences among groups in discontinuation rates or distribution of reasons for discontinuation.

Table I. Polyunsaturated fatty acid composition of study formulas (% by weight of total fatty acids)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Preterm formula</th>
<th>Discharge formula</th>
<th>Term formula</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study group</td>
<td>Control</td>
<td>Algal-DHA</td>
</tr>
<tr>
<td>18:2n-6 Linoleic</td>
<td>Control</td>
<td>18.7</td>
<td>18.6</td>
</tr>
<tr>
<td>18:3n-3 α-linolenic</td>
<td>Control</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>20:4n-6 ARA</td>
<td>Control</td>
<td>0.0</td>
<td>0.67</td>
</tr>
<tr>
<td>20:5n-3 EPA</td>
<td>Control</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>22:6n-3 DHA</td>
<td>Control</td>
<td>0.0</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Growth And Development Of Preterm Infants Fed Infant Formulas Containing Docosahexaenoic Acid And Arachidonic Acid
Sixty preterm infants (15 control, 23 algal-DHA, 22 fish-DHA) completing the first phase were not enrolled in the second phase for the following reasons: <80% of enteral feedings during hospitalization or <100% at 40 weeks PMA (n = 27); birth weight >1500 g (n = 19); formula intolerance (n = 6); parent (n = 4) or physician (n = 3) elected withdrawal; and >7 consecutive days off study formula (n = 1).

Two hundred forty-five preterm infants (83 control, 72 algal-DHA, 90 fish-DHA) and 105 breast-fed term infants were enrolled in the second phase. Three preterm infants with birth weight >1500 g who started the second phase before the protocol amendment remained in the study. Among the preterm groups, there were no significant differences in weight, length, head circumference, or gestational age at birth; gestational age when study formula was first consumed; sex or birth weight category (Table II); or racial distribution (data not shown). Compared with the algal-DHA group, the fish-DHA group had a higher incidence of multiple (>2) births (30% for fish-DHA vs 17% for algal-DHA) and a lower incidence of twins (11% for fish-DHA and 29% for algal-DHA; P < .01), with the control group intermediate (18% multiples and 20% twins). The distribution of infants across types of formula (premature, discharge, or term) did not differ among preterm groups at any time (data not shown). A total of 179 preterm (62 control, 52 algal-DHA, 65 fish-DHA) and 76 term infants completed the second phase. Discontinuation rates did not differ among study groups. Among the preterm groups, there were no differences in reasons for discontinuing the study during the second phase.

Growth

Mean weight, length, and head circumference growth rates in the first phase did not differ among preterm groups.

### Table II. Characteristics of study participants

<table>
<thead>
<tr>
<th>Infant characteristics</th>
<th>Control (n = 119)</th>
<th>Algal-DHA (n = 112)</th>
<th>Fish-DHA (n = 130)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)† (range)</td>
<td>1215 ± 33 (455–2340)</td>
<td>1189 ± 34 (490–2363)</td>
<td>1107 ± 31‡ (430–2280)</td>
</tr>
<tr>
<td>No. &lt;1000g/1000–1500 g/1500 g birth weight</td>
<td>30/73/16</td>
<td>27/77/8</td>
<td>44/79/7</td>
</tr>
<tr>
<td>Birth length (cm)† (range)</td>
<td>37.5 ± 0.4 (27–47)</td>
<td>37.4 ± 0.4 (28–48)</td>
<td>36.6 ± 0.4 (28–46)</td>
</tr>
<tr>
<td>Birth head circumference (cm)† (range)</td>
<td>26.7 ± 0.3 (19–41)</td>
<td>26.6 ± 0.3 (21–38)</td>
<td>25.9 ± 0.2‡ (21–33)</td>
</tr>
<tr>
<td>Gestational age at birth (weeks PMA)† (range)</td>
<td>29.6 ± 0.3 (23–35)</td>
<td>29.4 ± 0.3 (23–35)</td>
<td>28.8 ± 0.2‡ (24–34)</td>
</tr>
<tr>
<td>Age study formula first consumed (weeks PMA)† (range)</td>
<td>31.2 ± 0.2 (23–35)</td>
<td>30.9 ± 0.2 (23–35)</td>
<td>30.5 ± 0.2‡ (24–34)</td>
</tr>
<tr>
<td>No. male/female</td>
<td>67/52</td>
<td>54/58</td>
<td>73/57</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study group</th>
<th>Control (n = 83)</th>
<th>Algal-DHA (n = 72)</th>
<th>Fish-DHA (n = 90)</th>
<th>Breast-fed term (n = 105)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)† (range)</td>
<td>1179 ± 35 (455–2340)</td>
<td>1207 ± 37 (490–1590)</td>
<td>1110 ± 33 (430–1499)</td>
<td>3483 ± 39 (2724–4485)</td>
</tr>
<tr>
<td>No. &lt;1000g/1000–1500 g/1500 g birth weight</td>
<td>21/60/2</td>
<td>16/55/1</td>
<td>31/59/0</td>
<td>0/105</td>
</tr>
<tr>
<td>Birth length (cm)† (range)</td>
<td>37.5 ± 0.5 (27–46)</td>
<td>37.4 ± 0.5 (28–43)</td>
<td>36.7 ± 0.4 (28–44)</td>
<td>50.8 ± 0.2 (46–57)</td>
</tr>
<tr>
<td>Birth head circumference (cm)† (range)</td>
<td>26.6 ± 0.3 (19–41)</td>
<td>26.9 ± 0.4 (21–38)</td>
<td>26.0 ± 0.3 (21–30)</td>
<td>34.5 ± 0.1 (32–39)</td>
</tr>
<tr>
<td>Gestational age at birth (preterms; weeks PMA)† (range)</td>
<td>29.7 ± 0.3 (23–35)</td>
<td>29.7 ± 0.3 (23–34)</td>
<td>28.9 ± 0.3 (24–33)</td>
<td>—</td>
</tr>
<tr>
<td>Age study formula first consumed (weeks PMA)† (range)</td>
<td>31.4 ± 0.3 (27–36)</td>
<td>31.2 ± 0.3 (26–36)</td>
<td>30.7 ± 0.2 (25–34)</td>
<td>—</td>
</tr>
<tr>
<td>No. male/female</td>
<td>45/38</td>
<td>34/38</td>
<td>49/41</td>
<td>48/57</td>
</tr>
</tbody>
</table>

†Mean ± SEM (range).
‡P < .05, Fish-DHA vs control, algal-DHA.
§P < .05, Fish-DHA vs control.
Mean weight, length, and head circumference also did not differ among the three preterm groups at the completion of the first phase (40 weeks PMA; data not shown) or at the start of the second phase (Figure 2; data not shown for head circumference). Mean weights at the beginning of the second phase were (mean ± SEM) 2964 ± 56 g for the control group, 2954 ± 58 g for the algal-DHA group, and 3063 ± 51 g for fish-DHA group; mean lengths were 47.7 ± 0.31 cm for the control group, 47.6 ± 0.33 cm for the algal-DHA group, and 47.5 ± 0.28 cm for the fish-DHA group. Breast-fed term infants had greater mean weights (Figure 2, upper panel) than all preterm groups at all ages evaluated except the algal-DHA group at 118 weeks PMA. The algal-DHA group had greater mean weights than the control group at 66, 79, 92, and 118 weeks PMA and the fish-DHA group at 118 weeks PMA. Mean body length was greater for breast-fed term infants (Figure 2, lower panel) than all preterm groups at 40, 44, 48, 53, 57, and 66 weeks PMA and greater than the control and fish-DHA groups but not the algal-DHA group at 79, 92, and 118 weeks PMA. The algal-DHA group had greater mean lengths than the control group at 48, 79, and 92 weeks PMA and the fish-DHA group at 57, 79, and 92 weeks PMA. There were no differences in mean head circumference (data not shown) among the preterm and breast-fed term groups through 66 weeks PMA. Small differences (P < .05) were noted at 79 weeks PMA, with the algal-DHA group greater than the fish-DHA group and breast-fed term infants greater than the control and fish-DHA groups, and at 92 weeks PMA, with breast-fed term infants greater than the control and fish-DHA groups. Mean head circumference of the breast-fed term infants (mean ± SEM; 48.2 ± 0.29 cm) was greater than that of the control group (47.5 ± 0.30 cm; P = .052) and fish-DHA group (47.2 ± 0.32 cm; P = .004) at 118 weeks PMA, whereas the algal-DHA group (47.7 ± 0.36 cm) did not differ from any other group.

Intake and Tolerance

There were no differences in caloric intake from formula, daily gastric residuals, stool frequency, stool consistency, or abdominal distention among the preterm groups during hospitalization (data not shown). Based on parental reports, the algal-DHA group consumed more formula than did the fish-DHA group at 40 weeks PMA (mean ± SEM; 199.8 ± 8.5 vs 175.4 ± 7.5 mL/kg per day; P < .01) and the control and fish-DHA groups at 48 weeks PMA (215.9 ± 7.7 vs 188.3 ± 7.4 and 189.8 ± 6.9 mL/kg per day, respectively; P < .01), but there were no differences among preterm groups in reported mean formula intakes at 44, 53, or 57 weeks PMA. There were no differences among preterm groups with respect to parental reports of fussiness, diarrhea, or constipation at any time during the study (data not shown). Parents reported a greater incidence (P < .05) of “more gas than usual” for the algal-DHA group than the control group at 40 and 44 weeks PMA and for the fish-DHA group than the control group at 48 weeks PMA, but there were no differences at 53 or 57 weeks PMA.

General Development

Mean Bayley MDI and PDI scores of the breast-fed term infants at 118 weeks PMA (18 months after term) were near the reference norm of 100 and significantly higher than those for any preterm group (Figure 3). The algal-DHA and fish-DHA groups had higher MDI and PDI scores than did the control group. Similar differences were seen in a sub-analysis of the Bayley scores in which infants with organic
brain disease (eg, hydrocephalus, periventricular leukomalacia) were excluded (data not shown).

Safety Indexes

The only significant differences across 180 ICD-9-CM diagnostic categories22 diagnosed during initial hospitalization of preterm infants were “other conditions of the brain” (control, 9% vs fish-DHA 0%, P < .001) and “nonspecific low blood pressure readings” (algal-DHA, 4% vs fish-DHA, 0%; P = .019). Occurrences of specific medical conditions related to prematurity (Table III) were similar among groups during the first phase, with one exception. Incidence of intraventricular hemorrhage was similar at study enrollment (data not shown), but the algal-DHA group had a significantly lower occurrence at the end of the first phase. Among preterm groups, there were no differences in adverse events in the first phase and no differences in adverse events for any body system except the nervous system (control, 16% vs fish-DHA, 6%; P = .04) during the study. Two infants in the control group and 3 in the fish-DHA group died during initial hospitalization. Two infants in the control group died during the second phase of the study. Study site clinical investigators determined that the deaths were not related to study formula. There were significant differences among 3 of the 31 laboratory measurements: higher mean corpuscular hemoglobin for the fish-DHA group than for the control group (27.6 vs 27.0 pg/cell; P = .03); higher total cholesterol for the fish-DHA group than for the control and algal-DHA groups (3.85, 3.43, 3.48 mmol/L, respectively; P < .05); and lower serum potassium for the fish-DHA group than the control group (5.0 vs 5.3 mmol/L; P = .003).

DISCUSSION

This clinical trial demonstrated that feeding infant formulas with median worldwide human milk levels of DHA and ARA19,20 from single-cell algal and fungal oils can enhance growth of premature infants. A previous study18 found that very low birth weight (range, 846 to 1560 g; mean, ~1250 g) infants fed preterm formula with similar levels of DHA and ARA from the same sources gained weight faster during initial hospitalization than infants fed unsupplemented formula (34.7 vs 30.7 g/d). In addition, preterm infants fed the formula with DHA and ARA in the previous study had weights not different from breast-fed term infants at 48 and 57 weeks PMA, whereas those fed either unsupplemented formula or formula with DHA but no ARA remained significantly smaller than the term reference group at these ages. Our study did not find an effect of DHA and ARA on weight gain during initial hospitalization, which may be related to the inclusion of more extremely low birth weight infants with greater concomitant medical complications. It did demonstrate, however, longer-term growth enhancement associated with the tested levels of DHA and ARA from the algal and fungal oil sources. The very and extremely low birth weight infants fed formulas with DHA and ARA from algal and fungal oils from the start of enteral feeding to 12 months after term achieved body weights and lengths comparable to breast-fed term infants by 18 months after term, whereas infants fed unsupplemented formulas or formulas with DHA from fish oil did not. These results are of considerable importance because very and extremely low birth weight preterm infants remain at risk for subnormal weight and height through childhood and perhaps into adulthood.23–25

The mechanism for the increase in growth with DHA and ARA from algal and fungal oils is not known. Innis et al18 reported significant positive correlations between red blood cell ARA levels and weight and length of preterm infants at 40, 48, and 57 weeks PMA and speculated on potential mechanisms. Lapillonne et al12 also discussed mechanisms whereby DHA, EPA, and/or ARA may influence growth, including effects on eicosanoid production, gene expression, and membrane characteristics. In the current study, increased weight and length were seen with DHA from algal oil but not from fish oil, which provides some EPA in addition to DHA. Whether EPA played a role in the lower growth seen with the fish-DHA formulas compared with the algal-DHA formulas, however, is not clear. In the first phase of the study, the fish-DHA group had a lower mean birth weight than the control or algal-DHA groups, possibly related to more multiple (>2) births, but the mean birth weights of preterm infants continuing into the second phase did not differ among groups. Parents reported that infants in the algal-DHA group consumed more formula than those in the fish-DHA group at 40 weeks PMA and those in the control and fish-DHA groups at 48 weeks PMA, but intakes did not differ significantly among preterm groups at other ages.

Other studies of preterm infants fed formulas with DHA and ARA have not found enhanced growth, which may
be related to the levels and/or sources of the fatty acids or other aspects of study population characteristics or study design. Foreman-van Drongelen et al 13 and Vanderhoof et al 14,15 fed formulas with algal and fungal-oil sources of DHA and ARA, at similar levels (0.3% to 0.35% by weight of total fatty acids as DHA and 0.5% to 0.61% as ARA), to 3 and 2 months after term, respectively. Both studies, however, included preterm infants with higher average birth weights of ~1500 g. O'Connor et al 16 included smaller (mean birth weight, ~1300 g; range, 750 to 1805 g) and less healthy infants and fed test formulas to 12 months after term. The formulas studied, however, used lower levels of DHA (0.15% to 0.27%) and ARA (0.41% to 0.43%); DHA sources were fish oil and egg-derived triglycerides, with ARA from fungal oil. Fewtrell et al 17 studied preterm formula with egg lipid providing 0.17% DHA, 0.04% EPA, and 0.31% ARA. Study formulas were fed to preterm infants with mean birth weights of ~1340 g for only 31 to 33 days on average. Although the first three studies above 13-16 did not find consistent effects of DHA and ARA on preterm infant growth, Fewtrell et al 17 reported significant reductions in weight and length at 18 months after term associated with supplementation. Whether this negative effect on growth was related to the source of DHA and ARA or to some other aspect of the study formula, population, or design is unknown. However, the studies with the algal and fungal oils noted above, 13-16,18 as well as the current study, show no consistent negative effects on preterm infant growth and suggest the potential for growth enhancement of smaller premature infants.

In addition to the growth decrements associated with prematurity, very and extremely low birth weight infants have an increased risk of neurologic deficits and overall greater morbidity. 23-25 Thus, it was not unexpected that the breast-fed term group would have significantly higher Bayley scores than all groups of preterm infants. The more important finding is that the addition of these levels of DHA and ARA to the diets of preterm infants improved their mental and psychomotor development at 18 months after term, compared with preterm infants fed unsupplemented formula. Other investigators have not found significant differences in MDI or PDI scores at 12 16 or 18 17 months after term with supplemented formulas, although results suggested that smaller 16 or more preterm 17 infants might benefit from supplementation. Studies of term infants also show that the relation between DHA and ARA supplementation and developmental benefits may depend on levels, sources, or other differences in experimental design.26-28

Despite hypothetical concerns about adding DHA and ARA to formulas for preterm infants 23,29 such as potential interference with host defense mechanisms or impact on hemostasis, we found no increase in morbidity associated with supplementation. Our analysis of a wide spectrum of clinical data, including serum chemistry and hematology values and incidence and severity of medical conditions related to prematurity, found no safety issues related to the supplemented formulas. The finding of a significantly lower incidence of intraventricular hemorrhage in the algal-DHA group was unexpected. It is of interest that this group also had a higher incidence of nonspecific low blood pressure readings. Other investigators 13-18 also did not report increases in incidence of adverse events among preterm infants fed formulas with DHA and ARA.

Our study is unique in comparing algal oil and fish oil sources of DHA, each in combination with ARA from fungal oil for the long-term feeding of preterm infants, including those with extremely low birth weights and concurrent medical conditions associated with prematurity. Both combinations of long-chain polyunsaturated fatty acid sources supported significant developmental benefits for these at-risk infants. We also confirmed the growth-promoting effect of algal DHA plus fungal ARA, as first reported in the study by Innis et al 18. Overall, these results demonstrate that feeding formulas supplemented with DHA and ARA from algal and fungal oils at these levels to 12 months after term results in meaningful growth and development benefits for formula-fed preterm infants, even beyond the first year of life.

We gratefully acknowledge all of the clinical investigators and their research staff (see Appendix, available online at www.us.elsevierhealth.com/peds), as well as the participating infants and their caregivers for their contributions to the study. We thank Julia Boettcher and Alissa Willis for assistance in preparing the manuscript.

Table III. Occurrence of specific medical conditions in first phase (initial hospitalization)

<table>
<thead>
<tr>
<th>Medical condition</th>
<th>Control</th>
<th>Algal-DHA</th>
<th>Fish-DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Intraventricular</td>
<td>32 (29)</td>
<td>14* (13)</td>
<td>33 (27)</td>
</tr>
<tr>
<td>hemorrhage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrotizing</td>
<td>3 (3)</td>
<td>6 (5)</td>
<td>7 (5)</td>
</tr>
<tr>
<td>enterocolitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confirmed sepsis</td>
<td>16 (13)</td>
<td>19 (17)</td>
<td>19 (15)</td>
</tr>
<tr>
<td>Bronchopulmonary</td>
<td>17 (15)</td>
<td>16 (15)</td>
<td>21 (17)</td>
</tr>
<tr>
<td>dysplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinopathy of</td>
<td>31 (42)</td>
<td>35 (47)</td>
<td>53 (58)</td>
</tr>
<tr>
<td>prematurity</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P < .01, Algal-DHA vs control, fish-DHA.
†Stage II or III.
‡Defined as the requirement for oxygen at 36 weeks PMA with severe or chronic changes to the lung demonstrated on chest radiography.
REFERENCES


APPENDIX

M. Thomas Clandinin, John Van Aerde, Cheryl Harris, Kim Merkel, Mary Alice Springer, James Hansen, and Deborah Diersen-Schade were primary contributors to the design and supervision of the study, interpretation of results, and preparation of the paper. Other clinical investigators and sites were Dean Antonson, MD, Omaha, Nebraska; Jatinder Bhatia, MD, Medical College of Georgia, Augusta; Niceta Bradburn, MD, St Vincent’s Research, Indianapolis, Indiana; Sue Broyles, MD, Arch Center, Dallas, Texas; Jonathan Davis, MD, Winthrop University Hospital, Minneapolis, New York; Robert Gibson, PhD, Flinders Medical Centre, Bedford Park, Australia; David Green, MD, National Physician Partners, PA, Dallas, Texas; Laura Hillman, MD, University of Missouri, Columbia, Missouri; Satish Kalhan, MD, Metro Health Medical Center, Cleveland, Ohio; Mary Lim, MD, University of Florida, Jacksonville; Kathleen Meyer, MD, Baystate Medical Center, Springfield, Massachusetts; Brenda Morris, MD, Hermann Hospital, Houston, Texas; Steve Speadale, MD, Woman’s Hospital, Baton Rouge, Louisiana; Dennis Stevens, MD, University of South Dakota Medical School, Sioux Falls, South Dakota; Tracy Stewart, MD, Little Rock, Arkansas; and Jonathon Whitfield, MD, Baylor University Medical Center, Dallas, Texas.
TRENDS IN SEVERE BRONCHOPULMONARY DYSPLASIA RATES BETWEEN 1994 AND 2002

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Objective
To examine temporal trends in the rates of severe bronchopulmonary dysplasia (BPD) between 1994 and 2002.

Study design
In a retrospective cohort study, all infants with a gestational age (GA) <33 weeks in a large managed care organization were identified. Annual rates of BPD (defined as an oxygen requirement at 36 weeks corrected GA), severe BPD (defined as respiratory support at 36 weeks corrected GA), and death before 36 weeks corrected GA were examined.

Results
Of the 5115 infants in the study cohort, 603 (12%) had BPD, including 246 (4.9%) who had severe BPD. There were 481 (9.5%) deaths before 36 weeks corrected GA. Although the decline in BPD in this period was not significant, the rates of severe BPD declined from 9.7% in 1994 to 3.7% in 2002. Controlling for gestational age, the odds ratio (95% CI) for annual rate of decline in severe BPD was 0.890 (0.841-0.941). Controlling for gestational age, deaths before 36 weeks corrected GA also declined, with the odds ratio (CI) for the annual decline being 0.944 (0.896-0.996).

Conclusions
In this study population, the odds of having of BPD remained constant after controlling for GA. However, the odds of having severe BPD declined on average 11% per year between 1994 and 2002. (J Pediatr 2005;146:469-73)

Bronchopulmonary dysplasia (BPD) affects as much as 35% of very low birth weight infants (VLBW <1500 grams). This disease is marked by radiographic abnormalities, respiratory compromise, and a prolonged oxygen requirement. BPD is the result of a complex interaction of lung inflammation, lung injury, and lung repair that is in part associated with mechanical ventilation for surfactant deficiency.

Since the 1980s, the treatment of infants born prematurely has changed dramatically with the routine use of surfactant, increased use of antenatal steroids, decreased use of postnatal steroids, and improvements in mechanical ventilation. From the early 1980s to the early 1990s, the rate of infant mortality and BPD development among premature infants decreased. During the 1990s, the rate of infant mortality among premature infants continued to decline. Concurrently, the rate of BPD increased until the end of the 1990s, at which point it appeared to stabilize.

Despite its persistence, the BPD of today has been noted anecdotally to be less severe than the BPD of the past. Such documentation of trends in the rate of BPD by severity, however, has not been substantiated with empirical studies. To clarify these trends, we examined the rates of severe BPD in a 9-year period in a cohort of premature infants. We hypothesized that the rate of severe BPD is declining.

METHODS

Study Population
The infants were cared for in the 6 level III neonatal intensive care units (NICUs) in the Northern California Kaiser Permanente Medical Care Program (KPMCP) between...
1994 and 2002. All infants were admitted alive to the NICU with a gestational age (GA) <33 weeks. Infants with major congenital malformations were excluded from the study.

Data were obtained from the KPMCP Neonatal Minimum Data Set (NMDS) by using previously described methods.14-16 The NMDS database captures >95% of level III NICU admissions from a population of approximately 3.2 million members and 30,000 births each year.16 The institutional review boards of KPMCP and the Beth Israel Deaconess Medical Center approved this study.

Variables

We had 3 outcome variables: BPD, severe BPD, and death before 36 weeks corrected GA. We defined BPD, according to the National Institute of Child Health and Human Development (NICHD), as the use of supplemental oxygen at 36 weeks corrected GA,17 and severe BPD as the use of respiratory support (in the form of mechanical ventilation, continuous positive airway pressure (CPAP), and/or supplemental oxygen) at 36 weeks corrected GA. Because an infant had to reach 36 weeks corrected GA to qualify for the aforementioned definitions, we also examined deaths in infants younger than 36 weeks corrected GA. Some of these infants may have met the definition for BPD or severe BPD had they lived to 36 weeks corrected GA. Retinopathy of prematurity was defined as Stage 1 or greater as documented by the ophthalmologist on an ophthalmologic examination.

Infants were considered to have necrotizing enterocolitis (NEC) when any of the following conditions were present: several progress notes stating the presence of NEC and simultaneously the infant was nothing by mouth (NPO) for a minimum of 5 days; the infant had surgery for necrotic bowel; necrotic or gangrenous appearance of baby's intestines found at surgery; a pathologist confirmed that NEC was present in a surgically obtained bowel specimen; a pathologist confirmed that NEC was present at autopsy; or an isolated ileal perforation was present. Infants with these conditions were not defined as having NEC: bowel surgery because of conditions other than NEC; the presence of abdominal distention and an NPO order (''NEC scare''); or the presence of bilious vomiting or residuals and an NPO order (''NEC scare'').

An infant was defined as having intraventricular hemorrhage when a radiologist documented any of the following: intraventricular blood present without ventricular dilation; intraventricular blood present with ventricular dilation; or parenchymal hemorrhage present.

Statistical Analysis

We examined the unadjusted observed rates of BPD, severe BPD, and death before 36 weeks corrected GA overall and by GA. We then examined individually the association of severe BPD with race, sex, and small for gestational age (SGA) status, which was defined as being less than the 10th percentile for GA. Finally, we controlled for GA as an ordinal variable in all of the bivariate and logistic regression analyses.

Bivariate analyses of trends were performed with the Cochran–Mantel-Haenszel (CMH) trend test controlled across GAs. We used logistic regression analysis, controlling for GA, to assess the direction and magnitude of the trend in all outcomes. These logistic regression models provided odds ratios (ORs) for the annual rate of decline. The analyses were
conducted first on the entire cohort. The analyses were then repeated on the portion of the cohort with a GA <29 weeks. Finally, the analyses were repeated on the portion of the cohort with a GA between 29 and 33 weeks. Statistical analyses were performed with the SAS software version 8 package (SAS Institute; Carey, NC; 2000).

RESULTS

Study Sample

Of the 5115 infants studied, 603 (12%) had BPD, including 246 (4.9%) who had severe BPD. There were 481 deaths (9.5%) before 36 weeks corrected GA. Table I shows that of the infants with BPD, infants with severe BPD were more likely to be male, have lower birth weights, have longer NICU stays, and have more medical complications.

Trends in BPD

The trends in BPD, severe BPD, and death before 36 weeks corrected GA were similar in subgroups of the study population defined by race, sex, or SGA status. We therefore did not control for these characteristics in any of our analyses. As shown in Table II, the rate of BPD declined from 14.5% in 1994 to 11.8% in 2002, a non-significant difference after controlling for GA (CMH chi-square = 0.59; \( P = .44 \)). This was also true in both of the cohort subdivisions, GA <29 weeks and GA between 29 and 33 weeks (CMH chi-square = 0.41; \( P = .52 \) and CMH chi-square = 0.27; \( P = .60 \), respectively).

Trends in Severe BPD

In contrast, severe BPD declined from 9.7% to 3.7% in this same period. The rate of severe BPD declined from approximately 26% to 8% in the subset of infants with a GA <29 weeks and from approximately 5% to 2% in the infants with a GA 29 weeks or greater (Table II). When controlling for GA, this decline in the rates of severe BPD in the study period was significant (CMH chi-square = 19.4; \( P < .0001 \)). The GA-adjusted odds ratio for the annual change in rate of severe BPD was 0.890 (95% CI, 0.841-0.941).

This trend was consistent in the portion of the cohort with a GA <29 weeks. When controlling for GA, this decline in the rates of severe BPD during the study period was significant (CMH chi-square = 19.4; \( P < .0001 \)). The GA-adjusted odds ratio for the annual change in rate of severe BPD was 0.890 (95% CI, 0.841-0.941).

Conversely, in the portion of the cohort with a GA between 29 and 33 weeks, when controlling for GA, this decline in the rates of severe BPD during the study period failed to reach significance (CMH chi-square = 1.43; \( P = .23 \)). The GA-adjusted odds ratio for the annual change in rate of severe BPD was 0.950 (95% CI, 0.872-1.036).

Trends in Death in Infants Younger Than 36 Weeks Corrected GA

The rate of death in infants younger than 36 weeks corrected GA was consistently between 6% and 13%. When controlling for GA, the rates of death before 36 weeks corrected GA were similar in subgroups of the study population defined by race, sex, or SGA status. We therefore did not control for these characteristics in any of our analyses.

Table II. Unadjusted annual rates of severe bronchopulmonary dysplasia, bronchopulmonary dysplasia, and death before corrected gestational age of 36 weeks for the entire cohort and subdivided by gestational age.

<table>
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<tbody>
<tr>
<td>Entire cohort (n = 5115)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>289</td>
<td>458</td>
<td>445</td>
<td>610</td>
<td>659</td>
<td>702</td>
<td>660</td>
<td>638</td>
<td>654</td>
</tr>
<tr>
<td>BPD (%)</td>
<td>42 (14.5)</td>
<td>63 (13.7)</td>
<td>48 (10.8)</td>
<td>47 (7.7)</td>
<td>81 (12.3)</td>
<td>70 (10.0)</td>
<td>94 (14.2)</td>
<td>95 (14.9)</td>
<td>77 (11.8)</td>
</tr>
<tr>
<td>Severe BPD (%)</td>
<td>28 (9.7)</td>
<td>32 (7.0)</td>
<td>23 (5.2)</td>
<td>20 (3.3)</td>
<td>31 (4.7)</td>
<td>30 (4.3)</td>
<td>33 (5.0)</td>
<td>31 (4.9)</td>
<td>24 (3.7)</td>
</tr>
<tr>
<td>Died before 36 weeks corrected GA (%)</td>
<td>22 (7.6)</td>
<td>38 (8.3)</td>
<td>27 (6.1)</td>
<td>78 (12.8)</td>
<td>62 (9.4)</td>
<td>70 (10.0)</td>
<td>74 (11.2)</td>
<td>60 (9.4)</td>
<td>55 (8.4)</td>
</tr>
<tr>
<td>GA &lt;29 weeks (n = 1441)</td>
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<tr>
<td>n</td>
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<td>116</td>
<td>173</td>
<td>200</td>
<td>195</td>
<td>198</td>
<td>193</td>
<td>195</td>
</tr>
<tr>
<td>BPD (%)</td>
<td>24 (34.3)</td>
<td>39 (38.6)</td>
<td>34 (29.3)</td>
<td>31 (17.9)</td>
<td>52 (26.0)</td>
<td>45 (23.1)</td>
<td>64 (32.3)</td>
<td>65 (33.7)</td>
<td>55 (28.2)</td>
</tr>
<tr>
<td>Severe BPD (%)</td>
<td>18 (25.7)</td>
<td>22 (21.8)</td>
<td>16 (13.8)</td>
<td>13 (7.5)</td>
<td>17 (8.5)</td>
<td>20 (10.3)</td>
<td>26 (13.1)</td>
<td>20 (10.4)</td>
<td>16 (8.2)</td>
</tr>
<tr>
<td>Died before 36 weeks corrected GA (%)</td>
<td>18 (25.7)</td>
<td>23 (22.8)</td>
<td>18 (18.1)</td>
<td>65 (37.6)</td>
<td>58 (29.0)</td>
<td>62 (31.8)</td>
<td>60 (30.3)</td>
<td>55 (28.5)</td>
<td>47 (24.1)</td>
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<tr>
<td>GA greater than or equal to 29 but less than 33 weeks (n = 3674)</td>
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<td></td>
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<td>n</td>
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<td>329</td>
<td>437</td>
<td>459</td>
<td>507</td>
<td>462</td>
<td>445</td>
<td>459</td>
</tr>
<tr>
<td>BPD (%)</td>
<td>18 (8.2)</td>
<td>24 (6.7)</td>
<td>13 (4.2)</td>
<td>16 (3.7)</td>
<td>29 (6.4)</td>
<td>25 (5.0)</td>
<td>30 (6.5)</td>
<td>30 (6.8)</td>
<td>22 (4.8)</td>
</tr>
<tr>
<td>Severe BPD (%)</td>
<td>10 (4.6)</td>
<td>10 (2.8)</td>
<td>7 (2.1)</td>
<td>7 (1.6)</td>
<td>14 (3.1)</td>
<td>10 (2.0)</td>
<td>7 (1.5)</td>
<td>11 (2.5)</td>
<td>8 (1.7)</td>
</tr>
<tr>
<td>Died before 36 weeks corrected GA (%)</td>
<td>4 (1.8)</td>
<td>15 (4.2)</td>
<td>6 (1.8)</td>
<td>13 (3.0)</td>
<td>4 (0.9)</td>
<td>8 (1.6)</td>
<td>14 (3.0)</td>
<td>5 (1.1)</td>
<td>8 (1.7)</td>
</tr>
</tbody>
</table>
corrected GA, however, declined significantly during the study period (CMH chi-square = 4.0; \(P = .045\)). The GA-adjusted odds ratio for death before 36 weeks corrected GA was significant at 0.944 (95% CI, 0.896-0.996).

This trend was not seen in either subdivision of the cohort. When controlling for GA, the rates of death before 36 weeks corrected GA failed to decline significantly during the study period for infants with a GA <29 weeks and GA between 29 and 33 weeks (CMH chi-square = 0.54; \(P = .46\) and CMH chi-square = 2.51; \(P = .11\), respectively).

Sensitivity Analysis

Because infants who died when they were younger than 36 weeks corrected GA by definition could not have BPD, we performed a “worst-case-scenario” sensitivity analysis to test the stability of our effect estimate for severe BPD in the face of death as a competing risk. When all deaths before 36 weeks corrected GA were coded as cases of severe BPD, the GA-adjusted odds ratio for the annual change in severe BPD remained significant at 0.905 (95% CI, 0.868-0.945). This observation was consistent for the portion of the cohort with a GA <29 weeks. In this group, the GA-adjusted odds ratio for the annual change in severe BPD remained significant at 0.92 (95% CI, 0.872-0.971).

DISCUSSION

In this population of premature infants born between 1994 and 2002, although the overall rate of BPD remained constant, the GA-adjusted odds of having severe BPD declined on average 11% per year (\(P < .0001\)). In uncontrolled analysis, most of the decline occurred between 1994 and 1998. The odds of an infant dying before 36 weeks corrected GA during this same period also declined 5.6% per year (\(P = .03\)). These data are consistent with and extend those from earlier reports.\(^8\) The rate of decline of severe BPD is similar to the decline seen for BPD overall between 1980 and 1990.\(^3\) The decline in mortality rate was the same as seen between 1991 and 1999.\(^11\)

In addition to its clinical significance, the finding of a declining rate of severe BPD has important economic implications. In one study, non-asthmatic chronic respiratory disease, consisting primarily of BPD and respiratory sequelae of prematurity, was associated with the highest mean per-patient costs and the second highest state-level Medicaid expenditure of 8 chronic conditions.\(^8\) Even a small reduction in the number of children with the condition would free resources for other important health conditions.

It is clear from this data and other data sets that infants with a GA <29 weeks are at a greater risk for developing severe BPD. The portion of the study cohort with a GA <29 weeks showed a decline in severe BPD similar to the undivided cohort. Alternatively, the infants with a GA between 29 and 33 weeks did not show this decline. This would imply that there may be a fundamental difference in the severe BPD in these groups. This difference could be in either their underlying physiology or their response to medical intervention.

In this study, we did not have reliable data on the use of interventions and were therefore unable to make inferences about the effect that technology had on the decline in severe BPD. It is possible that the well-documented interventions that contributed to the decline in BPD initially also ameliorated the disease. For example, surfactant use has become ubiquitous in modern treatment of premature infants with respiratory distress syndrome. Its benefit in reducing neonatal morbidity and mortality rates is well documented.\(^2\)

The early decline seen in severe BPD in this study could be associated with the implementation of surfactant therapy in the early 1990s. Studies have shown a reduction in BPD also associated with CPAP\(^2\) and High Frequency Ventilation (HFV).\(^2,9\) Studies have also shown a reduction in the mortality rate for infants who are at the greatest risk for developing BPD associated with the use of antenatal steroids.\(^11\) Antenatal steroids may have improved the response to surfactant therapy in premature infants with respiratory distress syndrome\(^6\) and thereby contributed to the decrease in the rate of severe BPD. HFV and CPAP may have reduced lung injury and allowed infants to be weaned from respiratory support more quickly. Moreover, recently introduced practices may ameliorate the severity of BPD, such as permissive hypercapnea and more reliance solely on early CPAP.\(^5,19\) With the recognition of the deleterious effects of postnatal steroids,\(^20-22\) the field of neonatology dramatically reduced their usage to treat pre-term infants. Although we do not have data on postnatal steroid use in this cohort, it is reasonable to assume that, with the rest of the field of neonatology, there was a decrease in the use of postnatal steroids in the latter years of the study. This change in practice had the potential to increase the number of infants receiving respiratory support at 36 weeks corrected GA. This observation adds even more credence to the findings in this study.

It is possible that the risk profile of the infants may have changed during the study period. Although KPMCP does not have a general policy for resuscitation of infants in the delivery room, choosing to leave those decisions to the physician in attendance, it is possible that the attitude for resuscitation of the lowest GA infants could have changed. If this were true, it would only affect an insignificant number of infants and would not likely have any bearing on the results reported in this report.

Despite these limitations in identifying the cause of the decline, the central finding of the study is robust. Although the odds of having BPD have remained stable in the past decade, the rate of severe BPD declined significantly. Further work could focus on how this less severe form of BPD varies from traditional BPD.\(^23,24\) Evaluating the impact of changing NICU technologies on the rates of severe BPD, and identifying preventable predictors of BPD, so that the overall rate of BPD can be reduced.

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REFERENCES


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Sample mailing label
This is your subscription account number 3-DIGIT 001
SJ P1
FEB00 J009 C: 1 1234567-89 U 05/00 Q: 1
J. H. DOE, MD
531 MAIN ST
CENTER CITY, NY 10001-001

Personal subscriptions to The Journal of Pediatrics Online are for individual use only and may not be transferred. Use of The Journal of Pediatrics Online is subject to agreement to the terms and conditions as indicated online.
Objectives  To assess insulin dynamics to oral glucose tolerance testing in obese children, denoting individual contributions of insulin hypersecretion versus resistance to racial and etiopathogenetic specificity.

Study design  We performed 3-hour oral glucose tolerance testing in 113 nondiabetic obese children (age 13.6 ± 3.1 years; 41 male, 78 female; 37 black, 41 white; 35 with central nervous system [CNS] insult). The corrected insulin response (CIRgp; measuring β-cell secretion) and the composite insulin sensitivity index (CISI) were computed and log-transformed, and each was modeled in terms of the other, plus race/etiology, age, sex, body mass index z score, glucose tolerance, pubertal status, and geographic location.

Results  A scatterplot of logCIRgp versus logCISI showed that racial and etiopathogenetic groups plotted in different areas. CISI (controlled for CIRgp and other variables) was only 13% lower in blacks than in whites (P = .32). Conversely, CIRgp (controlled for CISI and other variables) was 49% higher in blacks (P = .028). CNS insult exhibited a 40% higher CIRgp (P = .054) and 11% higher CISI (P = .42) than intact white subjects.

Conclusions  Insulin hypersecretion and resistance are distinct phenomena in childhood obesity. Insulin hypersecretion appears to be the more relevant insulin abnormality both in obese blacks and in CNS insult. (J Pediatr 2005;146:474-81)
a manifestation instead be a manifestation of the hypersecretion? For example, insulin hypersecretion caused by β-cell dysregulation may be a primary phenomenon in the genesis of weight gain in some obese children and adults.27-31 Controversy continues regarding which comes first: the hyperinsulinism, the insulin resistance, or the obesity.32,33 This study was performed to determine whether insulin hypersecretion and insulin resistance are primary and independent phenomena in obese children and to determine if they segregate by race and etiopathogenesis (ie, central nervous system [CNS] insult).

**METHODS**

This study was performed at the University of Tennessee (UT) General Clinical Research Center and the University of California San Francisco (UCSF) Pediatric Clinical Research Center. Institutional review board and scientific advisory committee approvals were obtained at each site. Inclusion criteria were children 2 to 18 years of age, BMI defined as weight \( / \text{height}^2 \) [kg/m\(^2\)] above the 95th percentile for the BMI-for-age curve (Centers for Disease Control, 1999), and with continued weight gain for 3 months after attempts at outpatient exercise and nutritional counseling. Exclusion criteria included voluntary weight maintenance or loss, diabetes mellitus, renal or hepatic disease (or significant elevation of liver enzymes >3 times the upper limit of normal), antidepressant medication, and any glucocorticoid therapy in intact children or supraphysiologic doses of glucocorticoid (elevations >3 times the upper limit of normal), or loss, diabetes mellitus, renal or hepatic disease (or significant elevation of liver enzymes >3 times the upper limit of normal), antidepressant medication, and any glucocorticoid therapy in intact children or supraphysiologic doses of glucocorticoid therapy (>11 mg/m\(^2\) per day) in children with CNS insult, while controlling for sex, pubertal status, glucose tolerance status, BMI, and race.

**Chemical Analyses**

Plasma glucose concentrations were measured by the glucose oxidase method.39 Plasma insulin concentrations were measured by radioimmunoassay at each site. The interassay and intra-assay coefficients of variation were 9.2% and 4.5%, and 8.4% and 4.3% at UT and UCSF, respectively.

**Insulin Indexes**

From the OGTT, the following insulin indexes were obtained or computed: (1) corrected insulin response at glucose peak (CIR\(_{gp}\)), an indicator of β-cell activity: The higher the CIR\(_{gp}\), the greater the insulin secretion for the same glucose stimulus: CIR\(_{gp}\) = [(Insulin\(_{glupeak}\) × 100) \( / \) (Glu\(_{glupeak}\) × [Glu\(_{glupeak}\) - 70]); (2) composite insulin sensitivity index (CISI), a measure of insulin sensitivity obtained from the OGTT: The higher the CISI, the higher insulin sensitivity: CISI = 10000 \( / \) \( \sqrt{\text{FG}} \) \( \times \) [mean insulin(0–120 min)] / [mean glucose(0–120 min)].

**Statistical Analysis**

We categorized patients by race/ethnicity as white or black, except that for descriptive and analytic purposes, those with CNS insult were considered a separate category (the overwhelming majority were white and were therefore not subcategorized by race). The insulin indexes CISI and CIR\(_{gp}\) were both skewed, so they were transformed by base 2 logarithm for all analyses. Although there are numerous reports denoting the inverse relation between insulin secretion and resistance,19,26,42 we instead wished to examine the effects of race and CNS insult, after accounting for this known relation and other possible confounding factors, which have not been previously considered. We therefore used multiple linear regression analysis to model CISI and CIR\(_{gp}\) in terms of each other and group defined as black, white, and CNS insult, while controlling for sex, pubertal status, glucose tolerance status, BMI, and race, and location. To enhance interpretability, we backtransformed the estimated effects and confidence intervals of all variables into percentage effects by using the formula 100 * ([\( b \) \( / \) \( b \) \( - \) 1]), where \( b \) is the estimated effect on the log base-2–transformed outcome.

**RESULTS**

We studied a total of 119 nondiabetic obese children. Their demographic and insulin data are listed in Table I, stratified by race and CNS insult. Eighty-nine were studied in...
Memphis from 1996 to 2001 and 30 were studied in San Francisco from 2001 to 2003. As the number of Pacific Islanders and Hispanics was too low to allow for statistical analysis, they are not included in further analyses, leaving 113 subjects. Blacks exhibited a higher CIRgp versus either whites or CNS insult and lower CISI than either whites or CNS insult.

Figure 1 illustrates the glucose and insulin responses to OGTT for the cohort, stratified by race and by CNS insult. Although the frequency of glucose intolerance was similar in each group, whites exhibited the greatest glucose excursion (Figure 1, A). On average, blacks exhibited a rapid rise in insulin (Figure 1, B) within the first 30 minutes, which plateaued for the course of the OGTT, consistent with rapid and excessive insulin secretion, insulin resistance, and reduced insulin clearance. On average, white children and those with CNS insult exhibited a rapid rise within 30 minutes, but of lower magnitude than blacks, followed by a slow but steady decline, consistent with improved insulin sensitivity and better insulin clearance.

A scatterplot of CIRgp versus CISI demonstrated a hyperbolic relation (Figure 2, A). Log base-2 transformation of each insulin index yields the scatterplot shown in Figure 2, B, which exhibited a negative linear correlation overall ($r = -0.54, P < .001$). Different racial and etiopathogenetic groups tended to plot in different areas. Arbitrary cutoffs (dashed lines) for CIRgp (1.5) and CISI (1.7) divide the plot into 4 quadrants. The majority of white children (open squares) plotted in the lower right quadrant, with a CIRgp of <1.5 and a CISI of >1.7, indicating lower insulin secretion and better insulin sensitivity. The preponderance of children with CNS insult (gray squares) plotted in the upper right quadrant, with a CIRgp of >1.5 and a CISI of <1.7, indicating insulin hypersecretion with better insulin sensitivity. Finally, the majority of black children (filled squares) plotted in the upper left quadrant, with a CIRgp of >1.5 and a CISI of <1.7, indicating both insulin hypersecretion and resistance.

Table II models the CIRgp and CISI in terms of each other, and the other variables are presented. The expected inverse relation between secretion and resistance was apparent in these multivariate models, with each doubling of CIRgp estimated to be associated with a 22% decrease in CISI (95% CI, 14% to 28%; $P < .0001$) and each doubling of CISI associated with a 36% decrease in CIRgp (95% CI, 24% to 45%; $P < .0001$). CISI, after controlling for CIRgp, averaged 19% lower in blacks than in whites ($P = .12$); when also controlled for all other variables, this racial dichotomy reduced to only 13% ($P = .32$). Conversely, CIRgp (controlled for CISI) was 58% higher in blacks than in whites ($P = .011$), and when controlled for other variables, CIRgp remained 49% higher in blacks ($P = .028$). Those with CNS insult exhibited a 17% higher CISI than whites ($P = .23$), and when controlled for the other variables, this difference reduced to 11% ($P = .42$). However, CIRgp was 51% higher in CNS insult versus whites ($P = .018$); when controlled for all other variables, CIRgp remained 40% higher in those with CNS insult ($P = .054$).

DISCUSSION

The IVGTT and EHC have been the gold standards for measuring insulin sensitivity over the past 20 years. Evaluation of β-cell function by measuring the AIR to glucose infusion by using each of these procedures is also routinely performed. In these procedures, the increase in AIR corresponds to a reduction in insulin sensitivity, hence
the hyperbolic relation, termed the “disposition index.” Decline in the AIR corresponds with β-cell failure and the development of impaired glucose tolerance. However, use of the AIR as a measure of excessive insulin secretion is complicated by the fact that the IVGTT and EHC bypass two important enteric stimuli to β-cell activity, those of vagal efferents and insulinotropic incretins such as glucagon-like peptide-1 (GLP-1) released from the L-cells of the distal small intestine.

Weight gain may be more attributable to insulin hypersecretion than to insulin resistance. For instance, in normal adults, weight gain over a 15-year period correlated best with the magnitude of the acute insulin response to IVGTT. Insulin hypersecretion exists in 38% of obese adults, whereas insulin resistance exists in an overlapping 25% of adults. In French children, insulin hypersecretion was shown to precede the obesity and the insulin resistance and with the molecular correlate of the variable N-terminal repeat (VNTR) of the insulin gene. In the United States, coexistence of insulin hypersecretion and insulin resistance has been noted in black children. Furthermore, children with hypothalamic obesity after CNS insult exhibit insulin hypersecretion, which responds to insulin suppression using the somatostatin analog octreotide with weight loss and improved quality of life. Similarly, suppression of acute insulin secretion in a subset of obese adults manifesting insulin hypersecretion without insulin resistance promotes weight loss. Indeed, others have speculated that insulin resistance may be a compensatory physiologic response to limit further weight gain.

Since we were interested in vagal modulation of β-cell function, we computed insulin secretion and sensitivity indexes from the OGTT. The OGTT has the advantage of mimicking the normal physiologic response, including neural response, to glucose ingestion. Compared with the IVGTT and EHC, measurement of β-cell activity and insulin sensitivity from the OGTT is less time-consuming and easier to perform in children and in large-scale and population studies. The CISI derived from the OGTT correlates better with insulin sensitivity than either the HOMA or QUICKI in adults. Yeckel et al validated the use of the CISI from the OGTT in obese children and showed that it provided the best concordance with the M-value from EHC data in this population. In adults, Kanauchi et al have suggested that a CISI >3 is indicative of normal insulin sensitivity.

Our data recapitulate the hyperbolic relation between insulin secretion and insulin resistance described by Bergman, termed the “disposition index” (Figure 2, A). Despite the clear inverse relation between secretion (log CIRgp) and resistance (log CISI) (Figure 2, B), we wished to determine factors that alter this relation. Although our data cluster along a line, there is considerable scatter, and each racial group and children with CNS insult tend to exhibit differing levels of secretion vs resistance, suggesting that these are under partially separate control. Blacks exhibit a CISI (when controlled for CIRgp) approximately 19% lower than whites, indicating only slightly worsened insulin resistance. This difference in CISI between blacks and whites decreases to 13% when the other potential confounding factors, for example, sex, puberty, BMI z-score, glucose tolerance, and location (Memphis versus San Francisco), are taken into account. Location played a minor role in CISI difference between blacks and whites. When controlling for location, the blacks are still more insulin-resistant than whites, although the CISI rises, suggesting improved insulin sensitivity.

Figure 2. Scatterplots of insulin secretion (CIRgp) versus sensitivity (CISI) in 113 obese nondiabetic children. A, CIRgp versus CISI; B, CIRgp versus CISI plotted logarithmically.
might be explained by the fact that 45% of subjects in Memphis are black, whereas only 7% of those in San Francisco are black. Thus, when all of these confounding factors are adjusted, the difference in magnitude of insulin resistance between blacks and whites is obviated.

Black children exhibit insulin hypersecretion compared with white children, as manifest by a 58% increase in CIRgp, when CISI is controlled. This finding is not explained by other confounding variables, as the difference remains at 49% (P = .028). Gower et al42 and Uwaifo et al62 also noted increased AIR in blacks that was not explained by either the degree of insulin sensitivity or adiposity. One possible explanation may be differences in GLP-1, as recent studies have demonstrated increased GLP-1 levels in obese blacks compared with whites.63,64 In addition, reductions in insulin clearance have been noted in blacks, which may also contribute to alterations in sensitivity.19 One acknowledged weakness of our study is that we did not measure C-peptide levels to compute insulin clearance; however, the rapid insulin secretion with a peak at 30 minutes is unlikely to be entirely due to poor insulin clearance. It is also possible that both genetic and nutritional factors, such as geographic differences in fat and soft drink consumption and degree of physical activity, may also play a role in this difference in CIRgp, even within blacks. It is conceivable that the exaggerated insulin secretion evident in blacks may lead to β-cell failure over the long term and an increased incidence of type 2 diabetes mellitus.65

Our findings are different than those seen in previous pediatric studies using IVGTT and EHC, which imply significant racial differences in insulin sensitivity between blacks and whites.17,18,20,22,66 The blacks in such studies have higher fasting insulin and acute insulin response to glucose (AIR), as well as decreased insulin sensitivity. However, in our study, after controlling for age, sex, puberty, and degree of obesity, the racial differences in insulin sensitivity are minimized but the differences in insulin secretion remain accentuated. Furthermore, these other studies may have underestimated the racial differences in insulin secretion. The glucose-stimulated insulin level in obese Latino children were a better correlate of insulin sensitivity than was the fasting level.67 Our findings support the use of the OGTT as a measure of both insulin secretion and sensitivity in children. These findings may also be associated with the racial differences in the prevalence of the Metabolic Syndrome.25 Furthermore, we have found that the degree of insulin resistance predicts the weight response to metformin therapy in whites but not in blacks,68 perhaps their insulin hypersecretion rather than resistance is the biological underpinning of this dichotomy.

Hypothalamic obesity from CNS insult is due to damage to the ventromedial hypothalamus, which leads to a disinhibition of vagal tone from the dorsal motor nucleus of the vagus59,70 and increased acetylcholine secretion at the pancreatic β-cell, which results in an inappropriate glucose-mediated insulin hypersecretion.71 The increased CIRgp and higher CISI (of borderline significance) seen in this study when compared with intact children imply that this phenomenon is due to β-cell dysfunction and insulin hypersecretion rather than to insulin resistance; however, despite the hypersecretion, these children remain insulin-sensitive. This is in contradiction to the black population. Such insulin hypersecretion can be suppressed with the somatostatin agonist octreotide,52,53 which binds to a somatostatin receptor-5 receptor on the β-cell, coupled to the voltage-gated calcium channel, resulting in decreased calcium influx and insulin release in response to glucose. Therefore, these three cohorts of obese children manifest very different insulin dynamics, suggesting that their obesity may be due to different pathologies.

Insulin hypersecretion may promote insulin resistance through alterations of glucose transport72,73 or through

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### Table II. Linear regression models of CISI and CIRgp: Effect of group

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Blacks vs whites</th>
<th>CNS insult vs whites</th>
<th>Overall P value*</th>
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</thead>
<tbody>
<tr>
<td>Percent difference between groups (95% CI)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CISI†</td>
<td>−19% (−38% to 6%)</td>
<td>17% (−9% to 51%)</td>
<td>.025</td>
</tr>
<tr>
<td>CISI‡</td>
<td>−13% (−33% to 14%)</td>
<td>11% (−14% to 44%)</td>
<td>.22</td>
</tr>
<tr>
<td>CIRgp†</td>
<td>58% (11% to 123%)</td>
<td>51% (8% to 111%)</td>
<td>.015</td>
</tr>
<tr>
<td>CIRgp‡</td>
<td>49% (4% to 112%)</td>
<td>40% (−0.6% to 98%)</td>
<td>.051</td>
</tr>
</tbody>
</table>

*Omnibus test of any difference among the three groups.  
†Percent effects estimated in multivariate models of logarithmically transformed CISI controlling for CIRgp (continuous, logarithmically transformed) and logarithmically transformed CIRgp controlling for CISI (continuous, logarithmically transformed).  
‡Percentage effects estimated in multivariate models of logarithmically transformed CISI, CIRgp, controlling for puberty (prepubertal [ Tanner stage I] versus pubertal [Tanner stage II-V]), glucose tolerance (impaired versus normal), sex, location (San Francisco versus Memphis), CIRgp (continuous, logarithmically transformed), CISI (continuous, logarithmically transformed) and BMI z score (continuous, linear, and quadratic term).
downregulation of the insulin receptor and can cause weight gain directly by increased lipogenesis. Conversely, insulin resistance may promote compensatory hyperinsulinemia through poorly understood hepatic reflex mechanisms and can cause lipogenesis through upregulation of the Glut4 transporter, the enzyme acetyl-CoA carboxylase, fatty acid synthase, and lipoprotein lipase. However, in distinction to the concept of the "disposition index," our study supports the hypothesis that insulin hypersecretion and insulin resistance are partially independent phenomena and may manifest differently in different ethnic and etiopathogenetic groups. In light of individual successes of leptin therapy for leptin deficiency, "octreotide for hypothalamic obesity," and metformin for insulin resistance, we conjecture that obesity is a phenotype of different pathogenetic factors and that insulin hypersecretion and insulin resistance are partially independent phenomena. We speculate that measurement of insulin dynamics may play a role in the future success of targeting pediatric obesity pharmacotherapy.

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REFERENCES


A POPULATION-BASED COMPARISON OF BMI PERCENTILES AND WAIST-TO-HEIGHT RATIO FOR IDENTIFYING CARDIOVASCULAR RISK IN YOUTH

HENRY S. KAHN, MD, GIUSEPPINA IMPERATORI, MD, PhD, AND YILING J. CHENG, MD, PhD

Objective Determine whether waist-to-height ratio (WHtR) or sex- and age-specific percentiles of body mass index (BMI) better identifies cardiovascular risk.

Study design The third National Health and Nutrition Examination Survey (NHANES III) provided measurements on 7657 participants statistically weighted to represent 50.05 million youth 4 to 17 years of age. We estimated the subpopulations corresponding to BMI strata that were normal (<85th percentile), at risk for overweight (85th to <95th percentile), and overweight (≥95th percentile). We chose WHtR cutoff points (0.490 and 0.539) so that subpopulation sizes in the three WHtR strata would equal those in the three BMI strata. For 13 cardiovascular risk factors we compared mean levels among youth discordant for their BMI and WHtR strata.

Results 726 participants (representing 3.69 million youth) were identified as having WHtR stratum >BMI stratum. Compared with the 603 participants (representing 3.70 million youth) who were discordant in the opposite direction, weighted analyses showed they had higher mean levels of heart rate, low-density lipoprotein (LDL) cholesterol, fasting triglycerides, and total cholesterol (P <.015, adjusted for sex, age, and race-ethnicity). Their mean systolic blood pressure was lower, but this difference was eliminated after adjustment for their shorter stature.

Conclusion WHtR, a simpler anthropometric index than sex- and age-specific BMI percentiles, better identifies youth with adverse cardiovascular risk factors. (J Pediatr 2005;146:482-8)

Recent increases in pediatric obesity1,2 suggest that children and adolescents should be assessed anthropometrically as a step toward managing their long-term cardiovascular risk status. Some authorities have recommended calculating the body mass index (BMI; weight/height2, conventionally expressed as kg/m2) and then employing standardized reference charts to interpret the BMI as a percentile value specific to the person’s age (in months) and sex.3 However, a BMI percentile value serves only as an indicator of relative weight. It cannot by definition distinguish between excesses in different tissues (eg, fat, muscle, bone) or the anatomic distribution of tissues (eg, upper/lower, central/peripheral). For this reason, assessments based on BMI may not provide the best estimates of metabolic or hemodynamic risk.

Researchers in Cyprus4 and Japan5 have proposed the use of waist circumference-to-height ratio (WHtR) to assess pediatric central obesity, and they compared this anthropometric index with BMI percentiles for its ability to identify children with unfavorable cardiovascular risk factors. These studies suggested that WHtR might be a superior tool for identifying the child at risk, but the researchers’ work included only narrow age ranges and did not evaluate dependent variables related to glucose metabolism. Among adults, large studies have found that WHtR6-8 or waist circumference alone9 served better than BMI to identify cardiovascular risk factors.

In this study, we used cross-sectional US survey data in a population-based sample to determine how these alternative anthropometric indices—BMI percentiles or WHtR—would perform for identifying youth with adverse levels of 13 cardiovascular risk factors.
METHODS

Our analytic population was drawn from the third National Health and Nutrition Examination Survey (NHANES III), a probability sample of the noninstitutionalized US population studied in 1988–1994. This complex, multistage survey oversampled non-Hispanic blacks and Mexican Americans. Each participant was assigned a sampling weight that accounted for unequal selection probabilities (clustered design, planned oversampling, and differential nonresponse).10 Our final analytic population contained 3777 boys and 3880 girls who were 4 to 17 years of age; had data on height, weight, and waist circumference; and were not pregnant. For each participant, a household respondent completed a detailed interview after which a standardized examination11 was performed that included barefoot standing examination that included barefoot standing (on a digital, electronic scale), waist circumference (in the horizontal plane at a point marked just above the right ilium on the midaxillary line, at minimal respiration12), right midthigh circumference, and skinfold thicknesses at the right subscapular and right triceps sites (by Holtain calipers). Heights and circumferences were recorded to the nearest 0.1 cm and skinfolds to the nearest 0.1 mm.

All chemical analyses were performed in a standardized laboratory as described in the NHANES III documentation.13 Assays for fasting serum glucose were restricted to participants ≥12 years of age who reported fasting 8 to 19 hours. Assays for serum triglycerides were restricted to participants who fasted ≥9 hours, and calculations of serum low-density lipoprotein (LDL)-cholesterol were limited to participants with triacylglyceride concentration <4.5 mmol/L (a requirement of the Friedewald equation14) who were ≥12 years of age. For estimates of effects on glucose and hemoglobin A1c (HbA1c, glycated hemoglobin), we excluded participants with diabetes. Analyses for serum apolipoproteins B and A were conducted only during phase 1 of NHANES III (1988–1991).

Statistics

We used NHANES III sampling weights along with SAS (release 8.02, SAS Institute Inc, Cary, NC) and SUDAAN (release 8.02, Research Triangle Institute, Research Triangle, NC) software to estimate the sizes of the represented child populations, to describe the distributions in the population of risk variables associated with various anthropometric strata, and to perform the analyses using multivariable linear regression. On the basis of the statistical weights assigned, we estimated that our analytic sample (N = 7657) represented 50,047,016 US youth 4 to 17 years of age, of whom 48.4% ± 1.0% (SE) were girls. The estimated population distribution by race-ethnicity was 65.8% ± 1.7% non-Hispanic white, 15.6% ± 1.2% non–Hispanic black, 8.8% ± 0.9% Mexican American, and 9.8% ± 1.4% other.

BMI was calculated in kg/m² and then converted to a sex- and age-specific BMI percentile value using a computerized formula15 derived from the 2000 Centers for Disease Control (CDC) Growth Charts.16 Based on the BMI percentile value, we assigned each participant to an overweight BMI stratum (≥95th percentile), an at-risk for overweight BMI stratum (85th percentile to <95th percentile), or a normal BMI stratum (<85th percentile). The estimated population percentages (± SE) in these three BMI-percentile strata, respectively, were 10.7% ± 0.7%, 14.0% ± 0.8%, and 75.3% ± 1.1% (Table I). The population’s median value for BMI percentile was 59.9, confirming that the CDC 2000 Growth Chart reference describes a youth population of lower weight than the population sampled for NHANES III.16

WHtR was calculated as a ratio without units, not specific either to sex or age. We estimated the population’s WHtR distribution (mean 0.463, SE 0.002; median 0.451) and determined that the mean values of this anthropometric index were not significantly different by sex (mean for boys, 0.461; girls, 0.464) or by age group (mean for 4–11 years, 0.465; 12–17 years, 0.460). We identified 2 cutoff points in the WHtR distribution such that percentages of the estimated population in three levels would be equivalent to the percentages in three strata established by sex- and age-specific BMI percentiles. These cutoff points were at WHtR ≥0.539 (yielded 10.7% ± 0.8% of the population in the high WHtR stratum) and at WHtR ≥0.490 (yielded 14.0% ± 0.7% of the population in the intermediate WHtR stratum and 75.3% ± 1.0% in the normal WHtR stratum) (Table I).

We identified survey participants whose BMI-percentile stratum (iii or ii) was greater than their WHtR stratum (ii or i), a discordant subsample (N = 603) representing

<table>
<thead>
<tr>
<th>WHtR strata</th>
<th>(iii) ≥0.539</th>
<th>(ii) ≥0.490 – &lt;0.539</th>
<th>(i) &lt;0.490</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI-percentile strata</td>
<td>729 (4.16)</td>
<td>209 (1.03)</td>
<td>49 (0.17)</td>
<td>987 (5.36)</td>
</tr>
<tr>
<td>(iii) ≥95th percentile</td>
<td>145 (0.84)</td>
<td>560 (3.07)</td>
<td>345 (1.82)</td>
<td>1050 (5.70)</td>
</tr>
<tr>
<td>(ii) 85th – &lt;95th percentile</td>
<td>28 (0.17)</td>
<td>553 (3.12)</td>
<td>5039 (26.72)</td>
<td>5620 (31.16)</td>
</tr>
<tr>
<td>(i) &lt;85th percentile</td>
<td>902 (5.37)</td>
<td>1322 (7.23)</td>
<td>5433 (30.69)</td>
<td>7657 (44.50)</td>
</tr>
</tbody>
</table>

Table I. Distribution of US youth by three strata of BMI percentiles and three strata of WHtR showing NHANES III sample size (N, bold) and population estimates (italics, in millions). Youth identified in the boxed cells are concordant for their stratum of each anthropometric index.
a subpopulation of 3.70 million youth **above** the diagonal boxes in Table I. We also identified 726 participants whose WHtR stratum (**iii** or **ii**), ie, discordant in the opposite direction, representing 3.69 million youth **below** the diagonal boxes in Table I. Taking both these subpopulations together, about 14.8% of the US youth population was discordant by their strata of the anthropometric indices.

We compared these two subpopulations that showed discordance in opposite directions by using weighted linear regression modeling with adjustments for sex, age, (terms for age and age^2) and race-ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other). Our comparisons primarily examined 13 dependent variables of cardiovascular interest (fasting triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, total cholesterol/HDL cholesterol, LDL cholesterol, apolipoprotein B, apolipoprotein B/apolipoprotein AI, uric acid, fasting serum glucose, HbA1c, systolic blood pressure, diastolic blood pressure, heart rate). We secondarily examined three dependent variables of ancillary anthropometric interest: height (a variable associated with blood pressure^{17,18}), midthigh circumference (an indicator of relative lower-body mass), and subscapular/triceps skinfolds (an index of relative subcutaneous central fat patterning).

We also prepared linear regression models that used BMI percentiles and WHtR as continuous variables, including adjustments for sex, age, and race-ethnicity. For these continuous analyses, the \( b \) coefficients were standardized to reflect the change in each outcome variable associated with an increment of 1 standard deviation from the mean (calculated for each sex and age group [4-11 years or 12-17 years]) of either anthropometric index.

### RESULTS

Our analysis of discordant subpopulations found that youth whose WHtR stratum was higher than their BMI-percentile stratum included a disproportionately high
percentage of girls (55.7% ± 3.2%), a disproportionately low percentage of non-Hispanic blacks (7.0% ± 0.8%), and a disproportionately high percentage of Mexican Americans (13.9% ± 1.5%) when compared with our estimated total youth population. Those subpopulations whose BMI-percentile stratum was higher than their WHtR stratum had relatively few girls (44.3% ± 3.2%), relatively more non-Hispanic blacks (23.7% ± 2.7%), and relatively fewer Mexican Americans (6.6% ± 1.1%) compared with the total population. The two discordant subpopulations had means of age that were similar to each other (P = .28) and to the mean age of the estimated total population (9.7 years).

After adjustment for sex, age, and race-ethnicity the youth whose WHtR stratum was higher than their BMI-percentile stratum had higher heart rates and higher concentrations of LDL cholesterol, fasting triglycerides, and total cholesterol (P < .015; Table II). For four additional cardiovascular risk factors (total cholesterol/HDL cholesterol, apolipoprotein B, fasting glucose, and apolipoprotein B/apolipoprotein AI), these same youth showed a nonsignificant trend in the same adverse direction (.05 < P ≤ .12). On the contrary, however, these youth had lower systolic blood pressure (P = .0023). No differences were found for HbA1c, HDL cholesterol, uric acid, and diastolic blood pressure (P > .25).

A comparison of ancillary anthropometric variables in these discordant subpopulations found that youth whose WHtR stratum was higher than their BMI-percentile stratum had relatively smaller midthigh circumferences, were shorter, and had a greater ratio of subscapular to triceps skinfold thickness, ie, increased central patterning of subcutaneous fat (Table III).

Re-analysis of the systolic blood pressure in these discordant subpopulations, after a further adjustment for height, resulted in a reversed, but nonsignificant, difference in the mean systolic blood pressures (103.8 vs 102.4 mm Hg; P = .24).

When we examined the effect of BMI percentiles and WHtR as continuous variables, our results (Table IV) generally confirmed our findings in the discordant subpopulations. Continuous WHtR provided a better estimate (greater proportion of explained variation, ie, higher R² value) than continuous BMI percentiles for heart rate, LDL cholesterol, triglycerides, total cholesterol, total cholesterol/HDL cholesterol, apolipoprotein B, and apolipoprotein B/apolipoprotein AI. For these outcome variables, introduction of BMI percentiles in to the model contributed little or nothing to the information already provided by WHtR alone. Similarly, continuous WHtR provided a larger standardized β coefficient for these same seven cardiovascular outcome variables. In other words, an increase of 1 standard deviation in the WHtR resulted in a more adverse cardiovascular risk profile than an increase of 1 standard deviation in the BMI percentile.

By contrast, for systolic blood pressure and fasting glucose there was an apparent advantage in using BMI percentiles. However, if our systolic blood pressure models included adjustment for height, then the two alternative indices performed equally well for prediction of blood pressure (R² = 0.389 for each model). For fasting serum glucose the distinctions between the anthropometric indices were small. If both WHtR and BMI percentiles were entered in to the adjusted model for serum glucose (R²= 0.088), neither anthropometric index had a slope that was statistically different from zero (P > .06).

We also performed the same analyses using BMI Z score in place of BMI percentiles. The results were only minimally different (data not shown) and did not eliminate the advantages seen for WHtR.

Continuous WHtR, compared with continuous BMI percentiles, was associated with slightly lower systolic blood pressure and with reduced height (Table IV). In an additional model for systolic blood pressure, adjustment for height reversed the difference in β coefficients between the alternative anthropometric indices (2.0 ± 0.2 [R² = 0.395] for WHtR vs 1.9 ± 0.2 mm Hg [R² = 0.392] for BMI percentiles).

**DISCUSSION**

Our population-based comparison of two anthropometric indices demonstrated that WHtR could serve better than sex- and age-specific BMI percentiles for identifying US youth with high heart rate or adverse concentrations of LDL cholesterol, triglycerides, and total cholesterol. There also was a suggestion (nonsignificant) that youth with elevated total cholesterol/HDL cholesterol, apolipoprotein B, or apolipoprotein B/apolipoprotein AI would be better identified by WHtR. For adverse levels of HDL cholesterol, glucose, HbA1c, uric acid, and diastolic blood pressure, there was no clear difference in the predictive ability of the alternative anthropometric indices.

On the other hand, BMI percentiles better identified youth who had relatively high systolic blood pressure. Youth with increased BMI percentiles were typically taller than those with increased WHtR, however, and adjustment for height eliminated the greater increment in systolic blood pressure associated with increased BMI percentile. This finding is consistent with the known physiologic association between height and blood pressure. It suggests that the higher blood pressures identified by an increased BMI percentile are physiologically appropriate to the participants’ taller stature and unlikely to represent a cardiovascular hazard.

What WHtR characteristics are likely to explain its observed association with cardiovascular risk factors? A large WHtR is an estimator of the visceral (intra-abdominal) mass of adipose tissue (documented for adults) along with the subcutaneous truncal fat mass. Visceral adipose tissue is minimally present in newborns and is usually sparse among children. Nevertheless, the emergence of visceral fat in children and adolescents could be interpreted as a specific marker of systemic lipid overaccumulation. That is, even among youth with low or normal weight by BMI-percentile criteria, the emergence of more or larger adipocytes in the
visceral compartment would indicate that these youth have already exceeded the capacity of their peripheral adipocytes and other tissues to buffer and store normal amounts of lipid fuels. This excess of lipid fuels also may be expressed by a parallel rise in circulating triglycerides. Of potential importance to future disease, the excess lipid fuels also may find their way into ectopic sites of lipid storage (eg, skeletal muscle, liver, and pancreatic β cells) where they can cause substantial metabolic disruption. It is less clear that enlargement of the subcutaneous truncal fat depots reflects the same pathophysiology.

In instances where BMI percentile and WHtR were discordant, an elevated WHtR preferentially identified youth who were not only shorter but who also had increased central subcutaneous fat patterning (subscapular/triceps skinfold ratio) and relatively small midthigh circumferences (Table III). Central subcutaneous fat patterning in children is associated with low birth weight (presumably an adverse fetal environment) and an adverse cardiovascular risk profile. Similarly, reduced thigh size has been shown to be associated with adverse cardiovascular risk in adolescents and adults.

If a child’s elevated WHtR identifies cardiovascular risk at least as well as his or her elevated BMI percentile, then the choice of anthropometric index might depend on the comparative ease and reliability of the two indices. Weight measurement is more familiar to patients and clinicians than waist measurement, but familiarity does not necessarily confer precision. Accurate weighing requires removal of shoes and most clothing, and correction for occasional appliances or casts. It also requires the use of a high-quality scale that is periodically recalibrated. On the other hand, waist circumference requires only the removal (or loosening) of clothing around the waist and an inexpensive tape measure made of nonstretchable material. The standardized bony landmark for waist measurement (see Methods) is usually simple to identify after a short training period, and measurements made at this level can be highly reproducible. Both WHtR and BMI require measurement of height. However, if height measurement is inaccurate the error will be squared in computing the BMI.

It is possible, but not yet demonstrated, that the concept of waist enlargement relative to height may be easier for patients and their families to grasp than the concept of a BMI percentile. BMI-percentile reference values vary by sex and with each month of age. Different systems of reference values have been proposed—not without controversy—at various points in time and in different populations. Our analysis suggests that WHtR, a simple ratio that does not require specification of sex or age, might replace or supplement the use of sex- and age-specific BMI percentiles for assessment of cardiovascular risk associated with overweight or central obesity. We note with concern that abdominal size among US adults may have increased substantially in the past 4 decades.

### Table IV. Linear regression models of the youth population comparing continuous BMI percentiles and continuous WHtR, including adjustment for sex, age, and race-ethnicity

<table>
<thead>
<tr>
<th>Observations (N) from the survey sample</th>
<th>Dependent variable, units</th>
<th>Increment in dependent variable per 1 SD change in BMI percentile</th>
<th>SE of increment</th>
<th>R² for model</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiovascular risk variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6374 Heart rate, bpm</td>
<td></td>
<td>0.54</td>
<td>0.29</td>
<td>0.202</td>
</tr>
<tr>
<td>6333 Systolic blood pressure, mmHg</td>
<td></td>
<td>2.3</td>
<td>0.2</td>
<td>0.355</td>
</tr>
<tr>
<td>821 LDL cholesterol, mmol/L</td>
<td></td>
<td>0.063</td>
<td>0.047</td>
<td>0.041</td>
</tr>
<tr>
<td>2228 Log of fasting triglycerides, mmol/L</td>
<td></td>
<td>0.119</td>
<td>0.020</td>
<td>0.105</td>
</tr>
<tr>
<td>6652 Total cholesterol, mmol/L</td>
<td></td>
<td>0.039</td>
<td>0.014</td>
<td>0.031</td>
</tr>
<tr>
<td>6617 Total cholesterol/HDL cholesterol</td>
<td></td>
<td>0.219</td>
<td>0.029</td>
<td>0.066</td>
</tr>
<tr>
<td>337 Apolipoprotein B, g/L</td>
<td></td>
<td>0.017</td>
<td>0.005</td>
<td>0.020</td>
</tr>
<tr>
<td>1281 Fasting serum glucose, mol/L</td>
<td></td>
<td>0.051</td>
<td>0.020</td>
<td>0.088</td>
</tr>
<tr>
<td>320 Apolipoprotein B/apolipoprotein AI</td>
<td></td>
<td>0.0225</td>
<td>0.0044</td>
<td>0.042</td>
</tr>
<tr>
<td><strong>Ancillary anthropometric variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7629 Midthigh circumference, cm</td>
<td></td>
<td>4.25</td>
<td>0.13</td>
<td>0.858</td>
</tr>
<tr>
<td>7657 Height, cm</td>
<td></td>
<td>1.44</td>
<td>0.14</td>
<td>0.900</td>
</tr>
<tr>
<td>7557 Subscapular skinfold/triceps skinfold</td>
<td></td>
<td>0.0406</td>
<td>0.0050</td>
<td>0.278</td>
</tr>
</tbody>
</table>

*bpm*, beats per minute.

*Data restricted to participants 12-17 years of age who met fasting and triglyceride criteria.

†Data obtained only during Phase 1 of NHANES III.

‡Data restricted to participants 12-17 years of age without diabetes.
experiences a rapid increase in pediatric waist size, such as was recently described in the United Kingdom.40,45 Nevertheless, our analyses of WHtR as a continuous variable indicate that revisions in cutoff points are unlikely to eliminate the relative benefits of WHtR as a simple risk indicator.

We would caution that WHtR may not be suitable for the assessment of children <4 years of age. Moreover, WHtR cannot be recommended to identify pediatric underweight at any age or for continuous evaluation of growth. Unfortunately, there is no published prospective evidence to confirm that WHtR truly predicts adult outcomes related to cardiovascular disease, type II diabetes, or premature mortality.46 Nevertheless, in our era of accelerating overweight among youth, clinical and epidemiologic activities may benefit from improving the simple assessments we use from day to day.

The authors acknowledge the extraordinary efforts of the field staff, laboratory personnel, and statisticians who collected and processed the information in NHANES III. Dr Deborah A. Galuska read and thoughtfully commented on an early draft of this manuscript.

REFERENCES


A Population-Based Comparison Of BMI Percentiles And Waist-To-Height Ratio For Identifying Cardiovascular Risk In Youth


COMBINING UNPROTECTED PANCREATIC ENZYMES WITH
PH-SENSITIVE ENTERIC-COATED MICROSPHERES DOES NOT IMPROVE
NUTRIENT DIGESTION IN PATIENTS WITH CYSTIC FIBROSIS

Daina Kalnins, MSc, RD, Mary Corey, PhD, Lyndia Ellis, RN, Peter R. Dure, MD, FRCPc, and Paul B. Pencharz, MB, CHB, PhD, FRCPc

Objectives To assess the efficacy of combining unprotected powder enzymes and oral enteric-coated microsphere (ECM) and to ECM alone in treating nutrient maldigestion in patients with cystic fibrosis.

Study design Patients were randomly assigned into 2 consecutive, 2-week phases: ECM alone, and ECM plus unprotected powder enzymes. Fecal fat, energy, and nitrogen output were compared with intake at the end of each phase. Two-tailed, paired t-tests were performed to compare outcomes.

Results The mean age of the 14 patients (3 girls) was 5.7 ± 3.2 years (range, 1.9 to 13.4 years). There was no significant difference in percent malabsorption of fat (15.6% vs 18.2%), energy (13.3% vs 13.4%), or nitrogen (11.8% vs 11.3%) between phases.

Conclusions The addition of powder enzymes to ECM did not improve nutrient maldigestion compared with ECM alone. (J Pediatr 2005;146:489-93)

Approximately 85% of patients with cystic fibrosis (CF) have exocrine pancreatic insufficiency (PI), as evidenced by an abnormal fat absorption. Treatment of PI in CF includes oral pancreatic enzyme therapy. Although nutritional status improves with enzyme therapy, fat absorption is rarely normalized. In our experience, >30% of treated patients have significant nutrient maldigestion (>20% fat malabsorption).

Oral pancreatic enzymes are available as powder contained in capsules that do not have a protective coating and as enteric-coated microspheres (ECM) with a pH-sensitive coating. Both types of enzymes have potential limitations in their efficacy. Unprotected powder enzymes may become inactivated by gastric acid with decreased duodenal enzyme recovery if exposure to gastric contents is prolonged. ECM dissolve at a pH of >5.5; however, luminal contents in the CF duodenum may not reach this pH. Evidence from intubation studies confirms that release of enzymes from ECM is delayed in CF and thus they are delivered beyond the duodenum, even as far distal as the ileum. As a result, nutrient digestion occurs in the more distal small intestine, not in the duodenum and proximal jejunum as in health. The ECM were developed to avoid inactivation of the enzymes by gastric acid but depend on sufficient bicarbonate being secreted in the duodenum to raise luminal pH to >5.5. Based on findings from their intubation studies of enzyme recovery in adults, Delchier et al suggested that a combination of ECM and powder enzymes may improve overall nutrient digestion. We have clinically treated several patients with a combination of uncoated, powder enzymes and ECM and have found an improvement in gastrointestinal symptoms, growth, and fat absorption. These clinical observations encouraged us to embark on a formal study to objectively determine if the combination of unprotected powder enzymes plus ECM improves nutrient digestion.

METHODS

Patients with CF and PI (n = 20) attending the CF clinic at The Hospital for Sick Children (HSC), Toronto, were recruited to take part in the study. The diagnosis of CF

<table>
<thead>
<tr>
<th>CF</th>
<th>Cystic fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>Carbohydrate</td>
</tr>
<tr>
<td>ECM</td>
<td>Enteric-coated microsphere</td>
</tr>
<tr>
<td>HSC</td>
<td>Hospital for Sick Children (Toronto)</td>
</tr>
<tr>
<td>IBW</td>
<td>Ideal body weight</td>
</tr>
<tr>
<td>PFT</td>
<td>Pulmonary function test</td>
</tr>
<tr>
<td>PI</td>
<td>Pancreatic insufficiency</td>
</tr>
</tbody>
</table>

From the Divisions of Respiratory Medicine, Population Health Sciences, Gastroenterology/Nutrition, and the Research Institute, The Hospital for Sick Children, and the Departments Paediatrics, Nutritional Sciences, and Public Health Sciences, University of Toronto, Toronto, Ontario, Canada. Supported by ORGANON, Canada, Ltd, and the Canadian Foundation for Dietetic Research. Submitted for publication Jun 1, 2004; revision received Sep 10, 2004; accepted Oct 28, 2004. Reprint requests: Dr P. B. Pencharz, Division of Gastroenterology/Nutrition, The Hospital for Sick Children, 555 University Ave, Toronto, Ontario, Canada M5G 1X8. 0022-3476/$ - see front matter Copyright © 2005 Elsevier Inc. All rights reserved. 10.1016/j.jpeds.2004.10.063
was confirmed by elevated sweat chloride (>60 mmol/L) on 2 occasions and characteristic clinical symptoms. PI was confirmed by documented fat malabsorption (>15% malabsorption for >6 months of age; >7% malabsorption for >6 months of age) or 2 or more signs of PI, including hypoalbuminemia, failure to thrive, and hypovitaminosis A and/or E.

Inclusion criteria included patients who were 1 to 19 years of age, who required enzyme therapy and had signs or symptoms of maldigestion or poor weight gain and/or documented severe steatorrhea (>20% fat malabsorption while on enzyme therapy). Exclusion criteria included patients with pancreatic sufficiency, those requiring nutritional support through enteral tube feeding, CF-associated liver disease, CF-related diabetes, and/or severe lung disease (forced expiratory volume in one second [FEV] 1 <30%). If patients were taking acid-suppressing agents, they were to discontinue their use at least 2 weeks before study entry and for the duration of the study.

Weight and height were recorded with the use of a weight scale (Scale-Tronix, Inc, Model 5005, Wheaton, IL) and a stadiometer for height measurements. Tanner growth charts were used to plot height and weight for age and sex and to determine ideal body weight (IBW), based on weight at same percentile as height. Percent IBW was calculated by dividing actual body weight by IBW. Patients >5 years of age performed pulmonary function tests (PFTs).

A randomized crossover study design was used. A double-blinded study was not feasible because patients were capable of opening the capsules and would recognize the ECM and powder forms. The study included 2 consecutive, randomized, 2-week phases. The phases were ECM alone and ECM plus unprotected powder enzymes (combination phase). A 2-week period allowed for 1 week of adjustment to the regimen, with monitoring during the second week.

The enzymes, of porcine origin, were provided by Organon, Canada. ECM (Cotazym ECS 8) and unprotected powder (Cotazym) capsules each contained 8000 U lipase, 30,000 U amylase, and 30,000 U protease. The same batch was used for each product. The research pharmacy dispensed the enzymes (100 capsules per bottle) and counted returns to determine compliance with treatment. Enzyme dose was based on the dose at the time of enrollment, as determined by age-adjusted dosing guidelines established at HSC and the US CF Foundation consensus statement on enzyme dosing, which take into account the variability in nutrient (fat) intake at different ages. Enzymes were ingested at the beginning or at the beginning and middle of meals and snacks. For the combination phase, patients were instructed to replace one third of the ECM dose with powder enzymes. Subjects received the same total enzyme dose during each phase of the study.

Patients kept a 7-day symptom record (number of bowel movements per day, episodes of abdominal discomfort or pain) during the second week of each study period. All food and enzyme intake was recorded during a 72-hour period, at the end of each 2-week phase. A gram scale (Hanson Dietetic Scale, Model 1440, Shubuta, MI) for food weighing was provided. Analysis of food records was done by using the SPA/Carex computerized nutrient assessment system (Vision Software Tech Inc, Goodlettsville, TN, 1997). Enzyme intake was expressed as U lipase/g fat per day and U lipase/kg body weight per day. Daily energy recommendations were determined by using equations described in the US CF Foundation nutrition consensus report. During the same 72-hour period, patients were instructed to collect all stools. For younger children in diapers, dental bibs were used with the nonabsorbable (shiny) side against the child's buttocks. Stools were stored at −20°C and sent to the HSC chemistry laboratory.

Fecal fat output, determined by the method of van de Kamer, was divided by fat intake to determine percent fat malabsorption. An aliquot was freeze-dried for nitrogen analysis and bomb calorimetry (Freezone 12L, Labconco Corp, Kansas City, MO). Kjeldahl digestion of freeze-dried stool was required for nitrogen analysis. The Parr 1266 Bomb Calorimeter (ASTM, Parr Instrument Co, Moline, IL) was used to determine the energy content of freeze-dried stool and the Antek 7000 Elemental Analyzer (Mandel Scientific Company, Ltd, Houston, TX) to determine nitrogen content of stool.

Statistical comparisons between the study phases were performed by using a 2-tailed, paired t test. Differences between means were considered significant at P < .05. Associations between variables were tested by the Pearson product-moment correlation coefficient. A 3-way repeated-measures of analysis of variance was used to assess the difference between the study phases (calories, protein, fat, carbohydrate [CHO]) within subjects by day, within and between phases using the following classes: subject, day, and enzyme phase.

The study was approved by the Research Ethics Board, the Research Institute at HSC. Patients/parents signed an informed consent, and an information (assent) form was reviewed with children older than 7 years of age.

RESULTS

Patients

Fourteen subjects (3 girls) completed the study between February 1998 and June 2000. Six subjects dropped out because of (1) anticipation of feeling unwell during the ECM-alone phase (4 subjects were receiving ECM plus powder enzymes before study entry), (2) change of mind (1 subject), and (3) acute illness (1 subject). Mean age was 5.7 ± 3.2 years (range, 1.9 to 13.4 years) and mean percent IBW was 99.6% ± 9.3% (range, 80% to 116%). At study entry, 6 subjects had poor weight gain or a reduced growth velocity and 13 had a history of frequent/oily stools and/or abdominal pain. Four patients who had completed a 72-hour fecal fat while on enzyme therapy before study entry had a mean percent fat malabsorption of 12% (range, 10% to 16%). Six subjects who were old enough to perform PFTs had a mean FEV1 of 83.3% ± 19.9% (range, 47% to 106%).
Two subjects were taking the combination of ECM plus powder enzymes at study entry. Two subjects repeated the second study phase within 2 to 4 months of the first phase because of loss of food records and inappropriate enzyme dosing. One subject acknowledged missing collection of 1 stool of 4 in the ECM-alone phase, and another subject missed collecting 1 stool of 7 at the very beginning of the combination phase. Since analysis without these subjects did not affect study outcome, data for all 14 subjects are included in results.

Energy, Nutrient, and Enzyme Intake

There was no significant difference between phases in daily energy, protein, fat, or enzyme intake (Table I). Subjects received 112% of recommended energy needs, and the nutrient distribution (same for both phases) was 38% fat, 48% CHO, and 14% protein. There was no significant within-subject difference in energy, fat, or CHO intake within phases. There was within-subject variation in daily protein intake within phases but not between phases (P < .03).

Stool Output: Wet and Dry Weight

There was no significant difference in daily wet weight, dry weight, or percent fecal water content between phases. Mean wet weight of stool was 138.0 ± 74.5 g/d (range, 66.7 to 346.3 g/d) for the ECM-alone phase and 177.2 ± 160.6 g/d (range, 48.3 to 688.7 g/d) for the combination phase (P = .18). The mean dry weight was 44.5 ± 21.0 g/d (range, 18.5 to 88.6 g/d) for the ECM-alone phase and 47.1 ± 21.7 g/d (range, 17.9 to 96.8 g/d) for the combination phase (P = 0.61). The mean percent fecal water content was 66.7% ± 7.7% (range, 53.1% to 83.1%) for the ECM-alone phase and 68.7% ± 6.2% (range, 61.6% to 86.0%) for the combination phase (P = .28).

Stool Energy and Nutrient Losses

The coefficient of variability for fat, bomb calorimetry, and nitrogen analysis was ≤6%, 1.79% ± 1.08% (range, 0.04% to 9.37%), and 2.13% ± 0.97% (range, 0.31% to 4.24%), respectively.

There was no significant difference between phases for daily fat, energy, or nitrogen losses in stool (Table II). There was no significant difference between phases for energy/g wet weight of stool, which was 1.86 ± 0.65 kcal/g (range, 0.85 to 3.03 kcal/g) for the ECM-alone phase and 1.76 ± 0.54 kcal/g (range, 0.87 to 2.57 kcal/g) for the combination phase. Stool wet weight was correlated with daily stool energy loss (ECM alone: r² = 0.65, P < .0005; combination phase: r² = 0.76, P < .0005), fat loss (ECM alone: r² = 0.76, P < .0005; combination phase: r² = 0.85, P < .0005), and nitrogen loss (ECM alone: r² = 0.35, P < .02; combination phase: r² = 0.84, P < .0005). There was no significant difference in the degree of fat, energy, or nitrogen malabsorption between phases (Table II). (While on the combination of enzymes, 8 subjects had decreased fat malabsorption and 6 had increased fat malabsorption compared with ECM alone).

### Table I. Nutrient and enzyme intake

<table>
<thead>
<tr>
<th></th>
<th>ECM alone</th>
<th>Combination</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/d)</td>
<td>1909 ± 460</td>
<td>1962 ± 763</td>
<td>.70</td>
</tr>
<tr>
<td>Median</td>
<td>1847</td>
<td>1727</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1324–2831</td>
<td>1363–4414</td>
<td></td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>66.5 ± 19.2</td>
<td>69.7 ± 32.0</td>
<td>.50</td>
</tr>
<tr>
<td>Median</td>
<td>68.8</td>
<td>62.6</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>36.6–109.2</td>
<td>42.7–161.6</td>
<td></td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>81.2 ± 30.1</td>
<td>85.9 ± 50.0</td>
<td>.63</td>
</tr>
<tr>
<td>Median</td>
<td>79.4</td>
<td>74.5</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>52.6–163.6</td>
<td>42.5–277.4</td>
<td></td>
</tr>
<tr>
<td>Units Lipase/g fat*</td>
<td>1830 ± 551</td>
<td>2080 ± 835</td>
<td>.25</td>
</tr>
<tr>
<td>Mean</td>
<td>1905</td>
<td>2049</td>
<td></td>
</tr>
<tr>
<td>Range (n = 13)</td>
<td>636–2715</td>
<td>461–3639</td>
<td></td>
</tr>
<tr>
<td>Units Lipase/kg**</td>
<td>7882 ± 2468</td>
<td>8384 ± 2483</td>
<td>.17</td>
</tr>
<tr>
<td>Mean</td>
<td>8108</td>
<td>9433</td>
<td></td>
</tr>
<tr>
<td>Range (n = 13)</td>
<td>3467–10,909</td>
<td>4267–11,775</td>
<td></td>
</tr>
</tbody>
</table>

*Grams of fat of dietary intake; **kg of body weight.

There were significant correlations between fat and energy malabsorption (ECM alone: r² = 0.80, P < .0001; combination phase: r² = 0.66, P < .0005), fat and nitrogen malabsorption (ECM alone: r² = 0.76, P < .0001; combination phase: r² = 0.62, P < .005), and energy and nitrogen malabsorption (ECM alone: r² = 0.73, P < .0001; combination phase: r² = 0.34, P < .05). Although correlations for the ECM-alone phase appear to be stronger than for the combination phase, a few outliers were responsible for this trend. There was no correlation between intake and degree of malabsorption for energy, fat, or nitrogen [ECM alone: r² = 0.003 (energy), 0.031 (fat), 0.154 (nitrogen); combination phase: r² = 0.001 (energy), 0.122 (fat), 0.07 (nitrogen)].

Effect of Enzyme, Energy, and Nutrient Intake on Energy and Nutrient Malabsorption

There was no correlation between the degree of fat malabsorption and enzyme intake (Figure) or between nitrogen malabsorption and enzyme intake (expressed as U Protease/g protein per day) (r² = 0.004 and 0.003 for the ECM-alone and combination phase, respectively).

Symptom Score

Thirteen subjects reported symptom scores. The number of daily bowel movements did not differ between phases. During the 72-hour stool collection, subjects reported 1.8 ± 0.9 (range, 1 to 4) daily bowel movements in the ECM-alone phase and 1.8 ± 0.7 (range, 1 to 3) in the combination phase. Two subjects reported mild episodes of abdominal pain in the ECM-alone phase and 4 subjects reported mild episodes of abdominal pain in the combination phase. The total number of episodes of pain was 11 in the ECM-alone phase and 7 in
bowel movements and U Lipase/g fat (phase, respectively). There was no correlation between daily

Nitrogen malabsorption (%) 11.8 ± 492 Kalnins et al The Journal of Pediatrics

Energy malabsorption (%) 13.3 ± 500 to 1000 U lipase/g fat per day, including age-related

Fat malabsorption (%) 9.9 ± 18.2 ± 418.4–2293.4 ± 1177.7 ± 385.8 ± 7.4–26.2 ± 4.3–26.2 ± 1.0–11.4 ± 1.1–13.8 ± 4.8–13.6 ± 5.2–13.4 ± 6.6 ± 15.0 ± 13.5 ± 6.6 ± 15.0 ± 13.5 ± 4.0–4.0 ± 2.0–2.0 ± 1.0–2.0 ± 15.0 ± 11.3 ± 8.7 ± 10.8 ± 5.8–21.7 ± 3.6–25.8 ± 589.5 ± 1193.2 ± 265.1 ± 136.6 ± 255.2 ± 150.3 ± 192.7 ± 92.9–551.2 ± 1193.2 ± 585.8 ± 996.1 ± 1042.0 ± 252.8 ± 1063–599.9 ± 11.3–110.6 ± 19.2 ± 15.9–101.3 ± 5.1–5.1 ± 8.7 ± 8.7 ± 5.1 ± 11.3 ± 11.7 ± 13.6 ± 13.4 ± 5.2 ± 13.3 ± 6.6 ± 15.0 ± 13.5 ± 8.7 ± 10.8 ± 5.8–21.7 ± 3.6–25.8 ± 589.5 ± 2000 U lipase/g fat,2 the same as used in this study, with a minimum of ~500 U lipase/g fat. Others have found that ~90% fat absorption can be achieved with 500 to 1000 U lipase/g fat.23,24 On the basis of these data, one may suggest 500 to 1000 U lipase/g fat per day as a minimum starting enzyme dose for patients with CF who are >1 year of age, with objective evaluation if compliance has been assessed and the patient remains unresponsive to treatment. A maximum safe dose of 4000 U lipase/g fat per day, including age-related adjustments, is recommended by the US consensus conference on enzyme dosing to prevent fibrosing colonopathy.14

It would seem rational to suggest that adjuvant therapy, with gastric acid suppression resulting in a rise in pH in the proximal intestine, may improve the efficacy of this enzyme combination.25,26 However, studies evaluating ECM and adjuvants have provided mixed results, with improvement in fat malabsorption in one study25 but not in another.27 Long-term consequences of gastric acid secretion inhibition has not been studied in children, so this is an important consideration. Providing ECM in combination with powder enzymes provides a low-risk approach versus adjuvant therapy. The addition of such a third arm to the study may have hindered enrolment because of the longer time commitment required.

Even if maximal digestive activity is achieved, it is now apparent that the absorptive phase may also be defective in subjects with CF. Results from recent studies, using breath tests and stable isotopes, suggest that fatty acid absorption as well as the digestion of triglycerides is impaired in subjects with CF.23,28,29 Factors that may influence nutrient absorption include incomplete lipid solubilization caused by the combination phase. There was no correlation between the degree of fat malabsorption and daily bowel movements ($r^2 = 0.008$ and 0.044 for the ECM-alone and combination phase, respectively). There was no correlation between daily bowel movements and U Lipase/g fat ($r^2 = 0.005$ and 0.154 for the ECM-alone and combination phase, respectively).

**DISCUSSION**

Despite our previous clinical impression of benefit, formal evaluation of the addition of unprotected powder enzymes to ECM showed no effect. This report compared ECM alone with ECM plus unprotected powder enzymes. We suggest several reasons that may explain why the combination enzyme therapy failed to improve nutrient malabsorption, despite the pathophysiologic support for this approach.

First, in this study, powder enzymes replaced ECM at an equal dose. However, unprotected powder enzymes are not dose-equivalent to ECM because of enzyme deactivation in the gastric milieu.19,20 If we had instructed patients to replace ECM dose with double the dose as powder to make up for enzyme deactivation, we may have invalidated our conclusions because we would not be able to distinguish between an increased absorptive surface area as a result of the combination of powder enzymes and ECM or because of an overall increase in total enzyme dose.

Second, the majority of the patients had mild-moderate fat malabsorption on enzyme therapy, despite meeting study criteria of poor weight gain and/or weight loss and other symptoms suggestive of malabsorption. This observation demonstrates that subjective symptoms such as more frequent bowel movements do not necessarily correlate with degree of fat malabsorption.2

The results of this study support the need for objective evaluation of therapy when treating patients with CF. Enzyme dosing is arbitrary, as evident in the Figure, reflecting no correlation between enzyme dose and nutrient digestion, and this finding is supported by other studies.21,22 The individual response to treatment and wide range of doses must be considered when prescribing an enzyme dose. Our clinic data suggest that reasonable fat absorption can be achieved at 1800 to 2000 U lipase/g fat,2 the same as used in this study, with a minimum of ~500 U lipase/g fat. Others have found that ~90% fat absorption can be achieved with 500 to 1000 U lipase/g fat. The combination of powder enzymes and ECM or because of an overall increase in total enzyme dose.

<table>
<thead>
<tr>
<th>Table II. Fat, energy, and nitrogen malabsorption</th>
<th>ECM alone</th>
<th>Combination</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat output (mmol/d)</td>
<td>43.1 ± 27.1</td>
<td>47.3 ± 23.8</td>
<td>.50</td>
</tr>
<tr>
<td>Median</td>
<td>34.0</td>
<td>39.6</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>11.3–110.6</td>
<td>15.9–101.3</td>
<td></td>
</tr>
<tr>
<td>Energy output (kcal/d)</td>
<td>255.2 ± 150.3</td>
<td>265.1 ± 136.6</td>
<td>.74</td>
</tr>
<tr>
<td>Median</td>
<td>192.7</td>
<td>252.8</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>92.9–551.2</td>
<td>1063–599.9</td>
<td></td>
</tr>
<tr>
<td>Nitrogen output (mg/d)</td>
<td>1177.7 ± 589.5</td>
<td>1193.2 ± 585.8</td>
<td>.91</td>
</tr>
<tr>
<td>Median</td>
<td>996.1</td>
<td>1042.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>418.4–2293.4</td>
<td>527.8–2372.8</td>
<td></td>
</tr>
<tr>
<td>Fat malabsorption (%)</td>
<td>15.6 ± 9.9</td>
<td>18.2 ± 11.4</td>
<td>.14</td>
</tr>
<tr>
<td>Median</td>
<td>13.5</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>4.0–40</td>
<td>8–48</td>
<td></td>
</tr>
<tr>
<td>Energy malabsorption (%)</td>
<td>13.3 ± 6.6</td>
<td>13.4 ± 5.2</td>
<td>.90</td>
</tr>
<tr>
<td>Median</td>
<td>11.7</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>4.3–26.2</td>
<td>7.4–24.3</td>
<td></td>
</tr>
<tr>
<td>Nitrogen malabsorption (%)</td>
<td>11.8 ± 6.5</td>
<td>11.3 ± 5.1</td>
<td>.70</td>
</tr>
<tr>
<td>Median</td>
<td>10.8</td>
<td>8.7</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>3.6–25.8</td>
<td>5.8–21.7</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.
Combination = ECM (60%) plus powder enzymes (40%).
Percent malabsorption = output/intake × 100.

The combination phase. There was no correlation between the degree of fat malabsorption and daily bowel movements ($r^2 = 0.008$ and 0.044 for the ECM-alone and combination phase, respectively). There was no correlation between daily bowel movements and U Lipase/g fat ($r^2 = 0.005$ and 0.154 for the ECM-alone and combination phase, respectively).
a depleted bile salt pool and thick intestinal mucus, which may affect the unstirred water layer, reducing absorption of fatty acids into the small intestine epithelium. 

In conclusion, at the doses prescribed in this study, enzyme supplementation with unprotected powder enzymes in combination with ECM for patients with CF and PI did not improve nutrient digestion when compared with ECM alone.

REFERENCES


NEED FOR QUANTITATIVE ASSESSMENT OF TRANSGLUTAMINASE AUTOANTIBODIES FOR CELIAC DISEASE IN SCREENING-IDENTIFIED CHILDREN

EDWIN LIU, MD, MARCELLA LI, MS, FEI BAO, MD, DONGMEI MAO, MD, MARIA J. REWERS, MD, PHD, GEORGE S. EISENBARTH, MD, PHD, AND EDWARD J. HOFFENBERG, MD

Objectives To assess several transglutaminase autoantibody (TGAA) assays in their ability to distinguish celiac disease (CD) in screening-identified children with abnormal intestine biopsy specimens from those with normal biopsy specimens.

Study design Children at risk for CD (n = 54) composed of type 1 diabetics, first-degree relatives of type 1 diabetics or CD, and HLA-DQ2+ individuals followed from birth received intestine biopsy. Sera obtained at the time of biopsy were tested for TGAA, using the radioimmunoassay and 5 other commercially available enzyme-linked immunosorbent assays.

Results False-positive rates ranged from 28% to 80%. The positive predictive value (PPV) of the tests ranged from 63% to 84% (lower than reported for symptomatic children). Setting a higher cutoff for each assay maximized PPV.

Conclusions There are significant quantitative differences among all TGAA assays that could affect interpretation of a positive test for CD. The overall false-positive rate for all assays was high in this population. Using the assay as a quantitative rather than qualitative tool by increasing the cutoff of positivity to indicate biopsy increases PPV. Multicenter workshops are needed to identify critical differences and to standardize TGAA assays among laboratories. (J Pediatr 2005;146:494-9)
intestine histology may also reflect that change. Thus, one may hypothesize a model of the intestine injury in CD characterized by relapse and remission along with tissue injury and healing, whether induced by dietary changes or intrinsic to the autoimmune process. Regardless, the much lower predictive value of a positive TGAA test in screening-identified children indicates that more stringent selection for intestine biopsy of children at genetic risk for CD is needed.

Deciding when to perform biopsy in an individual who is identified through screening with TGAA is a clinical dilemma. A normal intestine biopsy does not exclude the possibility of later development of CD and may reduce patient acceptance of future biopsies. The use of different forms of TGAA assays further confuses the picture. In our laboratory, we use the human recombinant TG radioimmunoassay (RIA), which has been suggested to be more sensitive and specific than traditional enzyme-linked immunosorbent assay (ELISA). We found that quantitative adjustment of TGAA threshold for biopsy could improve test performance. We hypothesized that similar cutoffs could be found for the ELISA assays and that using higher titers would minimize negative biopsies in screening-identified children. In this study, we analyzed a series of children at risk for CD who previously underwent serial testing for TGAA. Using stored sera obtained at the time of biopsy, we compared the RIA with several ELISA-based assays for TGAA levels.

### METHODS

#### Subjects

Subjects were children under the age of 18 years, identified to be at risk for CD, based on 1 of the 3 criteria: (1) having type 1 diabetes, (2) being a first-degree relative of someone with type 1 diabetes or CD, or (3) participating in a follow-up study after being identified at birth as expressing HLA-DQ2. In all, a total of 54 children were included in this study: 27 subjects with type 1 diabetes, 14 subjects with a first-degree relative having type 1 diabetes or CD, and 13 children having HLA-DQ2. Of these, 34 had biopsy confirmation of CD and 20 had intestine biopsies without evidence of villus atrophy. Sera from 10 healthy donors were used as negative control subjects, as identified by RIA. Exclusion criteria were prior diagnosis of CD and gluten-free diet. Subjects and parents provided informed signed consent. This study was approved by the Colorado Multiple Institutional Review Board.

#### Screening

Follow-up for all groups included serologic screening tests for CD obtained by blood draws at 9, 15, and 24 months of age and yearly thereafter. Any positive test (TGAA index by RIA >0.05) was repeated in 3 to 6 months. A patient was referred for evaluation and intestine biopsy when 2 blood samples drawn on separate visits were TGAA positive or sooner, if requested by the family. At the time of intestinal biopsy, TGAA was again measured and additional serum was stored.

#### Small-Intestine Biopsy

Subjects continued their usual gluten-containing diet before undergoing biopsy. At upper gastrointestinal endoscopy, our intent was to obtain 4 samples from the duodenum: 2 from the proximal duodenum and 2 from the distal duodenum. In a few cases, we were only able to obtain 1 biopsy from each site. A single pathologist, who was unaware of clinical and laboratory findings, interpreted the sample according to the system described by Marsh. Normal biopsy specimens were assigned a score of 0. A biopsy specimen with normal villous architecture (villus height to crypt depth ratio >2:1) but with increased numbers of intraepithelial lymphocytes was assigned a score of 1 and was considered indeterminate for CD. A biopsy specimen with any degree of villous atrophy was assigned a score of 3. Subjects with inadequate specimens and biopsies interpreted as indeterminate were excluded. Marsh 2 and 3 scores were considered to be consistent with CD.

#### Autoantibody Assay

Initial screening for TGAA positivity was done by RIA, using in vitro transcribed and translated human recombinant transglutaminase, as previously described. The full-length complementary DNA clone encoding transglutaminase was obtained from human umbilical vein endothelial cells and labeled with $^{35}$S. Samples were measured in the fluid phase with

### Table I. Characteristics of each TGAA assay studied

<table>
<thead>
<tr>
<th>TGAA test</th>
<th>Substrate</th>
<th>Detection</th>
<th>Assay format</th>
<th>Positive cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIA</td>
<td>hrTG</td>
<td>$^{35}$S</td>
<td>RIA by 96-well plate</td>
<td>0.05 index</td>
</tr>
<tr>
<td>Inova Quanta Lite</td>
<td>hRBC TG</td>
<td>HRP/TMB</td>
<td>ELISA</td>
<td>20 U</td>
</tr>
<tr>
<td>Eurospital Eu-tTG</td>
<td>hrTG</td>
<td>HRP/TMB</td>
<td>ELISA</td>
<td>7 AU/mL</td>
</tr>
<tr>
<td>IMMCO</td>
<td>hrTG</td>
<td>Alk Phos/pNPP</td>
<td>ELISA</td>
<td>20 EU/mL</td>
</tr>
<tr>
<td>Biofons</td>
<td>hrTG</td>
<td>Alk Phos/pNPP</td>
<td>ELISA</td>
<td>15 AU</td>
</tr>
<tr>
<td>Binding Site</td>
<td>hrTG</td>
<td>HRP/TMB</td>
<td>ELISA</td>
<td>4 U/mL</td>
</tr>
</tbody>
</table>

*Alk Phos, alkaline phosphatase; hRBC TG, human red blood cell transglutaminase; HRP, horseradish peroxidase; hrTG, human recombinant transglutaminase; TMB, tetramethylbenzidine; AU, arbitrary units; EU, ELISA units.*
duplicates in 96-well plates, using a Top Count beta-counter (Packard Instrument Company, Meriden, Conn). The results are expressed as an index defined as (unknown sample cpm − negative control cpm)/(positive control cpm − negative control cpm). Positive and negative control sera were included in every assay. The upper limit of normal for the immunoglobulin A TGAA radioassay was previously established as 3 times the 100th percentile in 184 healthy control subjects at an index of 0.05. The interassay coefficient of variation was 12.5% and intra-assay coefficient of variation was 4.8%. All positive results as well as a subset of negative results were retested. If the two results were discordant, a third aliquot was evaluated, and the status was determined by 2 of the 3 samples.

All sera obtained at the time of biopsy were stored at −20°C and placed in aliquots to minimize repeated freeze-thaw events. TGAA (immunoglobulin A) was remeasured in duplicate, using the following assays (Table I):

1. In-house RIA: human recombinant transglutaminase, 35S-labeled, in 96-well fluid-phase format,
2. Inova Quanta Lite tTG ELISA (Inova Diagnostics, Inc, San Diego, CA, Cat No. 708730),
3. Eurospital Eu-tTGsystem ELISA (Eurospital, Trieste, Italy, Cat No. 9763),
4. IMMCO hrTG ELISA (IMMCO Diagnostics, Buffalo, NY, Cat No. 1144),
5. Biofons hrTG ELISA (Biofons Diagnostics, Turku, Finland, Cat No. RTA4000), and

Protocols for each assay were followed as directed, and all samples were tested in duplicate. If there was concordance between the duplicate samples, then the average of the results were reported.

### Statistics

Statistical analysis was done by using GraphPad Prism version 4.00 and GraphPad InStat 3 for Windows, GraphPad Software (San Diego, CA).

### Role of Funding Sources

This study was investigator-initiated and was conducted without support from any company manufacturing the assays studied; they had no input on the reporting of data or decision to publish. All kits were purchased by the investigator through grant funding mentioned above. The authors have no disclosures to make.

### RESULTS

All samples had high concordance on duplicates for all assays, with the exception of one sample on an ELISA, which was judged to be from human error. This sample was excluded from the analysis. In all assays, normal control sera were confirmed as negative (10/10 duplicate samples). Since patients were selected for intestine biopsy on the basis of persistent TGAA positivity by RIA, all subjects were positive by TGAA. This was confirmed on repeat measurements. Even though all 54 samples were persistently positive by RIA, 5 of 54 were negative by the Inova assay, 17 of 54 by Eurospital, 17

![Table II. Performance of each TGAA assay based on designated cutoff for positivity](source_url)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioimmunoassay</td>
<td>0.05 0.5 0.75 1</td>
<td>100 82 68 38</td>
<td>63 80 95 100</td>
<td>68 68 44 49</td>
<td>2.35 12.35</td>
</tr>
<tr>
<td>Inova</td>
<td>20 60 124 140</td>
<td>97 82 65 56</td>
<td>20 70 95 100</td>
<td>67 82 95 100</td>
<td>80 70 61 57</td>
</tr>
<tr>
<td>IMMCO</td>
<td>20 40 90 121</td>
<td>91 79 62 59</td>
<td>70 80 95 100</td>
<td>84 87 95 100</td>
<td>82 70 59 59</td>
</tr>
<tr>
<td>Biofons</td>
<td>15 35 70 100</td>
<td>91 74 66 59</td>
<td>65 75 95 100</td>
<td>82 83 95 100</td>
<td>81 63 61 59</td>
</tr>
<tr>
<td>Binding Site</td>
<td>4 15 26 30</td>
<td>91 73 67 15</td>
<td>47 74 95 100</td>
<td>75 83 96 100</td>
<td>75 61 62 62</td>
</tr>
<tr>
<td></td>
<td>1.73 2.76 12.67</td>
<td>64 49 49 100</td>
<td>63 59 59 100</td>
<td>62 62 62 100</td>
<td>62 62 62 100</td>
</tr>
</tbody>
</table>

AUC, area under curve; LR, likelihood ratio; PPV, positive predictive value; NPV, negative predictive value.

*Indicates normal cutoff activity.

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This was the first indication of disparity among the assays. Sensitivity, when compared with the current gold standard of intestine histology, ranged from 91% to 97% for all ELISA assays, whereas specificity in this group of screening-identified children ranged from 20% to 70% (Table II). The Inova and Eurospital were slightly more sensitive compared with the IMMCO, Biofons, or Binding Site ELISA, although the Inova was much less specific when interpreted at the usual cutoff values.

Need For Quantitative Assessment Of Transglutaminase Autoantibodies
For Celiac Disease In Screening-Identified Children

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Figure 1. Direct comparison of TGAA RIA on the x-axis to commercial ELISA assays (y-axis). Dashed lines represent usual cutoff values for TGAA positivity. Closed symbols represent TGAA levels in subjects with normal intestine histology. Open symbols represent TGAA levels in subjects with abnormal intestine histology. RIA gives a wider range of TGAA levels compared with ELISA assays.

Figure 2. Comparison of commercial ELISA assays demonstrates variability in quantitative ability to detect TGAA. Dashed lines represent usual cutoff values for TGAA positivity. Closed symbols represent TGAA levels in subjects with normal intestine histology. Open symbols represent TGAA levels in subjects with abnormal intestine histology.
Direct quantitative comparison for each assay makes the differences between assays even more apparent. The RIA gave a wide range of signals for TGAA determination and thus appeared to be the most quantitative of all the assays (Figure 1). Inova and Eurospital were more sensitive than the other ELISA assays and better able to distinguish lower titers of TGAA. However, both the Inova and Eurospital assays gave a saturated signal when measuring high-titered antibodies and failed to distinguish between levels of very high-titered serum (ceiling effect). In contrast, the IMMCO, Biofons, and Binding Site assays were better able to separate higher titers of antibody but demonstrated a floor effect with TGAA levels that did not vary as much for lower signals (Figure 2). Therefore, sensitivity in these 3 assays was slightly reduced. The Inova and Eurospital ELISA assays correlated well with each other, and the IMMCO, Biofons, and Binding Site performed similarly. Because of differences in sensitivity, there was less correlation with Inova or Eurospital compared with IMMCO, Biofons, and Binding Site (Table III). Of all the ELISA assays, IMMCO, Biofons, and Binding Site had the best correlation among themselves. The RIA correlated best with the Inova assay as the result of high sensitivity but also had high correlation with the IMMCO, Biofons, and Binding Site assay, due to ability to quantify high-titered TGAA levels. It had the lowest correlation with the Eurospital assay.

The PPV for an abnormal biopsy of the RIA was only 63%, whereas the PPV for the ELISA assays ranged from 67% to 84%. This is much lower than that reported for symptomatic children ranging from 93% to 98%. The false-positive rate (1-specificity) ranged from 20% to as high as 80% for all assays. Even though the RIA provided the best quantitative assessment of TGAA at all levels, through the use of receiver operating characteristic curves, we determined that the ELISA assays could also be used quantitatively to determine optimal cutoff that enhanced PPV and minimized.

Use of the standard cutoff for antibody positivity for all assays (excluding the RIA) would have resulted in unnecessary biopsies in 7 to 16 of the 20 subjects with normal intestine histology, depending on the assay used. Increasing the cutoff sacrifices sensitivity for each assay, but for purposes of screening in our research population, long-term follow-up and repeated measurements is used with potential for biopsy at the time of high levels of TGAA. However, in clinical practice, use of higher cutoffs in ELISA assays to indicate timing of biopsy would have left 10 to 14 individuals out of the 34 celiac patients undiagnosed if repeated measurements were not performed. Therefore, there is no absolute antibody level that can completely exclude or predict celiac disease, and use of different cutoff values should be based on clinical/research objectives.

Finally, Figure 3 illustrates the differences in cutoff values for each assay relative to actual intestine biopsy result at the time the serum was obtained. TGAA levels for assays needed to be increased from 2 to 15 times the usual cutoff of positivity to maximize PPV in screening-identified children at risk for CD.

**DISCUSSION**

It is becoming more evident that children identified as having CD through screening tests have milder presentations often with subclinical disease. Even in screening of at-risk children for CD, where prevalence may be higher than in symptomatic children, a positive TGAA result often leads to intestine biopsies that are normal. Thus, the PPV of standard TGAA assays is lower in screening-identified children when held against the current gold standard of intestine biopsy. We
have found that quantitative assessment of the TGAA level is useful to help determine who should receive intestine biopsy during the screening process. In the field of type 1 diabetes, antibody workshops have also shown that autoantibodies measured in a radioimmunoassay format is usually superior to standard ELISA assays in both sensitivity and specificity. Bonamico et al have also reported that TGAA measured by RIA is more sensitive and specific than ELISA. However, in our series of screening-identified at-risk children, the ELISA assays overall are comparable to the RIA. The quantitative differences among different ELISA assays is striking. For example, the Inova and Eurospital assays are better at distinguishing lower levels of TGAA, while saturating at higher signals. The IMMCO, Biofons, and Binding Site assays are the opposite, giving relatively lower signals through low to medium titers of TGAA, while separating high titers better. However, the RIA is noted to give a wider range of TGAA values overall, being able to distinguish lower and higher TGAA levels equally well, without saturation. This might be true because the RIA, unlike the ELISA, measures TGAA in the fluid phase rather than solid phase. It is likely in the fluid phase the amount of autoantigen does not become a limiting factor, and high-affinity autoantibodies are readily detected.

Despite marked differences among TGAA assays, all were able to distinguish between children with positive celiac biopsies and children with normal biopsy specimens. This is encouraging because it indicates that TGAA assays can be used as a quantitative tool to select children who will be more likely to have intestine pathology. In our population prescreened with TGAA by RIA, we have identified higher cutoff values for several ELISA assays that could be used to help decide when to perform intestine biopsy. Proposed cutoff values for each assay are as follows, but should be confirmed with an independent set of screening-identified sera: Inova cutoff 124 Units, Eurospital cutoff 14.6 AU/mL, IMMCO cutoff 90 EU/mL, Biofons cutoff 70 AU, and Binding Site cutoff 26 U/mL. However, it should also be noted that in the research setting, our practice is to perform repeated measurements at-risk children over time. Thus, we increase our chances of “catching” a patient during a time when TGAA levels are higher before performing biopsy, indicating abnormal small-intestine histology. In the clinical scenario, where single-point testing is usually performed, setting higher cutoffs to minimize negative biopsy specimens may not be appropriate for the risk of underdiagnosis. Because of the observed fluctuations in TGAA that occur in prospectively followed at-risk children, we would suggest that biopsy be performed immediately after a positive TGAA test that exceeds the proposed cutoff for biopsy referral for that assay.

In summary, there is a need for careful selection of screening-identified children at risk for CD for intestine biopsy. Increasing the cutoff for a TGAA assay before performing intestine biopsy will minimize the number of negative biopsies being performed, although sacrificing sensitivity. Since there are marked variability among TGAA assays, researchers and clinicians should be aware of each assay’s limitations. A lack of standardization among the assays makes interpretations difficult. As we depend increasingly on serology for the diagnosis of CD, standardization in clinical and research laboratories becomes imperative. Such comparison and standardization could be aided through the use of a multicenter TGAA workshop.

REFERENCES


GASTRIC SENSORY AND MOTOR DYSFUNCTION IN ADOLESCENTS WITH FUNCTIONAL DYSPEPSIA

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Objectives Validated, noninvasive studies were used to compare sensation and motor function of the upper gastrointestinal tract in adolescents with functional dyspepsia (FD) and in control subjects.

Study design Fifteen adolescents with FD and 15 healthy participants underwent standardized symptom assessment, a satiation nutrient drink test, and 13C-Spirulina platensis breath test for gastric emptying of solids. Adolescents with FD also underwent measurements of fasting and postprandial gastric volume by means of single-photon emission computed tomography, and their results were compared with those from 15 healthy volunteers (age, 18 to 25 years).

Results Compared with control subjects, adolescents with FD had significantly higher postprandial symptoms 30 minutes after reaching maximum satiation with the nutrient drink test and significant delay in the T1/2 for gastric emptying of solids. Compared with healthy 18- to 25-year-old adults, adolescents had a diminished postprandial gastric volume response. By means of single-photon emission computed tomography, frequent baseline dyspeptic symptoms were associated with prolonged T1/2 for gastric emptying and higher postprandial aggregate symptom score. A baseline increased severity of dyspepsia symptoms was associated with prolonged T1/2 for gastric emptying.

Conclusions Adolescents with FD demonstrate increased postprandial symptoms after challenge, delayed gastric emptying, and a reduced gastric volume response to feeding. (J Pediatr 2005;146:500-5)

Symptoms of dyspepsia—that is, upper abdominal pain or discomfort with nausea, bloating, and early satiety—are reported by 20% of adolescents in the community. Most children with symptoms of dyspepsia have no identified cause at endoscopy and are designated as having functional dyspepsia (FD). Measurements that require upper gastrointestinal intubation such as gastroduodenal manometry and barostat suggest that adult patients with FD have heightened gastric visceral sensation, abnormal gastric and small bowel motor contraction patterns, and an impaired gastric accommodation response or meal induced relaxation of the stomach.

These invasive tests are impractical for clinical use and are difficult to perform or stressful in children and adolescents. Hence, the generalizability of the information presented to date is unclear, and current management of dyspepsia depends on empiric trials of medications in the absence of clinically applicable tests.

Our aim was to use validated noninvasive tests of gastric motor and sensory function such as the satiation nutrient drink test (NDT), 13C-Spirulina platensis breath test (13C-SPBT) for measurement of solid gastric emptying, and measurements of gastric volume through the use of single-photon emission computed tomography (SPECT) to investigate the pathophysiology that correlates with symptoms in adolescents with FD. Our secondary aims were to assess the association of these pathophysiologic disturbances and the frequency or severity of dyspeptic symptoms and to explore the validity of the 13C-SPBT to measure gastric emptying of solids relative to the gold standard, scintigraphy.
which was performed within a few days of the research breath test as part of the clinical evaluation of the patients.

**METHODS**

**Study Population**

After approval by the Mayo Clinic Institutional Review Board, the legal guardians of all volunteers gave written informed consent, and all participants provided informed assent. Patients (age, 13 to 17 years) were recruited from the pediatric gastroenterology clinic, based on the following inclusion criteria: (1) Rome II criteria for FD; (2) upper endoscopy within 6 months with no evidence of endoscopic esophagitis, Helicobacter pylori, or small-bowel disease by histology; (3) absence of food allergy and systemic organic and developmental disorders; and (4) underwent a scintigraphic solid gastric emptying study as previously described within 1 week for clinical purposes. Healthy adolescents (age, 13 to 17 years) were recruited from the local community.

**Study Design**

Both groups of adolescents completed the following: the questionnaire on pediatric gastrointestinal symptoms (QPGS), an age-appropriate questionnaire evaluating the frequency and severity of gastrointestinal symptoms associated with FD, the NDT, and 13C-SPBT for the measurement of gastric emptying of a solid meal. Only adolescents clinically diagnosed with FD underwent measurement of the fasting and postprandial gastric volumes through the use of 99mTc-SPECT. A group of young, healthy adults (age, 18 to 25 years) who had undergone measurements of fasting and postprandial gastric volume by means of 99mTc-SPECT for research purposes, using the same technique in the same laboratory, was used for comparison.

**Nutrient Drink Test**

Subjects ingested Ensure (Ross Laboratories, Columbus, OH) at a rate of 30 mL/min by refilling a glass at 120 mL every 4 minutes and scored their level of satiety every 5 minutes during ingestion using a graphic rating scale as previously described. The maximum tolerated volume ingested was recorded. Thirty minutes after completing ingestion of the Ensure, they scored their postprandial symptoms (nausea, bloating, fullness, pain) by using a 100-mm visual analog scale anchored with the words “unnoticeable” and “unbearable.”

**13C-SPBT for Solid Gastric Emptying**

This method has been described in detail elsewhere. The test meal consists of eggs dosed with 200 mg of 13C–S platensis (ABDiagnostics, Inc, Brentwood, TN). The egg meal was cooked and placed on a slice of whole wheat bread and eaten with a glass of skimmed milk, for a total caloric value of 220 kcal. After an overnight fast, breath samples were taken at baseline before the meal and at 45, 90, 105, and 120 minutes after meal ingestion. When emptied from the stomach and metabolized, the S platensis gives rise to respiratory CO2 that is enriched with 13C. The 13CO2 breath content was determined in a centralized laboratory (Dr Stanley Konopka, ABDiagnostics, Inc) by isotope ratio mass spectrometry. The T1/2 for gastric emptying with 13C Spirulina was calculated as previously described by a method that was validated by simultaneous estimates of solid gastric emptying by means of scintigraphy.

**99mTc-SPECT Method to Measure Gastric Volume**

This method has been described in detail elsewhere. Intravenous 99mTc–sodium pertechnetate is taken up by the gastric mucosa; 10 minutes after intravenous injection of 99mTc–sodium pertechnetate, dynamic tomographic acquisition of the gastric wall was performed by using a dual-head gamma camera (SMV FX-80, General Electric Medical Systems, Waukusha, WI) in a multi-orbit mode system during fasting and for two 15-minute time periods after ingestion of 300 mL of Ensure. The system performs orbits of 360° at 15 min/orbit. A 3-dimensional rendering of the stomach and its volume was obtained using the AVW 3.0 image processing libraries (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN) as previously described. Gastric volume change from fasting to the postprandial period was assessed as a difference and as a ratio.

**Statistical Analysis**

The Wilcoxon rank-sum test was used to compare the maximum tolerated volume, individual and aggregate postprandial symptoms scores, and T1/2 of gastric emptying in FD and healthy adolescents. The same test was used to compare gastric volumes and ratios measured by 99mTc-SPECT in FD and healthy adolescents. The significance level was set at 5%.

A nonparametric analysis of variance test was used to compare the frequency (never to monthly, weekly, or daily) and severity of symptoms (“a little,” “a lot,” or “a very lot”) relative to T1/2 gastric emptying (SPBT), aggregate symptom score after the NDT, and postprandial/fasting gastric volume difference and ratio by means of SPECT. A Bland-Altman plot was constructed to compare the gastric emptying T1/2 results obtained a few days apart in FD patients using the gold standard (scintigraphy) and the 13C-SPBT. All statistical tests were 2-tailed, and the results are presented as median and interquartile range.

**Sample Size Assessment**

Before the study, the planned sample size of 15 individuals per group was predicted to have 80% power (a = 0.05) to detect clinically meaningful differences in the primary end points: maximum tolerated volume (40% difference), aggregate symptom score (34% difference), and accommodation volume ratio (8% difference).

**RESULTS**

**Patients and Control Subjects**

Fifteen adolescents who met the Rome II criteria for functional dyspepsia, age, 16 years (14,17); 67% female;
height, 1.67 m (1.63, 1.77); weight, 63.5 kg (48.2, 67.4)], and 15 healthy adolescents, age, 16 years (14, 17); 60% female; height, 1.67 m (1.61, 1.73); weight, 64.8 kg (60.1, 75.8)] underwent physiologic testing. Fifteen healthy young adults in the age range of 18 to 25 years, age, 22 years (19, 24); 80% female; height, 1.71 m (1.64, 1.77); weight, 75 kg (61, 90.6) underwent measurements of gastric volume by means of 99Tc-SPECT.

Baseline Gastrointestinal Symptoms

The majority of patients with FD (66%) had daily pain in the prior 3 months. Based on the QPGS, patients with FD had multiple sensations of upper abdominal discomfort at baseline including pain (85%), nausea (93%), bloating (65%), feeling of fullness (71%), and not being hungry after eating very little (50%). Seventy-one percent identified pain as their most bothersome symptom in the last 3 months, and the remaining 29% identified discomfort (nausea, bloating, fullness, or early satiety) as most bothersome.

Satiation Nutrient Drink Test

There was no statistically significant difference in the maximum tolerated volume between FD and healthy adolescents (Table I). However, the patients with FD had significantly higher aggregate postprandial symptoms 30 minutes after reaching maximum satiation ($P = .03$). The individual symptom scores for nausea ($P = .05$) and bloating ($P = .05$) were also higher in adolescents with FD. Seven of the 15 patients with functional dyspepsia had pain scores that were above the upper 99th percentile of the postprandial score for pain (>57/100 mm) in healthy adolescents.

13C-SPBT for Solid Gastric Emptying

Adolescents with FD had significantly prolonged $T_{1/2}$ for gastric emptying compared with healthy adolescents [123 (94, 149) minutes vs 97 (82, 108), $P = .03$, Figure 1, A]. Eight of the 15 patients had a $T_{1/2}$ for solid gastric emptying above the 90th percentile for healthy adolescents.

The Bland-Altman plot displaying the difference in estimated $T_{1/2}$ for gastric emptying with $^{13}$C–$S$ platensis versus the $T_{1/2}$ for gastric emptying by scintigraphy in the same individuals is shown in Figure 1, B. The plot illustrates the concordance of gastric emptying $T_{1/2}$ estimates by the 2 methods. Thus, 70% of the $T_{1/2}$ estimates are within 29 minutes of the $T_{1/2}$ by scintigraphy across a wide range of gastric emptying rates (80 to 163 minutes).

Gastric Volume Change with 99mTc-SPECT

Compared with healthy adults, age 18 to 25 years, adolescents with functional dyspepsia had a significantly higher fasting gastric volume ($P = .05$, Table II). After a meal, adolescents with FD had a significantly lower gastric volume change ($P = .01$) and a lower postprandial/fasting gastric volume ratio ($P = .005$, Figure 2). Eight of the 15 adolescents with FD had a gastric volume change that was <300 mL, the 5th percentile for the healthy adult control group.

Relation Between Frequency of Dyspeptic Symptoms and Gastric Functions

An increased frequency of dyspeptic symptoms (daily versus monthly to never) was associated with solid gastric emptying with $^{13}$C-SPBT ($P = .004$). Adolescents who had daily symptoms had a longer $T_{1/2}$ for gastric emptying compared with patients with weekly or less frequent symptoms (Figure 3, A). There was also a direct correlation between the frequency of symptoms and the postprandial aggregate symptom score by the NDT ($P = .04$; Figure 3, B). There was no association between the frequency of symptoms and the postprandial gastric volume change by SPECT ($P = .51$).

Relation Between Severity of Dyspeptic Symptoms and Gastric Function

Among adolescents who had dyspeptic symptoms in the last 3 months, severity of symptoms was associated with longer gastric emptying ($P = .007$). Those with pain or feeling uncomfortable “a very lot” had a longer $T_{1/2}$ for gastric emptying [$T_{1/2} = 147 (127, 170)$ minutes] compared with those whose symptoms affected them “a little” [$T_{1/2} = 102 (88, 102)$ minutes]. There was not a significant association between the severity of symptoms and the postprandial aggregate symptoms score ($P = .48$) or the postprandial gastric volume change ($P > .05$).

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**Table I. Maximum tolerated volume, time to satiation, and individual and postprandial aggregate symptoms score by the satiation nutrient drink test**

<table>
<thead>
<tr>
<th></th>
<th>Maximum tolerated volume (mL)</th>
<th>Time to satiation (min)</th>
<th>Aggregate symptoms</th>
<th>Nausea</th>
<th>Fullness</th>
<th>Bloating</th>
<th>Pain</th>
</tr>
</thead>
</table>

Values are median [interquartile range].
$^*P = .05$.
$^\dagger P = .03$. 

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Chitkara et al The Journal of Pediatrics April 2005
DISCUSSION

The current study documents the spectrum of sensory and motor functions of the stomach in adolescents with FD through the use of noninvasive methods. Although adolescents with FD were able to ingest a similar volume of a nutrient drink, they developed greater postprandial symptoms, in particular nausea and bloating, compared with healthy adolescents. Adolescents with FD also had a significantly higher T1/2 for gastric emptying of a solid meal and a decreased change in gastric volume after the meal relative to control subjects. These results suggest that adolescents with FD have abnormal gastric postprandial sensation, decreased gastric emptying, and diminished gastric volume responses after a meal, consistent with reports using invasive intubation studies in adults with FD. These observations also suggest that these novel noninvasive approaches may be effective to diagnose and select treatment for adolescent patients with dyspepsia rather than exposing patients to unnecessary investigations or empiric therapy.

Drink tests provide a simple method to assess and reproduce symptoms of dyspepsia as well as the response to medications used to treat this disorder. They have been used to assess satiation in healthy children using both water and nutrient drinks. In healthy children, ages 7 to 12 years, the amount of water consumed correlated with age, weight, and height. Chial et al demonstrated that healthy adolescents, age 13 to 17 years, had sex-related differences in the maximum

Table II. Gastric volume assessment by means of SPECT in adolescents with functional dyspepsia and young healthy adults (18 to 25 years)

<table>
<thead>
<tr>
<th></th>
<th>Fasting volume (cm³)</th>
<th>Postprandial volume (cm³)</th>
<th>Change in gastric volume (postprandial-fasting)</th>
<th>Postprandial/fasting volume ratio</th>
</tr>
</thead>
</table>

Values are median [IQR].
*P = .05.
†P = .0005.
‡P = .01.

Figure 1. A, Solid gastric emptying with 13C–Spirulina platensis breath test in adolescents with functional dyspepsia and health. (Dotted line represents upper 95% for T1/2 of gastric emptying in healthy adolescents). B, Bland-Altman plot comparing concordance between T1/2 of solid gastric emptying by the 13C–Spirulina breath test compared with the gold standard, 99mTc-scintigraphic–labeled meal (r = 0.37; P = .02).

Figure 2. Images of a patient with FD and control fasting-to-postprandial volume change measured by SPECT.
tolerated volume of Ensure to reach full satiation and in the postprandial symptom scores. Thus, age, sex, height, and weight are important covariates that need to be matched or controlled when assessing satiation by drink tests. There were no significant differences in these baseline characteristics between adolescents with FD and the healthy control group in the current study.

We observed that adolescents with FD had significantly delayed gastric emptying. Slow gastric emptying is thought to contribute to upper gastrointestinal symptoms and has been observed in 10% to 44% of adults with functional dyspepsia.23,24 The $^{13}$C-SPBT for gastric emptying of solids provides a fair estimate compared with the gold standard, gastric emptying by scintigraphy, though the correlation is not as robust as in adults ($r = 0.37$ in the current study versus $r = 0.88$).8 This is probably due to the fact that the two studies were performed on different days with different caloric content test meals in the current study. Thus, further validation of the $^{13}$C-SPBT for solid gastric emptying in children is necessary, but it has the advantage of avoiding radioactivity, allowing the performance of the study in healthy and minimally symptomatic children. Slow gastric emptying has previously been associated with severe postprandial fullness and vomiting in adults with functional dyspepsia.23,24 Because of the small sample size of the current study, the association between gastric emptying and individual dyspeptic symptoms was not explored. However, both an increased severity and increased duration of dyspeptic symptoms were associated with slower gastric emptying of solids.

The current study demonstrated a significant difference in the postprandial to fasting gastric volume difference in adolescents with FD compared with health, as previously shown in adult patients with functional dyspepsia,25 symptomatic postfundoplication,11 and other disorders such as diabetic dyspepsia.26 Our results show that the change in gastric volume is lower, in part, because fasting gastric volumes were higher in functional dyspepsia. This increased fasting volume may reflect the delayed gastric emptying observed in these patients. The mechanism of increased fasting and decreased postprandial volume change in adolescents with FD requires further study. Normalizing the gastric accommodation response may be a potential target for alleviating symptoms of individuals with FD with impaired gastric accommodation. Buspirone,27 sumatriptan, nitrites, and cisapride28 increase the volume of gastric accommodation in healthy adults or in patients with FD. Further studies in both children and adults are warranted.

An unavoidable limitation of this study is the lack of control data for gastric volume using $^{99}$Tc-SPECT from healthy adolescents. This is inevitable, given that the only validated imaging based study of gastric volume change is the $^{99m}$Tc SPECT test.11 Although age and weight were not considered to be significant covariates in previous analyses of gastric volume measurements in healthy adults,11,29 no studies in healthy children involving radioactivity could be performed to confirm this because of ethical considerations.30 However, newer nonradioactive techniques such as MRI31 or 3-dimensional ultrasound32 are being developed and validated as measures of gastric volume. Physiologic testing for functional gastrointestinal disorders in the pediatric age group may be practical in the near future.

**CONCLUSIONS**

Adolescents with functional dyspepsia demonstrate increased postprandial symptoms after challenge, delayed gastric emptying, and reduced gastric volume response to feeding. Identifying these physiologic targets by using a combination of noninvasive tests may lead to selective management based on pathophysiology.

The authors thank Mrs Cindy Stanislav for secretarial support, Ms Jennifer Pickett for nursing and scheduling support, and ABDiagnostics Inc, Brentwood, Tennessee, for providing the substrate and measurements of $^{13}$CO$_2$ for estimation of gastric emptying.
REFERENCES


IMPACT OF ZINC SUPPLEMENTATION ON MENTAL AND PSYCHOMOTOR SCORES OF CHILDREN AGED 12 TO 18 MONTHS: A RANDOMIZED, DOUBLE-BLIND TRIAL
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Objective  To evaluate the effect of zinc supplementation on mental and psychomotor scores in children aged 12 to 18 months.

Study design  In this double-blind, randomized, placebo-controlled trial, children aged 6 to 30 months received daily elemental zinc (10 mg for infants and 20 mg for others) or placebo for 4 months. Bayley Scales of Infant Development II were used for development assessment in the 12- to 18-month subgroup at enrollment and the end of the study.

Results  At the end of the study, the adjusted mean mental ($P = .36$) and psychomotor ($P = .28$) index scores were similar in the intervention and control groups. In a multivariate model, the baseline mental development index score was positively associated with the mother’s schooling, the child’s height for age, packed cell volumes, hospital birth, and attendance at a daycare center, and was negatively associated with the child’s age. Breastfeeding, the child’s weight for height, and packed cell volumes were positively associated with the baseline psychomotor index score.

Conclusion  Zinc supplementation did not affect the mental or psychomotor development index scores in a setting in which zinc deficiency is common. (J Pediatr 2005;146:506-11)

Zinc deficiency is common in developing countries because of diets rich in phytate and fiber, a low intake of animal foods, and intestinal zinc losses during diarrheal illnesses. Zinc supplementation reduces the incidence of pneumonia and diarrhea, and improves length gain in preschool years. The relationship between zinc deficiency and cognition is less clear. Zinc deficiency during rapid brain growth or during the juvenile and adolescent period is reported to affect cognitive development. The role of zinc in cognition in infants is biologically plausible. The high concentrations of zinc in the synaptic vesicles of the special “zinc containing” neurons in the forebrain, with its function in biochemical processes like myelination and the release of neurotransmitters like gamma-aminobutyric acid and glutamate, suggest that it may be a modulator of neuronal excitability.

METHODS

Study Setting  The setting and details of the methods have been previously published. The trial was conducted in the urban community of Dakshinpuri in New Delhi, which has 15,000 dwellings and 75,000 inhabitants. Childhood malnutrition, zinc deficiency, diarrhea, and lower respiratory tract infections are common in this setting.
Randomization and Blinding

Children were included when parental informed written consent was available. Eligible children for the main study were individually randomized by using a simple randomization scheme in blocks of 8. The randomization scheme was generated by a statistician at the Statens Serum Institute, who was not otherwise involved with this study, using SAS software (version 8.1; SAS Institute, Cary, NC). Zinc or placebo syrups, similar in appearance and taste, were prepared and packaged in unbreakable bottles by GK Pharma Aps, Koge, Denmark; they also labeled bottles with unique child identification numbers according to the randomization scheme. Six bottles, 1 for each of the 4 study months and 2 extra, per child were produced and labeled before enrollment commenced.

Enrollment and Intervention Delivery

Children aged 6 to 30 months were identified through a door-to-door community survey. We excluded children for non-consent, when they were planning to move within the next 4 months, when they required hospitalization on the enrollment day, or when they had received a massive dose of vitamin A within 2 months. Information was obtained on the socioeconomic characteristics of the family, the child’s feeding practices, recent morbidity experience, birth weight, access to television, and consumption of alcohol by the father. Diarrhea and lower respiratory tract morbidity were monitored after randomization in all enrolled children, as described previously. Development assessments at enrollment and study end were limited to children aged 12 to 18 months at enrollment.

Zinc was supplemented as zinc gluconate, with daily doses of 10 mg (6 mL) of elemental zinc for infants and 20 mg (12 mL) for older children for 4 months by a study attendant. One bottle containing 250 mL was kept in the child’s home and replaced monthly. Immunizations and treatment for acute illnesses were provided at the study clinic as per the World Health Organization guidelines. The study was approved by the All India Institute of Medical Sciences ethics committee. Informed verbal consent from community leaders and written consent from parents was obtained, and a copy of the form was left with the family.

Sample Size Calculations

Sample sizes were estimated with data from development assessment practice sessions conducted in the same area. Using value of α as 0.05 (95% confidence) and that of β as 0.1 (90% power) to detect a 5% improvement in the mean mental development score and in the mean psychomotor development score, 105 and 204 children, respectively, were required to be enrolled per group. To allow for about 20% attrition, we assessed mental and psychomotor development in 250 children per cell.

Development Assessments

Mental and psychomotor development scores were assessed at enrollment and 4 months after supplementation with the Bayley Scales of Infant Development II, strictly according to the instruction manual. Children were tested in the presence of their mothers at the clinic. When the child was uncooperative, crying, or sleepy during the assessment, the test was abandoned and the child was re-assessed the next day. Three attempts on different days were made before the child was labeled as uncooperative. Children who were sick at the time of assessment were treated and tested after recovery.

Assessments were done in a well-lit, ventilated room, free of distractions. Rapport was established with the child, and the assessment was initiated only when the child was comfortable. The chronological age was first estimated by subtracting the date of birth from the date of testing. For premature births, the months and days by which the child was premature were subtracted from the chronological age to compute the corrected age. Children were given credits for administered items only when they were able to complete them.

The Bayley Scales II were administered by the author and a clinical psychologist, who were both trained. After several practice sessions, standardization exercises were conducted in 100 children. Ten children were assessed on a day, independently by the 2 assessors with a 1-hour interval between the 2 assessments; these exercises were completed within 10 days.

Interobserver agreement during the study was ascertained by testing in duplicate, 10% of the baseline and end study assessments and correlating the mental developmental index and psychomotor developmental index score obtained by the first and the second assessor with the Kappa statistic (mental development index Kappa = 0.88; psychomotor development index Kappa = 0.86).

Plasma Zinc

Blood samples were collected in all children at baseline and for 30% of randomly selected children at study end for the analysis of plasma zinc and copper levels and packed cell volume. The methods and results for these samples have previously been published.

Data Management and Analysis

Double data entry followed by validation was completed within 48 hours of filling out the form in the field. A child’s raw scores on the mental and psychomotor scales were computed by adding the total number of items for which the child received credit on each of the scales. The raw mental and psychomotor scores were converted into age-adjusted index scores with US norms. Mean mental and psychomotor development scores were compared between groups with the Student t test.

A regression analysis was performed to adjust the effect of zinc on mental and psychomotor scores for selected baseline characteristics. For this, the effect of each baseline factor was determined on the regression coefficients of zinc/placebo treatment on the end study mental and psychomotor scores in multiple linear regression models with 2 independent variables (ie, treatment assignment and the characteristic). The factors that changed the crude coefficient by ≥10 percent were included in the final model to provide estimates of zinc.
treatment adjusted for potential confounders, and the adjusted and unadjusted results are presented. For mental development scores, the baseline characteristics in the model included baseline mental index score, packed cell volume, age in months, father’s alcohol consumption, and years of schooling of parents. In the case of psychomotor development scores, the explanatory variables in the model included baseline psychomotor index score, height for age, packed cell volume, and years of schooling of parents.

Additionally, a secondary analysis was performed to identify covariates associated with baseline mental and psychomotor scores with multiple regression. All factors that were significantly ($P < .05$) associated with the outcome in the univariate analysis were included in the model in addition to

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**Figure.** Trial profile of the main study with outcomes of diarrhea and pneumonia and the substudy on development assessments.
age as an explanatory variable. Analyses were conducted with Stata software, version 6 (Stata Corp, Union Station, Tex).

**RESULTS**

A total of 3802 children aged 6 to 30 months were available through the door-to-door survey. Of these, 2482 were randomized (Figure). Those children who were 12 to 18 months old at enrollment were subselected for baseline and end-study mental and psychomotor assessment, 327 in the zinc group and 323 in the placebo group. Of these, 283 (86.5%) in the zinc group and 288 (89.2%) in the placebo group were available for end-study assessments (Figure).

**Baseline Characteristics**

The children in the 2 groups were similar for most variables, but there were a significantly higher proportion of children in the zinc group whose mothers (\( P = .07 \)) and fathers (\( P = .004 \)) were literate (Table I). At baseline, there was a significant difference between the mental development index scores of the zinc and placebo groups (\( P = .0009 \)), but the psychomotor development scores were similar (\( P = .12 \)).

As reported previously, 45% of the study subjects had low plasma zinc levels at enrollment, and the end-study mean serum zinc level was significantly higher in the zinc group than in the placebo group.\(^8\,^9\)

**End Study Mental and Psychomotor Development Index Scores**

After 4 months of supplementation, the mean mental development index was similar in the intervention and placebo groups (\( P = .09 \); Table II). The mean psychomotor development index (\( P = .12 \)) was similar in the 2 study groups (Table II). There was no interaction between baseline zinc status and the effect of zinc supplementation on the end-study mean mental development index or psychomotor development index\(^9\) (\( P = .5 \) for psychomotor and \( P < .5 \) for mental index scores). Zinc supplementation, therefore, did not have a differential effect among subjects who were zinc deficient at baseline.

**Factors Associated with Baseline Mental and Psychomotor Index Scores**

The child’s age was negatively associated with the mental development scores, and the mother’s years of schooling, height for age, packed cell volume, birth at hospital, and attendance at the village Anganwadi (a daycare facility for children aged <6 years, where a daily dietary supplement is also given) were positively associated with the mental development scores. For the psychomotor development index scores, a positive association was found with breastfeeding, weight for height, height for age, and packed cell volume, and a negative association was found with the age of the child. Zinc had no significant effect on the mental (\( P = .35 \)) or psychomotor development index score (\( P = .28 \)) after adjustment for several potential confounding factors (Table III).

**DISCUSSION**

Optimizing intakes of zinc did not lead to improved mental or motor development scores in this study. Some limitations of the study need consideration. Assessments were done only in the 12- to 18-month age subgroup of children. This could potentially have resulted in baseline differences in the intervention and control groups, and although the intervention effects were adjusted for potential confounding factors, there may be others that were not measured. Although the Bayley Scales II were developed and standardized on the basis of a sample representative of a US population, we believe that for the purpose of comparing the impact of zinc versus placebo on cognition, this instrument is valid.

The findings on mental development are consistent with earlier randomized trials in which zinc was supplemented during early infancy.\(^{17,18}\) In another trial, in which term infants were supplemented with zinc for 5 months, the mental development score was lower in the zinc group than in the
placebo group, although the effect was small.19 One of the 2 zinc supplementation trials in low birth-weight infants showed significant improvement in motor development with the Griffiths scale, but the other did not find such an effect.17,18 Two trials in India and Guatemala found significant improvement in the physical activity of infants aged 6 to 9 months who received zinc supplementation; in these trials, activity was assessed with the time sampling observation method.20,21 In a recent zinc supplementation trial conducted among infants in India who were small for their gestational age, no direct effects of zinc supplementation were seen on the infants’ development or behavior.22

The conclusion from these studies is that zinc supplementation in infants and young children in developing countries failed to improve mental development. Although a few studies found an impact on physical activity,20,21 the implications of these benefits are unclear.

Our results confirm previous findings that low maternal education level, stunting, and iron deficiency anemia are associated with impaired mental and psychomotor index scores. Children born in hospitals had better scores, which is probably an indirect indicator of socioeconomic status. Attendance at the day care facility was associated with higher development index scores. This may be related to the stimulation provided by the caregivers or peers or the supplement provided to these children at the facility. The decline in mental development index scores with an infant’s age may be because existing care practices and other environmental factors are inadequate, and these may worsen during this period because of the cessation of breastfeeding by most mothers and the increasing demands on the child for interaction at this age. The importance of care practices is also reflected in the positive association between a mother’s education level, child’s height, and packed cell volume and the psychomotor development scores. Weight for height was directly related to the psychomotor scores, but not to the mental development scores. Malnourished children, particularly those who are wasted, are frequently described as lethargic, possibly because they reduce their activity as a protective strategy to conserve energy.

The lack of benefit of zinc on mental and psychomotor index scores may have several explanations. There may truly be no effect of zinc deficiency on cognition; 4 months may be insufficient time for treatment effects to unfold; or zinc deficiency may affect specific behaviors or processes that are not detected with the Bayley Scales II.23 It is also possible that the effect of zinc on mental and psychomotor development may have been limited by its interaction with other trace elements, such as iron, copper, folate, and possibly others.24 Zinc deficiency exists as a part of overall malnutrition and in combination with other micronutrient deficiencies. Concurrent supplementation with other limiting micronutrients may be necessary for the effect of zinc repletion to manifest. Improving zinc intakes in developing countries, such

### Table II. Adjusted and unadjusted end-study mental and psychomotor scores of children aged 12 to 18 months

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Zinc</th>
<th>Placebo</th>
<th>Difference in means or difference in proportions (95% CI)*</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>283</td>
<td>288</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mental Development</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD) index score</td>
<td>92.8</td>
<td>91.3</td>
<td>1.5 (-0.3 to 3.3) †</td>
<td>.358</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-0.7 (-2.1 to 0.8) ‡</td>
<td></td>
</tr>
<tr>
<td>Psychomotor Development</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD) index score</td>
<td>93.9</td>
<td>92.2</td>
<td>1.6 (-0.4 to 3.6) †</td>
<td>.284</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.8 (-0.7 to 2.3) ‡</td>
<td></td>
</tr>
</tbody>
</table>

*All estimates are unadjusted, except those marked with † and ‡, which are values after adjusting for potential confounders.
† Adjusted effects in multiple linear regression models adjusted for baseline mental index score, packed cell volume, age in months, father consuming alcohol, and years of schooling of parents. Model R square = 40%.
‡ Adjusted effects in multiple linear regression models adjusted for baseline psychomotor index scores, age in months, height for age, packed cell volume, and years of schooling of parents. Model R square = 39%.

### Table III. Factors associated with baseline Mental and Psychomotor Index Scores in multiple regression models

<table>
<thead>
<tr>
<th>Predictor variable</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mental Development</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Index Score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td>-2.21</td>
<td>-2.6--1.80</td>
<td>0</td>
</tr>
<tr>
<td>Years of schooling of mother</td>
<td>0.48</td>
<td>0.29–0.67</td>
<td>0</td>
</tr>
<tr>
<td>Height-for-age Z Score</td>
<td>1.82</td>
<td>1.01–2.63</td>
<td>0</td>
</tr>
<tr>
<td>Baseline packed cell volume (%)</td>
<td>0.38</td>
<td>0.13–0.63</td>
<td>.003</td>
</tr>
<tr>
<td>Children born in hospital</td>
<td>2.21</td>
<td>0.50–3.92</td>
<td>.011</td>
</tr>
<tr>
<td>Children attending Anganwadi</td>
<td>4.51</td>
<td>0.09–8.94</td>
<td>.046</td>
</tr>
<tr>
<td>Psychomotor Development</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Index Score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age in months</td>
<td>-0.94</td>
<td>-1.51–0.37</td>
<td>.001</td>
</tr>
<tr>
<td>Children breast fed</td>
<td>3.43</td>
<td>0.87–5.98</td>
<td>.009</td>
</tr>
<tr>
<td>Weight-for-height Z-score</td>
<td>1.55</td>
<td>0.15–2.94</td>
<td>.030</td>
</tr>
<tr>
<td>Height-for-age Z-score</td>
<td>4.09</td>
<td>3.02–5.14</td>
<td>.000</td>
</tr>
<tr>
<td>Baseline packed cell volume (%)</td>
<td>0.57</td>
<td>0.23–0.92</td>
<td>.001</td>
</tr>
</tbody>
</table>

*Model R square = 27%.
† Model R square = 16%.
as India, is, however, a priority because of its effects on diarrhea and respiratory infections.

The authors thank Dr. Martin Frigg, Task force SIGHT AND LIFE, Basle Switzerland, for providing vitamin A and placebo capsules, the Indian Council of Medical Research and the Norwegian Council of Universities’ Committee for Development Research and Education for their core support, Renu Bhatia for help in administering Bayley Scales II, and Sandeep Saxena for statistical analysis.

REFERENCES

SOCIAL CONSEQUENCES IN ADULT LIFE OF END-STAGE RENAL DISEASE IN CHILDHOOD

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Objective  To describe employment achievement and social independence of adults with childhood end-stage renal disease (ESRD) and to explore determining factors.

Study design  Employment, occupational level, living arrangements, social engagements, and subjective health perception were cross-sectionally established between 1998 and 2000 in 144 of all living 187 adult Dutch patients with ESRD with an onset at age 0 to 15 years between 1972 and 1992. Potential clinical determinants were established by means of a review of all medical charts.

Results  Compared with age-matched Dutch citizens, patients were more often involuntarily unemployed (19.4% vs 11.1%), had a lower occupational level, more often still lived with their parents, and more often had no partner. A low occupational level was associated with a dialysis duration >8 years (OR, 9.6; 95% CI, 1.9-47.6); living at the parental home was associated with the male sex (OR, 3.4; 95% CI, 1.5-7.8) and with a dialysis duration >8 years (OR, 3.7; 95% CI, 1.3-10.2).

Conclusion  Prolonged dialysis during childhood may decrease the ability to gain high-skilled professions and social independence. Unemployment is twice as high in adult patients with childhood ESRD than in healthy persons, but more than twice as low as compared with young ESRD patients with an adult onset of the disease, according to previous reports. (J Pediatr 2005;146:512-7)

As life expectancy has increased in patients with end-stage renal disease (ESRD), concern has risen about its late sequelae. Until now, few data existed on the effect of intensive, chronic therapy in childhood on physical and social development in adulthood. Although previous studies report high percentages of unemployment and social dependency in this group, most of these studies are small, are based on registry reports with mostly incomplete data, or are now >10 years old.1-6 No data exist on the occupational level of this particular patient group.

Between 1998 and 2000, we conducted a national long-term follow-up study to evaluate Late physical, social, and psychological Effects of Renal Insufficiency in Children (LERIC) in all Dutch children who had commenced renal replacement therapy between 1972 and 1992 and who were born before Jan 1, 1979. In this paper, we report on the social outcome. Our aim was to establish the employment status, the occupational level, and the domestic and marital status as measures of social independence. Our second aim was to explore the association of potential determinants with these outcomes. Finally, we measured the impact of somatic and psychosocial factors on the subjective health perception in these young adult patients.

METHODS

Study Design

LERIC was designed as a cohort study and consisted of a cross-sectional and a retrospective part. The aim of the cross-sectional study was to establish the current health status and social status. The aim of the retrospective part of the study was to evaluate the relationship of a set of predefined determinants to health outcome. The study covered the total period of renal replacement therapy for each patient. The medical ethics committees of all participating centers approved the study.
Formation of the Cohort

The LERIC cohort comprises all Dutch patients who had started chronic renal replacement therapy at age of 0 to 15 years between 1972 and 1992 and who were born before 1979. Patients in whom renal function recovered within 4 months after commencing dialysis were excluded. Patients who underwent pre-emptive transplants were included. Patients who started renal replacement therapy after 1991 were excluded so that there was a potential follow-up period of at least 6 years. Patients were recruited from the database of The National Dutch Registry of patients on renal replacement therapy (RENEINE, Rotterdam, The Netherlands) and the database of all centers for pediatric nephrology in The Netherlands.

Pediatric Dialysis and Transplantation Centers

Programs for chronic dialysis and for renal transplantation in children were started in the Netherlands in the early 1970s in 4 University hospitals, in Utrecht, Nijmegen, Rotterdam, and Amsterdam. By law, renal replacement therapy in children remained restricted to these 4 centers. All centers have followed approximately the same treatment strategy in trying to transplant children as soon as possible and keeping the period of dialysis as short as possible. After 1980, peritoneal dialysis became gradually the favorite mode of chronic dialysis in children, leaving hemodialysis as a second option.

Data Collection

Between November 1998 and August 2000, members of the LERIC-team visited 37 hospitals in the Netherlands, including the 4 centers for dialysis and transplantation in children. They collected data on clinical characteristics and potential determinants in relation to social outcome by reviewing all available medical charts. Emigrated patients were located, and medical information was obtained from their current physicians. The predefined determinants were: sex, other ethnicity, period of onset of renal replacement therapy (1972-1981 vs 1982-1992), age at onset of renal replacement therapy, total duration of renal replacement therapy and of dialysis, the occurrence of disabilities, co-morbidity, and a short stature at adult age (defined as height <−2SD). Co-morbidity was considered present in the case of the presence of 1 or more of the clinical diseases as defined by Davies et al (ie, malignancy, clinical apparent ischemic heart disease, peripheral vascular disease, clinical apparent left ventricular dysfunction, diabetes mellitus, systemic collagen vascular disease, cerebral vascular disease, chronic obstructive airway disease, or other significant pathology). Disabilities were considered to be present in the case of severe deafness, blindness, or being disabled by motor function disorders. The procedure of the cohort formation and the method of data collection have been described elsewhere in detail.

We collected data on current subjective health perception with the RAND-36 Health Survey, the level and percentage of employment, educational attainment, on the level of engagement (married/partner, no partner), on the domicile situation (ie, whether patients were living alone, with parents, or a with partner), and on having offspring. The RAND-36 Health Survey is almost identical to the MOS SF-36. It is made up of 36 questions and standardized response choices, which measure 8 distinct mental and physical health domains. Overall physical health and mental health are assessed by aggregation of all domain scores according to an algorithm, leading to the so-called Physical Component Summary and Mental Component Summary.

Occupational level was established with a 5-point categorization scale, used by the National Dutch Bureau of Statistics (CBS; Centraal Bureau voor de Statistiek, The Hague, The Netherlands; www.CBS.nl). This categorization of employment is based on the educational attainment of skills required for a given job and distinguishes the following levels: elementary level (no schooling needed), low level (basic school and low vocational training, “Lager Beroeps Onderwijs”), intermediate level (intermediate vocational training, “Middelbaar Beroeps Onderwijs” or “HAVO/VWO”), high level (high level vocational training, “Hoger Beroeps Onderwijs”), and scientific level (university). If a job could be categorized in more than 1 category, we used the level of educational attainment of the patient for the final categorization. Occupational level of both parents was established by means of a questionnaire and categorized as aforementioned. Unemployment was defined as <20% of time spent on paid work and not attending a full scholarship; homemaking was defined as employment.

We compared patient data on educational attainment, employment, and marital status with data from an age-matched Dutch population, which were obtained from the Central Dutch Bureau of Statistics CBS (www.cbs.nl). We used the figures from 1999, and compared the employment status among different age groups: 18 to 24 years, 25 to 34 years, and >34 years. Finally, we analyzed associations between the previously described clinical determinants and unemployment, low occupational level, having a partner, and domestic situation.

Statistical Analysis

Data are presented as means and percentages. To compare the difference between patients and the age-matched Dutch population and to examine the relationship between disease characteristics and levels of employment, levels of employment were dichotomized as follows: elementary and low level employment (LOW) versus intermediate, high, and scientific occupational level (HIGH). We used a stratified analysis for linear trend in proportions (Epi_Info ®, www.cdc.gov/epiinfo) to analyze the difference in occupational level between patients and the general Dutch population. The Chi-square test was used to compare the proportion of patients living with a partner versus the proportion of patients living alone or with their parents, as a measure of social integration, and to compare the proportion of patients living with their parents versus the proportion of patients living alone or with a partner, as a measure of social dependence. The Pearson correlation test was used to analyze the univariate association between unemployment, a low level (LOW) of employment, living at the parental home, and having no partner on one side, and the previously mentioned clinical determinants on the other. All significant determinants of the univariate analysis (set at a level of P ≤0.2; data not shown) were then entered into a logistic regression model with forward conditional inclusion to assess their independent impact on the outcome measures. For this multivariate analysis, the following determinants were dichotomized, on the basis of mean values or clinical importance: total duration of renal replacement therapy into a group of patients treated <18 years and a group treated >18 years,
total duration of dialysis into a group that was on dialysis <4.4 years and a group that was on dialysis >4.4 years, and age at the time of investigation into a group <30 years old and a group >30 years old. To analyze the effect of a long period of dialysis on outcomes, we also dichotomized the total dialysis duration in periods of more or less than 8 years. Apparent significant associations of dichotomized variables with outcomes were checked with a linear regression model. Only results with significance level of \( P \leq 0.05 \) are reported. Patients who followed an educational program for more than 50% of their time were defined as “employed” in the correlation and regression models.

The same method was used to analyze the effect of somatic and social factors on the quality of life; the Pearson correlation test was used to analyze the univariate association between the Physical Component Summary and the Mental Component Summary of the RAND-36 and clinical and social determinants (ie, unemployment, low occupational level determinants, having no partner, and living at the parental home; data not shown). All significant determinants of the univariate analysis (set at a level of \( P \leq 0.2 \)) were then entered into a logistic regression model with forward conditional inclusion to assess their independent impact on the outcome measures.

## RESULTS

### Cohort

Of the 249 patients in the cohort, 62 had died at the time of investigation. Details on mortality and causes of death have been reported previously.\(^8\) Of the 187 living patients at the time of investigation, 144 (77%) participated in this part of the LERIC study. Clinical characteristics of participants and non-participants at baseline were similar. At the time of investigation, 30 patients were treated with chronic dialysis and 114 had a functioning renal graft. The mean age at time of investigation was 29.3 years (range, 20.7-41.8 years). The mean total duration of renal replacement therapy, chronic dialysis, and living with a renal graft were 18.0, 4.4, and 13.6 years, respectively. Details on clinical characteristics of all patients are presented in Table I.

### Employment, Domestic Situation, Partnership, and Offspring

A total of 97 patients (67.4%) had paid work. Of the patients who were unemployed, 28 (19.4%) were involuntarily without work or found to be medically unfit to work, compared with 11.1% of all age-matched Dutch inhabitants (Table II). The percentage of unemployment between patients who underwent a transplant and patients who received dialysis was not significantly different (17.7% vs 25.8%; Chi-square \( P = .2 \)). The percentage of involuntary unemployment was similar in all age groups (18-24 years, 21.1%; 25-34 years, 17.6%; 35-44 years, 23.8%; Chi-square \( P = .9 \)). The occupational level was significantly lower in patients than in the general population (Chi-square for linear trend = 18.4; \( P = .00002 \); Table III).

Of all 144 patients, 46 (31.9%) lived alone, 49 (34%) lived with a partner, and 49 (34%) still lived with their parents. The odds ratio of living with parents, as a measure of dependency, versus living alone or with a partner was 3.3 (95% CI, 2.3-4.7) for LERIC patients compared with age-matched Dutch inhabitants. The odds ratio of living with a partner was 0.3 (95% CI, 0.2-0.4) for LERIC patients compared with age-matched Dutch control subjects (Figure).

Twelve women and 11 men had offspring; 1 woman was pregnant at the time of investigation for the first time. In 4 women, a pregnancy was terminated early on medical grounds. One woman had given birth to 4 children.

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**Table I. Clinical characteristics of participants and non-participants of the original LERIC cohort in this study**

<table>
<thead>
<tr>
<th></th>
<th>Participants</th>
<th>Non-participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>144</td>
<td>43</td>
</tr>
<tr>
<td>Male/Female</td>
<td>76/68</td>
<td>27/16</td>
</tr>
<tr>
<td>Age at onset RRT (years)</td>
<td>10.9 ± 2.8</td>
<td>11.0 ± 3.1</td>
</tr>
<tr>
<td>Age at investigation (years)</td>
<td>29.3 ± 5.4</td>
<td>30.0 ± 5.4</td>
</tr>
<tr>
<td>Duration of RRT (years)</td>
<td>18.0 ± 5.4</td>
<td>18.8 ± 5.0</td>
</tr>
<tr>
<td>Duration of dialysis (years)</td>
<td>4.4 ± 4.8</td>
<td>3.7 ± 4.2</td>
</tr>
<tr>
<td>Duration of transplantation</td>
<td>13.6 ± 6.4</td>
<td>15.1 ± 5.2</td>
</tr>
<tr>
<td>Patients on dialysis at investigation</td>
<td>30 (20.8%)</td>
<td>10 (23.2%)</td>
</tr>
<tr>
<td>Comorbidity</td>
<td>63 (43.8%)</td>
<td>21 (48.8%)</td>
</tr>
<tr>
<td>Disabilities</td>
<td>26 (18.1%)</td>
<td>7 (16.3%)</td>
</tr>
<tr>
<td>Mean Physical</td>
<td>45.7 ± 11.2</td>
<td>ND</td>
</tr>
<tr>
<td>Health Component*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Mental</td>
<td>49.8 ± 9.4</td>
<td>ND</td>
</tr>
<tr>
<td>Health Component*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table II. Employment status at follow-up**

<table>
<thead>
<tr>
<th>Employment status</th>
<th>Patients (n = 144)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Employed for time equivalent</td>
<td>97 (67.4%)</td>
</tr>
<tr>
<td>100%</td>
<td>53 (36.8%)</td>
</tr>
<tr>
<td>50%-99%</td>
<td>29 (20.1%)</td>
</tr>
<tr>
<td>20%-49%</td>
<td>15 (10.4%)</td>
</tr>
<tr>
<td>Unpaid work</td>
<td></td>
</tr>
<tr>
<td>Unpaid “profession”</td>
<td>10 (6.9%)</td>
</tr>
<tr>
<td>Social project</td>
<td>11 (7.6%)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>47 (32.6%)</td>
</tr>
<tr>
<td>Student</td>
<td>15 (10.4%)</td>
</tr>
<tr>
<td>Homemaker</td>
<td>4 (2.8%)</td>
</tr>
<tr>
<td>&gt;80% Unemployed &amp; no current educational training</td>
<td>28 (19.4%)</td>
</tr>
<tr>
<td>Medically rejected</td>
<td>14 (9.7%)†</td>
</tr>
</tbody>
</table>

*RRT, renal replacement therapy; ND, not determined.

*Summary scales of the RAND-36 quality of life survey.

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8 Dutch population aged 20 to 45 years. A total of 381,900 people receiving unemployment benefits or employment unfitness benefits of a population of 5,975,845 (6.39%).

†A total of 283,260 people receiving employment unfitness benefits (4.74%). Data from the Central Bureau for National Statistics, 1999 (Centraal Bureau voor de Statistiek: www.cbs.nl).
Associations Between Determinants and Outcome

By means of logistic regression analysis, unemployment was revealed to be independently associated with co-morbidity (Table IV). We found no correlation between the occupational level of patients and that of their parents (father–patient $R = 0.1, P = .3$). A low occupational level was associated with a total period of dialysis both $>4.4$ years and $>8$ years in the past, but not with the modality of renal replacement therapy at the present time, the total treatment duration, the age at onset of renal replacement therapy, or with the appearance of co-morbidity or disabilities (Table V). A linear regression model confirmed a linear inverse association between time on dialysis and the level of occupation ($\beta = 0.24; P = .01$).

Living at the parental home was associated with a total duration of dialysis $>8$ years, but not $>4.4$ years, and with the male sex (Table IV). More patients who started renal replacement therapy in the era of 1972 to 1982 still lived at the parental home or did not have a partner at the time of investigation than patients who started renal replacement therapy between 1982 and 1992 (Table IV).

Impact of Psychosocial and Somatic Factors on Quality of Life

A low Physical Summary Component was only associated with the existence of comorbidity; a low Mental Summary Component was associated with having disabilities and, more strongly, with unemployment status (Table V).

DISCUSSION

This study provides information on job achievement and social development of young adult patients with ESRD since childhood. These outcomes are influenced by the attitude toward renal replacement therapy for children in the early days. For instance, only 3 patients in our cohort were developmentally disabled because of the policy followed before 1990 not to accept all children for chronic renal replacement therapy. It is only a recent development that these children are generally accepted for chronic dialysis treatment. This, of course, will have a great impact on the long-term social outcome of this group of patients.

Employment

Our 20% unemployment figure is in line with the 15% reported by Offner et al in a study of patients who had received a renal transplant during childhood. It is also fits with data from a very recent report by Broyer et al on children who received a renal transplant. They found a percentage of 25.1% professionally inactive patients who were either just unemployed or received an unfit-for-work benefit for medical reasons. The figure is, however, much lower than the one reported by the European Dialysis and Transplant Association (EDTA) in the early 1990s for pediatric ESRD patients aged 21 to 35 years. In their report, the authors mention an unemployment rate between 25% and 65%, dependent on dialysis status and the existence of disabilities. Unfortunately, they do not provide nation-specific information on this item nor do they describe their definition for unemployment.

The current data suggest that employment seems to have become more accessible for dialysis patients with pediatric ESRD in the last 10 years. This observation is consistent with data from Gamperli et al, whose research showed a striking geographical difference in employment rate in favor of the northern European countries. Yet, the current data suggest that employment seems to have become more accessible for dialysis patients with pediatric ESRD in the last 10 years. This observation is consistent with data from Gamperli et al, who found an employment increase from 39% to 62% in 10 years in their patients with pediatric ESRD.

Our data are in contrast with data from recent studies on employment in patients with adult onset of ESRD, which report...
unemployment rates varying between 49% and 77%. Van Manen showed a further loss of work of approximately 20% within 1 year after dialysis onset in a Dutch cohort of patients with adult-onset ESRD, although the number of employed patients was already half the expected percentage at dialysis onset. In a large longitudinal study of 2,533 persons, Van de Mheen et al showed that this mobility out of employment is related to chronic health problems in adult patients. More than the physical inability itself, the relatively sudden onset of a life with a chronic disease and the inability to accept this situation and to adjust to it appear to be related to the employment status, at least in patients with ESRD. This could explain the differences in employment rates found between our patients with ESRD of pediatric onset and patients with adult onset of ESRD. Patients with a chronic disease since childhood have grown up with little or no perception of a life without disease. Consequently, their lives may meet their lower expectations, despite the physical disabilities. Thus, good mental health as a result of early adjustment might help patients to succeed in finding a job, as our data suggest. Like others, we found an association between physical impairment and unemployment. However, this association was not very strong, and we found no association between mode of renal replacement therapy and employment status. These findings indicate that a careful psychosocial guidance at childhood into acceptance and adjustment may prevent later unemployment.

We found that patients with a long duration of dialysis were at risk for achieving only low-skilled professions. The occupational levels of patients and their parents were absolutely not correlated. The most plausible explanation is the poor educational attainment that we also found in our patients, as we previously have described. It contrasts with findings of Van de Mheen et al in patients with adult onset of chronic disease, which found no association between health problems and changes in occupational class. It is particularly important to improve schooling in children on dialysis to enhance their employment prospects.

In a recent report, Broyer et al found an association between a low educational level and final body height. An explanation for this observation might be that in this study the final body height actually reflects the time spent on dialysis at youth. Growth retardation in children with ESRD is directly related to the extent and duration of renal failure. That is, patients with a low final body height will most probably also have spent a relatively long time on dialysis during their youth as compared with patients with a normal height. Indeed, Broyer et al mention in their discussion that they previously found an inverse association between the waiting time on dialysis and educational attainment. Unfortunately, they do not mention the influence of graft failure in the >50% patients who lost their first graft or the influence of the total time on dialysis on the level of educational attainment.

### Independence

We found that significantly more patients still lived with their parents, compared with the age-matched Dutch population. This finding is consistent with other reports on patients with

<table>
<thead>
<tr>
<th>Table V. Association between Subjective Health Perception and medical and social determinants in all patients; results of the logistic regression analyses</th>
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<tr>
<td><strong>Physical health perception</strong></td>
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<tr>
<td><strong>Odds ratio</strong></td>
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<tr>
<td>Comorbidty</td>
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<td>Disabilities</td>
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<td>Unemployment*</td>
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</table>

The Physical and Mental Component Summary scales of the Rand-36 Quality of Life survey were used.

*Students excluded.

†P<.001.

‡P<.05.

§P<.01.

n.s., not significant.

*P<.05; **P<.01; ***P<.001. Note: only significant associations are shown (set at P<.05).

††students excluded.

‡‡only employed patients.

unemployment.
pediatric ESRD.1-4 Male sex and a very long duration of dialysis in the past were associated with living in the parental home. Like others, we found that parental dependency and not having a partner were most prominent in the first generation of patients with childhood ESRD, those who started renal replacement therapy between 1972 and 1982.6 We found no relation between subjective health assessment and marital status or living arrangement. This contrasts with the effects of not having a partner and the development of health problems in previously healthy subjects. Joung et al found in a large Dutch cohort study that people who lived with a partner had lower subjective morbidity perception than those who lived alone. They also found an increased risk for developing health complaints in people who never got married.18 Reynolds confirms the lack of relation between quality of life and social functioning on these indicators in young adults with pediatric ESRD.2 Like others, he found overall more “social immaturity” in their domestic situations and partnerships.1,4,6 However, these adverse outcomes were predominantly related to objective current health problems.2 All these studies conclude that renal patients were less prepared for the physical demands of marriage than control subjects. This is in line with our findings.

Limitations

As a result of the study design and of the limited number of patients, we were unable to investigate the effect of the different center strategies and of the changed therapeutic strategies with time, such as the inclusion criteria for renal replacement therapy at onset and the therapy protocols, on outcomes. We could not obtain reliable data on psychological treatment strategies for rehabilitation. The subjective health assessment and outcome measures were cross-sectionally established at the same time in this study. Therefore, we cannot answer the question of whether low subjective mental health predisposes these patients for unemployment or whether it is (partly) caused by the unemployment. A prospective study is needed to reveal the direction of the causal relationship of quality of life and employment state.

We thank Mariken Gruppen, Hannah Coutinho, Bella Drost, Janneke van den Broek, and Anouk van der Graaf, medical students who contributed to the data collection. Data collection was made possible by the co-operation of these physicians: R.J. Hene, Medical Centre University, Utrecht; J.J. Homan van de Heide, Academic Hospital, Groningen; M.R. Lilien, Wilhemina Children’s Hospital, Utrecht; N.J. van der Kar, St. Radboud Hospital, Nijmegen; M. Kooistra, Dianet, Utrecht; J.W. van der Pijl, Medical Centre University, Leiden; E.J. Rischen-Vos, Dijkzigt Hospital, Rotterdam; S. Surachno, Academic Medical Centre, Amsterdam; E.D. Wolff, Sophia Children’s Hospital, Rotterdam; A.J. Apperloo, St. Elisabeth Hospital, Tilburg; M. Beekhuis, Rijnland Hospital, Leiderdorp; J. Boonakker, Reinier de Graaf Gasthuis, Delft; M.H.L. Christiaans, Academic Hospital, Maastricht; P.P.N.M. Diderich, St. Franciscus Gasthuis, Rotterdam; M.A. van Dorp, St. Clara Hospital, Rotterdam; W.T. van Dorp, Kennemer Gasthuis, Haarlem; W.J. Fagel, Medical Centre, Leuwarden; P.G. Gerlag, St. Joseph Hospital, Veldhoven; A. van Es, Dialysis Centre ‘t Geoo, Helvoirt; A.B. Geers, St. Antonius Hospital, Nieuwegein; E.G. Hagen, Hospital De Lichtenberg, Armersfoort; S.J. Hoornjte, Catharina Hospital, Eindhoven; R.M. Huisman, Dialysis Centre, Groningen; K. Jie, Groene Hart Hospital, Gouda; G.M.Th. de Jong, Drechtsteden Hospital, Dordrecht; A.J. Hoitsma, St. Radboud Hospital, Nijmegen; G. Kolster, Isala Clinics, Zwolle; I. Keur, Dianaet Buitenveldert, Amsterdam; W.A.H. Koning-Mulder, Medical Spectre Twente, Enschede; A.G. Lieveer, Diagonessenhuis, Eindhoven; P.B. Leurs, Oosterschelde Hospital, Goes; N. vd Lely, Reinier de Graaf Gasthuis, Delft; M.J. Nubié, Medical Center, Alkmaar; C. Öldenbroek, Westfries Gasthuis, Hoorn; M.J.M. Smit, Juliana Children’s Hospital, The Hague; G. Vastenburg, Schepen Hospital, Emmen; R.M. Valentijn, Red Cross Hospital, The Hague; A.E. v Wijk, Hospital Free University, Amsterdam.

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IMPACT OF SEXUAL NETWORKS ON RISK FOR GONORRHEA AND CHLAMYDIA AMONG LOW-INCOME URBAN AFRICAN AMERICAN ADOLESCENTS

JONATHAN M. ELLEN, MD, BETH A. BROWN, SHANG-EN CHUNG, ScM, JOHN J. POTTERAT, BA, STEPHEN Q. MUTH, BA, THOMAS W. VALENTE, PhD, AND NANCY S. PADIAN, PhD

Objective  To determine whether African American adolescents, whose recent sex partners reported having another sex partner, are at increased risk for exposure to genital chlamydial infection or gonorrhea.

Study design  A household sample of low-income urban African American adolescents 14 to 19 years of age, up to two of their close friends, and their sex partners were interviewed and tested for gonorrhea and chlamydial infection.

Results  Thirty-four of 145 adolescents had at least one recent sex partner infected with Neisseria gonorrhoeae and/or Chlamydia trachomatis. The adjusted model showed that adolescents, whose recent sex partners reported having another sex partner, were more likely to have a recent sex partner with gonorrhea and/or chlamydial infection.

Conclusion  In addition to individual factors, network factors may explain why African American adolescents are at increased risk for exposure to sexually transmitted infections (STIs). Multi-level community-based interventions may need to address network factors along with personal behaviors in order to prevent STIs among low-income urban African American adolescents. (J Pediatr 2005;146:518-22)

The prevalence of sexually transmitted infections (STIs) among adolescents remains unacceptably high. In the United States, African American adolescents and young adults are at higher risk for acquiring gonorrhea and chlamydial infection relative to other youth. Increasingly, investigators are seeking to identify determinants of STI acquisition among adolescents beyond failure to use condoms and number of sex partners. Neither of these individual risk factors has been found consistently to predict STI diagnosis, in part because these risk factors are contingent on exposure to an infected partner. Investigators have thus begun to focus attention on understanding exposure to infected partners by exploring characteristics of adolescents’ sexual networks.

There has been some success in identifying sexual network characteristics that increase adolescents’ risk for exposure to an STI. Research suggests that adolescents whose sex partners are having sex with other partners are at higher risk for exposure to an STI. However, these studies are limited. First, these studies focused on adolescent perceptions about whether a sex partner had other partners rather than partner self-report. Because individuals are often unaware that their partners have other partners, partners may have been misclassified as not having another sex partner when in fact they did, biasing the results. Second, these studies conducted their analyses at the level of sex partners and not networks. In other words, they examined the independent influence of each sex partner. In the first study, the investigators included every sex partner of each adolescent in the analysis but used statistical methods to treat sex partners from the same adolescent as independent observations. In the second study, the investigators only focused on the most recent sex partner, eliminating data about other partners. As such, both studies failed to account for the combined risk from all the adolescents’ recent sex partners.

In the current study, we focus on what we term the “local network” of the adolescent. The local network includes the adolescent and all his/her recent sex partners as confirmed by interview. We limited our definition of network to recent sex partners because any...
missing data about recent sex partners is compounded when trying to find the sex partners of sex partners. We used report of adolescents’ recent sex partners about their other sex partners and did not rely on adolescents’ perceptions about whether their sex partners had other sex partners.

Our primary objective was to determine whether African American adolescents, whose local network of sex partners had sex partners from outside the local network, were at increased risk for exposure to an STI. Our secondary objective was to determine whether having sex partners outside the local network was associated with age differences between adolescents and their local networks of sex partners, ie, age discordance within local networks. Several studies have shown that adolescents whose sex partners are older than they, termed age-discordant sex partners, are more likely to be diagnosed with an STI, including HIV. However, a competing or complementary hypothesis is that older sex partners are more likely to have a higher prevalence of STIs than adolescents with similarly aged sex partners.

We hypothesize that local networks with a higher number of sex partners from the outside are more likely to contain at least one person with gonorrhea and/or chlamydial infection, and that networks with greater age discordance are more likely to have a higher number of sex partners from the outside.

**METHODS**

**Participants**

Between June 2000 and September 2002 we recruited a household sample of African American adolescents 14 to 19 years of age residing in census tracts of the San Francisco Bay Area with the highest reported prevalence of gonorrhea and genital chlamydial infections. The prevalence of gonorrhea and chlamydia cases among 15- to 19-year-olds was 4572 per
100,000 and 6971 per 100,000, respectively.\textsuperscript{11} We also recruited one to two close friends of the household adolescents, 14 years of age and older living in the same neighborhoods (see Figure 1). For this analysis, we focused on index adolescents who had had vaginal or anal intercourse at least once in the 3 months before their interview.

**Procedures**

The household sample of youth was recruited by telephone; numbers were generated using a procedure called list-assisted random-digit sampling, in which telephone exchanges with higher coverage for the target community were over sampled. Approximately 40,000 telephone numbers were dialed, and about 22,000 of those were identified as households. Ninety-seven percent of households were ineligible either because they were out of the targeted area or because there was no African American 14 to 19 years of age residing in the household. The names and addresses of up to two close friends or social contacts were elicited from each participating household youth. The social contacts were then contacted and asked to participate in the study.

Study procedures were the same for both household sample and the social contacts. Consent was obtained from parents for all participants younger than 18 years of age and directly from participants 18 years of age or older. For interviews conducted by telephone, parental consent was obtained and witnessed by telephone, and a copy of the consent was mailed to the parent for signature. Once parental consent was obtained, minors recruited as study participants could volunteer to participate.

Any person not able to be contacted by telephone was referred to field outreach staff. Members of this staff attempted to identify and locate these potential participants using a variety of tracking methods, including home visits, word-of-mouth queries, use of public records, and the assistance of key informants and community leaders in making contact with participants and their families (for minors). Once contact was made, if the participant was less than 18 years of age, the field-worker first contacted the participant’s parent(s). Once contact was made with a parent, the field-worker described the study and read an Institutional Review Board-approved consent form to them. If the parent consented to the child’s participation, the field staff re-contacted the minor and obtained his or her assent. An interview date, including time and place, was then planned.

Participants were interviewed either over the telephone or in person. They were queried about age, gender, and date of

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**Figure 2.** In this example of two local networks, a household respondent (at left) is asked to nominate recent (3 months) sex partners as well as up to two friends (here only one was enrolled). Attempts are made to interview all persons. Each interviewed sex partner is asked to estimate his/her number of other recent sex partners. The number of outside sex partners for each local network is the sum of the sex partner estimates of their other recent sex partners subtracting one for each time the index person was named by one of the sex partners. For example, in local network 2 at left, the interviewed household youth names four sex partners. Only one sex partner was found and interviewed. That sex partner claimed to have 3 sex partners (one of which is the original respondent), for a net total of two outside sex partners.
last sexual exposure with each partner, and about the number of sex partners during the preceding 3 months. For each sex partner, they were asked to provide the name and locating information. The interviewer entered responses directly into a computer running the CASES software program (Computer-assisted Survey Methods, USC, Berkeley, Calif) that is distributed and supported by the Computer-assisted Survey Methods Program at the University of California at Berkeley. The interview lasted approximately 45 minutes, and respondents received $25 for their participation.

All participants also were asked to supply a vaginal swab (females) or urine specimen (for males, or offered to females who refused a vaginal swab); specimens were tested for evidence of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections using a commercial ligase chain reaction kit (LCx, Abbott Laboratories, Abbott Park, NJ). Participants testing positive were notified and offered treatment free of charge.

Using standard public health procedures, we attempted to contact and enroll all named sex partners using the same procedures described earlier. Once enrolled, the sex partners were similarly interviewed and tested for gonorrhea and chlamydial infection.

### Analysis

In this article, we refer to both household youth and social contacts as index persons. The unit of analysis is an index person’s local network, which includes the index person and his or her interviewed sex partners (see Figure 2). Because there were 145 index persons with at least one interviewed sex partner, there were 145 local networks. The number of outside sex partners was calculated by summing the sex partners’ self-report of the number of sex partners they had in the past 3 months and subtracting one for each time the index person was named by one of the sex partners. None of the 145 index persons estimated more partners than they nominated during the interview; therefore, no outside sex partners emanated from the index persons. The number of outside sex partners was then dichotomized into any outside the local network and none outside the local network.

We calculated age discordance by determining the age difference between the index person and each partner, then we calculated the mean age difference for each local network. We used a hierarchical generalized linear model using Hierarchical Linear Modeling version 5 (SSI, Inc., Lincolnwood, Ill) to calculate odds ratios and 95% confidence intervals. Because the outcome was measured at the index person’s level and was nested within “index-social contact” networks (termed a cluster), multi-level modeling was the appropriate method of analysis. This model recognized the correlation within “index-friend” network, the variance between “index-friend” network, and allowed cluster-specific interpretations. Because the number of persons interviewed in the index-friend network could be an independent predictor of STI diagnosis and number of outside sex partners, we controlled for number of interviewed sex partners in each cluster in each analysis. We also adjusted our findings for demographics, such as sex, age, and race/ethnicity, to help minimize the influence of other potential risk factors when conducting our analysis.

### RESULTS

A final sample of 673 eligible adolescents living in 470 eligible households was identified through telephone screening of households. After excluding 93 duplicated respondents, 580 household youth were offered the interview. Three hundred and fifty of the 580 (60.3%) were interviewed; 305 social friends were referred. After excluding 57 duplicate social networks, the staff attempted to contact, enroll, and interview 248 social contacts; 177 (71.4%) completed the interview. Among the 527 household youth and social contacts, 159 (30%) reported never having had sex, 82 (16%) reported not having sex at least 3 months before the interview, and the remaining 286 (54%) reporting having had sex during the previous 3 months.

Each participating household youth and social contact who had been sexually active in the past 3 months was asked to nominate up to six partners; none refused to nominate partners. Two hundred and eighty-six named at least one sex partner, and 145 (51%) had at least one nominated sex partner participate in the study. When we compared sexually active participating household youth and social contacts with at least one interviewed sex partner with those with no interviewed partners (N = 141), we found no difference in age, race, or rate of STI diagnosis. However, those with sex partners were more likely to be male (OR = 1.75; 95% CI = 1.09, 2.81).

### Table. Characteristics of adolescents and interviewed sex partners

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<thead>
<tr>
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<th>Adolescents N = 145</th>
<th>Sex partners N = 183</th>
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<tbody>
<tr>
<td>Mean age, years (standard deviation)</td>
<td>17.9 (1.8)</td>
<td>19.0 (3.6)</td>
</tr>
<tr>
<td>Male, n (percent)</td>
<td>71 (49.3)</td>
<td>100 (43.5)</td>
</tr>
<tr>
<td>Race/Ethnicity, n (percent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>125 (86)</td>
<td>143 (78)</td>
</tr>
<tr>
<td>White</td>
<td>3 (2)</td>
<td>4 (2)</td>
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<tr>
<td>Latino</td>
<td>4 (3)</td>
<td>6 (3)</td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>2 (1)</td>
<td>6 (3)</td>
</tr>
<tr>
<td>Mixed/Other</td>
<td>11 (8)</td>
<td>24 (13)</td>
</tr>
<tr>
<td>History of an STI, n (percent)</td>
<td>23 (16)</td>
<td>26 (14)</td>
</tr>
<tr>
<td>Mean number of sex partners last 3 months (standard deviation)</td>
<td>1.7 (1.1)</td>
<td>1.5 (1.7)</td>
</tr>
<tr>
<td>Mean number of sex partners in lifetime (standard deviation)</td>
<td>5.6 (3.5)</td>
<td>5.4 (4.0)</td>
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</table>
four (mean = 1.3; sd = 0.6). Thirty-four of 145 local networks (23.4%) contained at least one interviewed person infected with *N. gonorrhoeae* and/or *Chlamydia trachomatis*.

Adjusting for the number of persons interviewed in a network, local networks with any sex partners outside this network were not more likely to have at least one person in the network with gonorrhea or/and chlamydial infection ($P < .1$; OR = 2.16; 95% CI = 0.91, 5.11). However, when we adjusted our model for important known confounders, including characteristics of index persons (sex, age, and race/ethnicity), we found a statistically significant association between outside sex partners and infection status in the local network ($P < .05$). Local networks having outside sex partners were more likely to have at least one person with gonorrhea or chlamydial infection in network (OR = 2.77; 95% CI = 1.10, 6.95), after adjusting for the number of persons interviewed in the network.

Finally, when we tested whether age discordance was associated with presence of outside sex partners, controlling for number of partner interviews, we found a significant association. Local networks where the mean age difference between the index person and each of his or her partners was $\geq 2$ years were more likely to have a sex partner outside the network compared with local networks with mean age discordance $< 2$ years (OR = 3.67; 95% CI = 2.88, 4.47).

**DISCUSSION**

We found that urban adolescents—most of whom are African American and reside in a high STI prevalence community—whose sex partners have other sex partners outside their local network, are more likely to have at least one sex partner in their local network who is infected with *N. gonorrhoeae* and/or *Chlamydia trachomatis*. In other words, the likelihood that an urban African American adolescent residing in a high STI prevalence community will be exposed to an STI is related to the presence of sexual links between his or her recent sex partners and the community. This supports the empirical findings of Potterat et al but in data collected on a household sample of youth and their social contacts rather than on adult contact tracing networks.12

We also found that members of local networks with greater age discordance between adolescents and their sexual partners are more likely to have sex with persons who have other sex partners outside the local network. This may explain why age discordance is associated with many adolescents being diagnosed with an STI.

Although this study cannot address whether age discordance is associated with subsequent infection and whether there is a higher prevalence of age discordance or greater risk of STI associated with age discordance among African American adolescents compared with other youth, it is a hypothesis worth exploring in subsequent studies, especially those with nonhomogeneous race/ethnicity in study participants.

The major limitation of any network study is the incompleteness of the data. First, it is not possible to know characteristics of unreported sex partners. Second, not all reported sex partners could be located for an interview. There was no difference in rates of STIs reported by those adolescents who named at least one sex partner and those who named none. Additionally, by focusing exclusively on other sex partners of interviewed sex partners, we reduced potential bias associated with the existence of un-interviewed sex partners. However, un-interviewed sex partners affect the application of findings beyond this study population because they might pose risk of STI to the adolescent who we did not describe.

The implication of this study is that network data are useful for assessing which youth are at elevated risk for an STI. These data might be useful in high-risk communities where identification of highly connected persons may prove to be important to reduce the burden of STIs within that community.

**REFERENCES**

OUTBREAK OF ADENOVIRUS TYPE 30 IN A NEONATAL INTENSIVE CARE UNIT

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Objectives To describe an outbreak of adenovirus, type 30, in a neonatal intensive care unit (NICU).

Study design This was a retrospective, observational study.

Results An outbreak of adenovirus infection occurred in an NICU. It lasted 6 months and involved 21 of 333 (6.3%) infants. The introduction of infection control measures controlled the outbreak; however, premature discontinuation of the measures resulted in a second wave of cases. The virus caused pneumonia in 7 infants, conjunctivitis in 7 infants, pneumonia and conjunctivitis in 1 infant, and upper respiratory tract illness in 1 infant. Infection was asymptomatic in 5 infants. Six infants died. Death was associated with the presence of pneumonia ($P = .0001$), administration of steroids ($P = .003$), and mechanical ventilation ($P = .02$). Investigation into the origin of the outbreak suggested that the virus may have been introduced and spread during ophthalmologic procedures.

Conclusions Adenovirus type 30 can cause severe disease among premature infants in an NICU. Infants with severe bronchopulmonary dysplasia requiring mechanical ventilation are more likely to have development of adenovirus pneumonia and die. Standard infection control measures are effective in controlling an outbreak. Ophthalmologic procedures continue to be a potential source of adenovirus outbreaks. (J Pediatr 2005;146:523-7)

adenovirus is a common respiratory virus. It has the capacity to cause upper respiratory tract and lower respiratory tract illnesses, conjunctivitis, gastroenteritis, cystitis, and rash. Adenoviruses are transmitted primarily by direct contact and by the fecal-oral route. Adenoviruses are unusually stable and can persist for prolonged periods in the environment.\(^1\)\(^-\)\(^3\) There are 49 serologically distinct types of adenoviruses that can cause human disease.\(^1\) Some types, such as 1, 2, 5, and 6, are endemic in various parts of the world, whereas other types, such as 7, 8, 19, and 37, are associated with outbreaks.

Although the mechanism of protection against adenovirus is not fully understood, severe, often fatal, or chronic infections in individuals with severe combined immunodeficiency, organ transplantation, and AIDS and in neonates suggest that cellular immune mechanisms may play a major role in limiting infection.\(^4\)\(^,\)\(^5\) Available data suggest that neonates have diminished cellular immunity. In particular, T-lymphocytes and natural killer cells exhibit reduced cytotoxic activity.\(^6\) Since antibody also may contribute to the defense against adenoviruses, the limited antibody repertoire displayed by the B-lymphocytes of neonates and the markedly reduced concentrations of passively acquired maternal antibodies found in premature infants make the premature infant highly susceptible to adenovirus.\(^5\) The current report describes an outbreak of adenovirus type 30 among 21 infants in a neonatal intensive care unit (NICU).

METHODS

This is a retrospective, observational study. Data were obtained from medical records and infection control records. The fluorescent antibody tests for the rapid detection of adenoviruses (Imagen Adenovirus by Dako with a sensitivity of 86% and a specificity of 100%) and cultures on human lung carcinoma and monkey kidney cell monolayers were performed in the virus laboratory of the hospital. Infants were screened when symptomatic, and routinely on a weekly basis. Typing of adenovirus isolates were performed by neutralization assays in the New York State Laboratory in Albany, New York. Typing was performed in the New York State Laboratory in Albany, New York.
RESULTS

Cases

Twenty-one infants became infected with adenovirus. There were 11 girls and 10 boys. Sixteen infants were white and 5 infants were black. Almost all (20 of 21) of the infants were premature. Birth weights ranged from 444 to 2568 g, with a mean of 937.9 g; gestational age of the infants ranged from 24 to 37 weeks, with a mean of 26.7 weeks. Postnatal age at the time of adenovirus infection ranged from 3 to 22 weeks, with a mean of 9.1 weeks. Adenovirus infection was manifest primarily as pneumonia (a significant increase in respiratory support, a new infiltrate, and a change in the quantity and quality of secretions) in 7, conjunctivitis in 7, pneumonia with conjunctivitis in 1, asymptomatic in 5 (diagnosed during surveillance studies), and upper respiratory tract infection in 1.

Six infants received therapy directed specifically at the infection. Intravenous immunoglobulin (IVIG) was given as a one-time dose of 400 mg/kg. Ribavirin was administered intravenously according to the manufacturer's suggestions for 3 to 7 days under a compassionate use protocol. Five of these infants died. The lone survivor was a relatively healthy infant who would not have been selected for treatment except for the death of her twin, who was infected with adenovirus. In addition to specific antiviral therapy, all infants were supported with conventional or high-frequency ventilation or pressor agents or both, as needed.

Characteristics of the infants who died and who survived are compared in the Table. The two groups were similar with respect to sex, race, birth weight, gestational age, age at time of infection, presence of bronchopulmonary dysplasia (BPD), and number of eye examinations. However, corticosteroid use, the diagnosis of adenovirus pneumonia, and being on a mechanical ventilator at the onset of the adenovirus infection were each significantly more common in the infants who died. Three of the four infants who received corticosteroids and died received hydrocortisone (1.5 to 10 mg/kg per day IV) after they had become acutely severely ill with adenovirus pneumonia but before the diagnosis of adenovirus had been made. Two of three had prior exposure to hydrocortisone, but it was at least 1 month before their adenovirus infection. Overall, these infants had respiratory failure, and, in addition to corticosteroids, other treatments such as albuterol, ipratropium, terbutaline, DNase, acetylcysteine, surfactant, and nitric oxide were used in life-saving attempts. The fourth patient was considered to be receiving chronic steroids. He had been receiving long-standing systemic dexamethasone 1 month before his adenovirus infection. He was given inhaled budesonide in an attempt to decrease systemic corticosteroids 2 weeks before his adenovirus infection. He continued to receive inhaled budesonide at the time of adenovirus illness. Five infants who died were being mechanically ventilated for severe BPD before the adenovirus infection.

Epidemiology of the Outbreak

The NICU consisted of 3 rooms designated A, B, and C. Typically, room A housed up to 18 infants who were moderately to severely ill; room B, 22 infants who were moderately ill; and room C, 14 infants who were mildly ill or were recovering premature infants. The average daily census in the NICU during the outbreak varied between 32 and 43, with a median of 42. A total of 333 infants were cared for in the NICU during the outbreak and were at risk of being infected. Thus, the overall attack rate was 6.3%.

Case 1 was identified on day 1 of the outbreak during an evaluation for pneumonia in a ventilated infant (Figure). A rapid fluorescent antibody screen for respiratory viruses was positive for adenovirus. The baby was moved from the open area in room B to one of the two isolation rooms and placed in contact precautions with gowns and gloves. Three days later, on day 4 of the outbreak, case 2 was identified during an evaluation for pneumonia in a ventilated infant located in the open area of room B. A rapid screen for respiratory viruses was positive for adenovirus. The baby was moved to the second isolation room, on contact precautions. Two days later, on day 6 of the outbreak, case 3 was identified in room A during an evaluation for pneumonia. A rapid screen for respiratory viruses was positive for adenovirus. Case 3 was moved to room B to begin the process of cohorting adenovirus-positive infants. Room B was closed to new admissions, and none of the infants in room B were moved to other rooms in the NICU. However, infants in room B were moved to other units in the hospital or discharged to home whenever possible. Cases 4 to 6 occurred from day 9 through day 13. They exhibited conjunctivitis, upper respiratory tract symptoms, or pneumonia.

Mandatory use of gowns and gloves for handling all infants in room B was introduced on day 18; only gloves were mandatory in rooms A and C. Cases 7 and 8 presented on days 19 and 25 with conjunctivitis or pneumonia. All
adenovirus-positive infants had been discharged to their homes or had become virus-negative by respiratory screen by day 45. The routine use of gowns and gloves in room B and gloves in rooms A and C were discontinued on day 66, 48 days after their introduction and 41 days after the last case, number 8, appeared.

Case 9 appeared in room A on day 71, 46 days after case 8. Case 10 also appeared in room A, 10 days later, on day 81. Forty-two days later, on day 123, case 11 appeared in room B. Because these last 2 cases were asymptomatic, and they occurred many days after the first cluster of 8 cases, infection control measures were not reinstituted. However, 31 days later, on day 154, case 12 appeared, quickly followed by 7 other cases, cases 13 through 19, over a period of 15 days. Cohorting of adenovirus-positive infants in room B was quickly reinstituted. Gowns and gloves for all patient and bedside contacts were instituted throughout the NICU; in addition, masks were required for contact with any adenovirus-positive baby in room B. This included parents as well. Room B was divided into 3 areas: (1) adenovirus-positive infants, (2) newly admitted infants, and (3) infants of room B who had never been adenovirus-positive but who were believed to be high-exposure risks and therefore not moved out of room B. Nurses caring for infants with adenovirus were not permitted to care for any noninfected baby.

Any nurse, physician, or baby in the NICU who had a respiratory illness or conjunctivitis was screened for adenovirus: a fluorescent antibody screen for respiratory viruses when respiratory symptoms were present and a viral culture when conjunctivitis was present. Healthcare workers with conjunctivitis were not allowed to work until asymptomatic. Any healthcare worker with an adenovirus-positive upper respiratory infection (URI) was not allowed to work until asymptomatic. Healthcare workers with mild non-adenovirus URIs could work while masked, gloved, and gowned. Healthcare workers with moderate or severe non-adenovirus URIs were not allowed to work until the illness resolved. A total of 11 healthcare workers with URIs were screened, and none was positive for adenovirus. Eight healthcare workers with conjunctivitis were cultured, and 2 were positive for adenovirus (1 nurse and 1 ophthalmologist). All infants in the NICU were screened by means of antigen detection on nasopharyngeal specimens 1 week, alternating with cultures of stools the next week; thus, all infants were screened on multiple occasions. These screening procedures detected adenovirus infection in 5 asymptomatic infants by respiratory antigen screens in 4 infants and by stool culture in 1 baby.

Case 20 appeared 12 days after case 19 on day 178. Case 21, the final case in the outbreak, was identified on day 190. Adenoviruses from 10 infants were typed, and all were type 30. Three of the virus isolates came from the first wave of cases and 7 came from the second wave. Adenoviruses from the 2 healthcare workers were typed and were also type 30. Infection control measures were continued for 55 days or 47 days after the last case was identified.

Factors Possibly Contributing to the Outbreak

Seven days before the first case of the adenovirus outbreak, one of the ophthalmologists who performed retinopathy of prematurity (ROP) examinations and ROP surgery had an upper respiratory tract illness (Figure). Although he wore a mask, he continued to examine infants for the ensuing week. His colleague, ophthalmologist 2, had conjunctivitis on day 3 of the outbreak and continued to work while symptomatic through day 11 of the outbreak. After discovery of the initial NICU adenovirus infections, ophthalmologist 1 underwent a respiratory culture for virus 21 days after the onset of his URI, day 14 of the outbreak, and the culture was negative. Ophthalmologist 2 had culture performed 13 days after the onset of his conjunctivitis, day 15 of the outbreak, and his culture was positive for adenovirus type 30. It is possible that the virus was initially introduced into the unit by one or both ophthalmologists. It is most probable that
ophthalmologic examinations and laser therapy for ROP contributed to the spread of the virus in the unit. The first baby to have adenovirus infection had undergone laser eye surgery and ROP examinations 1, 5, and 8 days before becoming ill with adenovirus, corresponding to 1 day before and 2 and 6 days after ophthalmologist 1 became ill.

Investigation into the ROP examination process revealed that (1) ophthalmologists did not wear gloves during routine ROP examination procedures but did wash their hands between examinations, (2) eyelid retractors and eyeball depressors used for the examinations were disinfected for less than 5 minutes in 70% alcohol, and (3) the stock of 70% alcohol was not routinely changed on a daily basis. However, cultures of the instruments did not demonstrate adenovirus. On day 92 of the outbreak, the ROP examination process was changed to meet the guidelines of the American Academy of Ophthalmology: (1) gloves worn for the examination and changed between examinations, (2) instruments soaked in the 70% alcohol solution for 5 to 10 minutes, and (3) 70% alcohol changed twice daily. On day 164 of the outbreak, sufficient numbers of instruments for the ROP examinations became available to provide individual sterilized pieces. During the outbreak, a total of 116 ROP examinations had been performed on the 21 affected infants.

**DISCUSSION**

Adenovirus has long been associated with hospital-acquired infections. In the past 20 years, there have been at least 8 outbreaks reported in pediatric facilities.8-15 Four of these outbreaks occurred in neonatal intensive care units and 3 occurred in chronic care facilities. Only 2 adenovirus types were involved in 7 of these outbreaks. Adenovirus type 8 caused 3 of the 4 NICU outbreaks and involved 38 patients. All 3 of the chronic care facility outbreaks were caused by adenovirus type 7 and involved 66 patients. Mortality rates in the outbreaks ranged from 0% to 39%. Whereas 33% of adenovirus type 7–infected patients died, only 1 of 31, 3%, of adenovirus type 8–infected patients died, even though all 31 patients with adenovirus type 8 were premature infants. This suggests that severity of disease depends, in large part, on the serotype of the virus. Adenovirus type 30 was the serotype involved in the present report, and 6 of 21 (29%) infants died. Adenovirus type 30 has only been reported previously in 1 neonatal case report; the infant died, and not as part of an outbreak.16

Since the severity of disease varied markedly in the current report from asymptomatic infection to fatal pneumonia, it was important to identify risk factors that may have affected on outcome. We compared gestational age, birth weight, age at time of infection, presence of BPD, diagnosis of adenovirus pneumonia, ventilator use at the start of the adenovirus infection, and corticosteroid use during the adenovirus infection between the 6 infants who died and the 15 infants who survived. The diagnosis of pneumonia, ventilator use, and corticosteroid use were all associated with increased risk of death from the adenovirus infection. Birenbaum et al13 previously suggested that the use of corticosteroids may be responsible for systemic manifestations and deterioration of premature infants infected with adenovirus. In our outbreak, no infant received corticosteroids after adenovirus was diagnosed; however, corticosteroids were used to treat severe respiratory decompensation in ventilated infants with BPD before knowledge of the adenovirus infection.

Treatments with IVIG, Ribavirin (ICN Pharmaceuticals, Inc, Costa Mesa, Calif), or both were administered to 6 infants, 5 of whom died. It is difficult to assess the value of either treatment, since they were given to the infants who were extremely ill. Ribavirin previously has been given to a limited number of children with adenovirus infections, with mixed results.17-19 One might suspect that IVIG might be efficacious in viral infections, since it contains a variety of antiviral antibodies. Ironically, Piedra et al12 reported the occurrence of an outbreak of adenovirus type 8 in an NICU while conducting a study evaluating the efficacy of IVIG to prevent viral infections in premature infants. In our situation, the typing of the adenovirus was not immediately known; in retrospect, type 30 is rare, and therefore it is unlikely that commercially available preparations of IVIG would contain significant amounts of antibody to this serotype. More recently, cidlovir was shown to be beneficial in several stem cell transplantation recipients infected with adenovirus.20

Ophthalmologic examinations have been implicated in at least 2 outbreaks of adenovirus in NICUs.13,15 Both outbreaks were due to adenovirus type 8, a virus known to cause conjunctivitis frequently. The number of examinations for retinopathy of prematurity in one of the studies was directly related to the incidence of infection.13 Hered21 queried nurse supervisors of NICUs in the United States about instrument management for examinations for ROP and found that only 36% used “best practices” as recommended by the American Academy of Pediatrics. The present report suggests that the adenovirus may have been introduced into and, possibly, spread through the NICU during ophthalmologic examinations. The timing of examinations and the development of symptomatic disease were consistent with the expected incubation period. Investigation into the procedures used in our NICU identified several deficiencies that may have contributed to the outbreak. These included the absence of glove use and inadequate disinfection of instruments. As seen in the present report, the number of examinations performed may be large in a typical NICU, and each infant may have multiple opportunities to become infected; the number of ROP examinations performed on the 20 premature infants in the current report totaled 116, a mean of 5.8 per infant. It should be mentioned, however, that it is also possible that the ROP examinations were not necessarily themselves the cause but that in performing an ROP examination, the conjunctiva was injured and became more susceptible to infection. Similarly, the lung may become more susceptible to infection while undergoing mechanical ventilation. Six of 8 infants who had pneumonia had been intubated and mechanically ventilated. This emphasizes the need to examine critically the procedures performed on premature infants who may be
exposed to or who could possibly be incubating viruses, especially during outbreaks.

Droplet precautions (with the use of masks) were not used initially because adenovirus was thought to be spread primarily by contact with respiratory tract secretions or stool. We did not think that respiratory spread through small or large droplets from the premature infants would be significant. Logistically, it would have been difficult for NICU personnel to wear masks continuously. The infection control strategy was discussed extensively with the New York State Department of Health before implementation. In retrospect, the use of masks for droplet precautions earlier may have been beneficial. Overall, the outbreak responded to the infection control procedures instituted. Possibly because procedures were discontinued prematurely, a second wave of cases resulted. In between the two peaks, 3 cases occurred, which were believed to be insignificant until the second wave of cases occurred. In retrospect, the 3 cases suggested persistence of the virus in the unit.

Adenovirus is known to persist on environmental surfaces for weeks. In addition, and perhaps more importantly, it may be shed intermittently in stool for months to years. However, in our unit, only one of several hundred stool cultures yielded adenovirus. Screening of asymptomatic infants identified 5 cases; 4 were shedding virus in the nasopharynx and only 1 in the stool. The use of screening procedures, the detection of asymptomatic shedding of the virus, and heightened infection control procedures may have prevented further spread of the virus. It is possible that asymptomatic healthcare workers may have contributed to the persistence of the virus in the unit. However, unlike other outbreaks of adenovirus, relatively few healthcare workers in the present outbreak had respiratory tract symptoms or conjunctivitis caused by the adenovirus.

**SUMMARY**

We report an outbreak of 21 cases of an unusual serotype, type 30, of adenovirus in an NICU. Standard infection control measures were successful in controlling the outbreak but may have been relaxed prematurely. Based on our experience, we recommend that infection control measures be continued for a prolonged period to ensure elimination of the virus from the NICU. Asymptomatic infants found on screening tests should be considered as an alert to the persistence of virus. Infants infected with adenovirus should remain in isolation while they are in the NICU.

**REFERENCES**


Objective  Plasma assay for very long-chain fatty acids has made it possible to perform large-scale screening of at-risk individuals to identify asymptomatic patients with X-linked adrenoleukodystrophy (X-ALD). We evaluated the burden of undiagnosed adrenal insufficiency in 49 such patients (age, 4.5 ± 3.5 years).

Study design  Serum adrenocorticotropic hormone (ACTH) and standard-dose ACTH stimulation test were performed at the baseline and followed prospectively until initiation of adrenal replacement therapy (follow-up, 2 ± 1.7 years).

Results  At baseline, 39 (80%) patients had impaired adrenal function, serum ACTH levels were elevated in 34 (69%) patients, and ACTH stimulation test was abnormal in 21 (43%) patients. There was a moderate association between Serum ACTH and age at baseline, \( r = 0.32, P = .05 \). By the end of follow-up, 86% of patients had borderline or overt adrenal insufficiency (age of onset, 4.8 ± 3.7 years).

Conclusions  We detected a high prevalence of unrecognized adrenocortical insufficiency in asymptomatic boys with X-ALD. It is known to be a frequent cause of morbidity and can be prevented by careful monitoring, early identification of impaired adrenal reserve, and timely initiation of therapy. It manifests early and before onset of neurologic symptoms, suggesting X-ALD as a candidate disorder for neonatal screening. (J Pediatr 2005;146:528-32)

X-linked adrenoleukodystrophy (X-ALD) is a progressive disorder that affects the nervous system, adrenal gland, and testes due to a defect in ABCD1, a gene that codes for a peroxisomal membrane protein. 1 Its incidence is estimated to be 1:17,000. 2 It is associated with the accumulation of saturated very long-chain fatty acids (VLCFA) in brain and adrenal and in plasma. Demonstration of increased VLCFA levels in plasma is a reliable diagnostic assay in affected male subjects. 3 Results of the plasma assay are abnormal on the day of birth. 3

At least 70% of X-ALD patients have Addison disease with primary adrenocortical insufficiency, with glucocorticoids usually more affected than mineralocorticoids. 4 X-ALD is estimated to be the cause of adrenal insufficiency in approximately 35% of patients with idiopathic Addison disease. 5-7 Prior studies have demonstrated Addisonian crisis as a common cause of acute presentation of childhood X-ALD. 8

It is possible to identify asymptomatic patients with X-ALD by conducting large-scale screening of at-risk relatives of known X-ALD patients. 2 This enables early identification of patients who are at high risk of developing adrenal insufficiency, which, if untreated, can lead to life-threatening complications. We report prospective evaluation of adrenal function in a cohort of 49 young patients identified by family screening with no clinical/radiologic evidence of neurologic abnormalities or known adrenal insufficiency.

METHODS

Asymptomatic male patients (n = 49; mean age, 4.5 ± 3.5 years) with X-ALD were identified by screening at-risk members of the family of known X-ALD patients 2 with the
plasma VLCFA assay. All patients were neurologically asymptomatic, and their brain MRIs were normal. None had been previously diagnosed with Addison disease. Adrenal function in all patients was evaluated within 4 months of diagnosis of X-ALD (baseline visit) and was followed prospectively at yearly intervals until the time of initiation of adrenal replacement therapy (mean ± SD follow-up duration, 2 ± 1.7 years). Patients lost to follow-up were also included in the analyses, with the assumption that the last complete visit is representative of their current adrenal status. Serum adrenocorticotropic hormone (ACTH) and response to standard-dose ACTH stimulation test were used for evaluation of adrenal function. At baseline, results of these tests were available for 38 patients for serum ACTH and 40 patients for ACTH stimulation test; other results were missing because of sampling or reporting errors. Adrenal function was rated as abnormal if serum cortisol <6 μg/dL (160 nmol/L) or ACTH >500 pg/mL (110 pmol/L) and ACTH stimulation test was abnormal and borderline if ACTH was between 70 to 500 pg/mL (15 to 110 pmol/L) and ACTH stimulation test was normal (see text for criteria). Mineralocorticoid deficiency was considered to be present only when the baseline aldosterone (standing up measurement) was <5 ng/dL. The protocol was approved by the institutional review board, and signed informed consent was obtained for each patient.

### Statistical Analyses

The data are presented in the form of mean ± SD unless specified otherwise. The unpaired Student t test and χ² test of homogeneity were used for baseline comparison. A paired t test was used to compare baseline and end of follow-up characteristics. The nonparametric Kruskal-Wallis test for median comparison was used when parametric assumptions were not met. Spearman rank correlation was used for evaluating the relation between age and plasma ACTH at baseline and end of follow-up, respectively. Association between repeated measurements of plasma ACTH and age was evaluated by using longitudinal regression analyses [generalized estimating equations (GEE)] with robust standard errors. Plasma ACTH levels were defined as the dependent variables, and age was the only predictor used in the model. A value of P < .05 was considered statistically significant. All statistical analyses were done by using STATA version 8.0 (Stata Corporation, Austin, Tex).
RESULTS

Overall Adrenal Function

At baseline, 39 (80%) patients had some form of adrenal insufficiency (details described in Table I). Adrenal function of patients with borderline or normal adrenal function at baseline was considered eligible for prospective evaluation. Of 25 eligible patients, 14 were prospectively studied; others were started on adrenal replacement therapy (ART) by their local physicians or were lost to follow-up.

Of 6 patients with normal baseline adrenal function, two developed borderline adrenal function and 1 developed overt adrenal insufficiency during follow-up. Three patients with borderline abnormalities in baseline adrenal function worsened to have development of overt biochemical adrenal insufficiency during follow-up. Detailed comparisons are provided in Table II. Hyperpigmentation was seen in 28%. Only 1 patient reported previous episodes suggestive of adrenal crisis.

In all patients, the neurologic examination and brain MRI remained normal at the time biochemical evidence of adrenal insufficiency developed. Mineralocorticoid levels were normal in all of the 18 patients in whom they were tested.

By the end of the study, 42 (86%) patients had either biochemically definitive or borderline impairment of adrenal function.

Age and Adrenal Function

Figure 1 shows that adrenal function was already impaired to some degree in most of the asymptomatic boys at the time that X-ALD was diagnosed by VLCFA assay, irrespective of age. Pearson $\chi^2$ test did not demonstrate association between age and status of adrenal function at baseline or end of follow-up ($P = .5$). Mean age of onset of adrenal insufficiency (criteria for which are defined above) was $4.8 \pm 3.7$ years, ranging from 5 months to 13 years. The distribution of age at diagnosis of adrenal insufficiency in this cohort is shown in Figure 2. There was no significant association between age and repeated measurements of plasma ACTH levels when using the GEE model, ($P = 0.2$).

Serum ACTH and ACTH Stimulation Test

At baseline, 34 (89.5%) patients had elevated plasma ACTH (>70 pg/mL). Figure 3 shows a modest association between baseline plasma ACTH levels and age ($r = 0.32$, $P = .05$). Plasma ACTH (pg/mL) was significantly different between those who had normal cortisol response to ACTH stimulation and those who did not, although it was greatly elevated in both of these categories (171.1 ± 164 vs 965 ± 692, $P < .0001$).

Figure 4 shows the proportion of patients with abnormal ACTH stimulation tests (Figure 4, A) and abnormal baseline Plasma ACTH levels (Figure 4, B) at the time of diagnosis of X-ALD as a function of age. No significant association was seen between the former two variables and age at the time of baseline visit ($P > .5$).

Although baseline cortisol was significantly reduced in patients with abnormal response to ACTH stimulation test ($11.5 \pm 3.7$ vs $17.8 \pm 5.8$, $P < .0001$), it was within normal range for both groups. Only 2 patients had baseline serum cortisol less than 6 µg/dL. There was no difference in baseline cortisol between patients, who had normal plasma ACTH versus those with abnormal plasma ACTH ($P = .9$).

Stages of Adrenal Dysfunction

On the basis of plasma ACTH, response to ACTH stimulation test and serum cortisol measurements, patients were classified into 4 stages: stage 0 (no adrenal dysfunction) comprised patients with normal plasma ACTH, normal serum cortisol, and normal response to ACTH stimulation test; stage 1 (adrenal insufficiency) with isolated abnormalities in plasma...
ACTH; stage 2 (adrenal insufficiency) with abnormalities in plasma ACTH, and ACTH stimulation test; and stage 3 with abnormalities in all 3 measures. Figure 5 shows the age composition of patients in these stages of adrenal dysfunction. The Kruskal-Wallis test of difference of medians demonstrated a trend of difference in median age across these stages \((P = .07)\); because of just 1 patient in stage 3, a definitive association cannot be established.

**DISCUSSION**

This study shows that approximately 80% of 49 asymptomatic boys with X-ALD, identified by plasma VLCFA screening of at-risk relatives, already had biochemical evidence of otherwise clinically silent adrenal insufficiency at the time of diagnosis of X-ALD. Neurologic examination and brain MRI were normal at the time of diagnosis of adrenal insufficiency in all patients, indicating that biochemical evidence of adrenal insufficiency precedes clinical evidence of overt neurologic involvement. This does not rule out presence of subclinical metabolic changes detectable by 1H-MRSI; however the predictive ability of these changes remains uncertain. Increased plasma ACTH levels in neurologically involved and asymptomatic X-ALD patients without clinically evident adrenal insufficiency has been reported previously.11-16 Such patients can be identified only by VLCFA screening or mutation analysis. A key finding is that 70% of the patients studied by 2 years of age already showed increased serum ACTH levels (Figure 4, A). Impaired cortisol response to ACTH appears to develop somewhat later (Figure 4, B), but the difference is not statistically significant.

As described previously, the progression of adrenal dysfunction in X-ALD can be subdivided into four stages (0 to 3). Figure 5 shows that median age tends to increase across stages 1 and 2; however, because of just 1 patient in stage 3, a definitive trend could not be demonstrated statistically. Despite this moderate age effect, biochemical evidence of adrenal impairment may already be present very early. The ACTH level was already increased in 2 boys at 6 months of age. Because our test sample included only these 2 patients, who were younger than 6 months old, we do not know at which age these abnormalities commence. Pathologic changes in the adrenal gland were already present in two 22-week-old fetuses with X-ALD.17

The data presented here indicate that impaired adrenal function in X-ALD patients can be identified before the development of overt adrenal insufficiency. Careful monitoring and appropriate provision of hormone therapy should make it possible to prevent or reduce the risk of adrenal crises, which in the past have caused significant morbidity and mortality in X-ALD.8 In the past, some clinics have used serum cortisol levels to monitor adrenal function in asymptomatic patients. Our data show that this assay is not sufficiently sensitive and that measurement of ACTH levels and ACTH stimulation test should be added. To our knowledge, clinically evident adrenal insufficiency has not been reported in X-ALD patients before 1 year of age, and we do not know precisely at what age biochemical evidence of impaired adrenal function first develops. However, since in some of our patients the serum ACTH levels were already increased at 5 months of age, it would be prudent to begin...
monitoring by 3 months. The time that steroid replacement therapy should be initiated involves clinical judgment. We recommend that it be considered in stage 1 and 2 patients and certainly in stage 3 patients. It is also important to note the previously reported lowering of dehydroepiandrosterone (DHEA) and its sulfate conjugates (DHEA-S) in X-ALD patients, even in the absence of cortisol and ACTH abnormalities. It is thus desirable to monitor these hormones closely in conjunction with cortisol and ACTH in boys with X-ALD.

The key to starting timely ART is early recognition of X-ALD patients, which constitute a high-risk subset of clinically silent adrenal dysfunction. Timely ART is imperative to prevent occurrence of potentially life-threatening adrenal crisis previously reported in these patients; however, there is no evidence to indicate that it delays, halts, or reverses neurologic progression. At this time, the most effective way to accomplish is to screen genetically at-risk relatives of known X-ALD patients with the plasma VLCFA assay. By using this approach, we identified 250 asymptomatic patients. However, this screening procedure has important limitations. For various reasons, not all at-risk relatives can be tested; unidentified X-ALD patients represent a high-risk subset of undiagnosed and untreated adrenal insufficiency, thus subjecting them to unnecessary and largely avoidable risk of adrenal crisis. Neonatal screening would overcome these limitations. Our previous demonstration that plasma VLCFA are already increased on the day of birth suggests that biochemical analysis as the most promising approach. The techniques in current use for plasma VLCFA assay are not suitable for mass screening. A technique that can be applied in filter paper blood spots, now used in many states for tandem mass spectrometry for metabolic screening of newborn infants, would be of immense value.

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REFERENCES

Objective  To determine the incidence, natural history, and clinical characteristics of Hashitoxicosis (Htx) in pediatric patients with autoimmune thyroiditis.

Study design  Medical records of children diagnosed with Hashimoto thyroiditis between 1993 and 2002 were reviewed. The clinical course of patients presenting with hyperthyroidism was determined. Variables including sex, age, family history, thyroid hormone levels, anti-thyroid antibody titers, $^{123}$I thyroid scan results, and presenting features were investigated as possible predisposing factors for the development of Htx.

Results  Out of 69 patients with autoimmune thyroiditis, 8 were diagnosed with Htx. The duration of hyperthyroidism ranged from 31 to 168 days. Three patients became hypothyroid after an average of $46.3 \pm 13.2$ days, and 5 patients became euthyroid after an average of $112.8 \pm 59.8$ days. Additional findings included an elevated thyroid stimulating immunoglobulin (TSI) titer in 3 of the 8 patients with Htx, and increased uptake on $^{123}$I scan in 2 patients.

Conclusion  Htx is an uncommon yet important cause of hyperthyroidism in children that has a variable clinical course. The diagnosis may be complicated, as presenting features sometimes exhibit significant overlap with Graves’ disease. No factors predisposing to the development of Htx were identified. (J Pediatr 2005;146:533-6)

Hashitoxicosis (Htx) refers to the presence of biochemical hyperthyroidism in patients with autoimmune thyroiditis. It is believed to result from unregulated release of stored thyroid hormone during inflammatory-mediated destruction of the thyroid gland. The incidence and typical clinical course of Htx in pediatric patients has not been well characterized. In addition, it is unknown whether features of the disease differ in patients with thyroiditis who develop hyperthyroidism compared with those who do not, which could be of predictive value. This study investigated Htx in a large cohort of children with Hashimoto thyroiditis.

METHODS

Following institutional review board approval, medical records of patients followed for Hashimoto thyroiditis in the pediatric endocrine clinic at Riley Hospital for Children between July 1993 and August 2002 were reviewed. Hashimoto thyroiditis was diagnosed by a pediatric endocrinologist on the basis of anti-thyroid antibodies. Patients with Hashimoto thyroiditis who presented with biochemical hyperthyroidism that subsequently spontaneously resolved were considered to have had Htx. Hyperthyroidism was defined as a suppressed thyrotropin level in association with elevated or normal thyroid hormone levels. Variables examined included sex, age, family history, anti-thyroid antibody titers, thyroid scan results, and presenting features. The clinical course in patients diagnosed with Htx was determined through follow-up visits and serial measurements of thyroid hormone levels.

<table>
<thead>
<tr>
<th>Htx</th>
<th>Hashitoxicosis</th>
<th>TSI</th>
<th>Thyroid stimulating immunoglobulin</th>
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Table I. Summary of patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tr>
<td>Age (y)</td>
<td>10.9 ± 3.1</td>
</tr>
<tr>
<td>Gender</td>
<td>Girls: 63 (91%); boys: 6 (9%)</td>
</tr>
<tr>
<td>Family history</td>
<td>Positive: 40 (58%); negative: 29 (42%)</td>
</tr>
<tr>
<td>Goiter</td>
<td>Present: 51 (74%); absent: 18 (26%)</td>
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<tr>
<td>Anti-thyroid antibodies</td>
<td>AMA: 559.3 ± 1019 (range, 1-5650); ATA: 142.6 ± 379 (range, 0-2174)</td>
</tr>
<tr>
<td>Thyroid function</td>
<td>Hyperthyroidism: 49 (71%); euthyroidism: 12 (17.4%); hypothyroidism: 8 (11.5%)</td>
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Normal ranges: Anti-thyroglobulin antibody (ATA): <2 U/mL; anti-microsomal antibody (AMA): <2 U/mL.

Statistical Analysis

Statistics were done using Microsoft Excel 2000 (Microsoft Corp., Redmond, Wash) (for descriptive statistics) and Statistical Package of the Social Sciences, version 11.5 (SPSS Inc., Chicago, Ill) (for other analyses). Data are expressed as means ± standard deviations. Fisher’s exact test (for categorical variables) and t tests (for continuous variables) were used for comparisons between groups when the data were normally distributed. The Mann–Whitney U test was used for data that were not normally distributed.

RESULTS

Of 104 patients identified, 69 were included (6 boys; 63 girls). Of the 35 patients excluded, 25 had other forms of acquired hypothyroidism and 10 had congenital hypothyroidism. The patients included in the study had an average age of 10.9 ± 3.1 years (range, 1.3-17.3 years). A goiter was noted in 51 patients (74%). Forty subjects (58%) had a known family history of thyroid disease. All patients had an elevated antithyroglobulin antibody titer, anti-microsomal antibody titer, or both. The average anti-thyroglobulin antibody titer was 142.6 ± 379 U/mL (normal, <2 U/mL; range, 0-2174), and the average anti-microsomal antibody titer was 559.3 ± 1019 (normal, <2 U/mL; range, 1-5650 U/mL). Of the 69 patients, 49 (71%) had hypothyroidism at presentation and were started on levothyroxine. Twelve children (17.4%) were euthyroid at presentation, 10 of whom subsequently became hypothyroid. Eight patients (11.6%) were initially hyperthyroid, and were eventually diagnosed with Htx. These results are summarized in Table I.

Details of the patients with Htx are provided in Table II. No difference was seen in the average anti-thyroid antibody titers in patients with Htx compared with those who were hypothyroid or euthyroid at presentation. Similarly, no statistically significant correlations were identified between patient characteristics and the presence of hypothyroidism. Thyroid stimulating immunoglobulin (TSI) titer was measured in 7 patients with Htx, and it was found to be elevated in 3. One had a TSI of 137% (normal, <130%) in association with an elevated thyrotropin of 7.5 uIU/mL, whereas another had a TSI titer of 137% in association with a suppressed thyrotropin of <0.01 uIU/mL but a low uptake on 123I scan (1%). This patient later became hypothyroid 54 days after the diagnosis of Htx. The third patient had a TSI of 187% concurrent with normalization of his thyropropin of 0.915 uIU/mL. 123I thyroid uptake scan was obtained in 4 patients. Of these, 2 patients had increased uptakes of 61% and 53% (normal, 10%-35%), respectively, although thyroid function tests performed on the same day as the scans were entirely normal. In the other 2 patients, low uptakes of 1% in one patient while hyperthyroid and 12% in the other patient while euthyroid were noted. No patient had both an elevated TSI titer and increased uptake on 123I scan. Three patients had classic symptoms of hyperthyroidism including tremor, tongue fasciculations, and palpitations.

Two children required pharmacological treatment for hyperthyroidism. One patient received beta blocker therapy and 1 month later became hypothyroid, whereas another was treated with methimazole for 3 months, after which the decision was made to stop the medicine. This patient became euthyroid 1 month after stopping methimazole, and has remained so after 28 months. One subject was referred to Ophthalmology for possible proptosis, which was ruled out by exophthalmometry. Because of persistence of eye pain magnetic resonance imaging was ordered to evaluate the ocular muscles. The findings were normal.

The duration of hyperthyroidism in subjects with Htx ranged from 31 to 168 days after diagnosis. Three patients became hypothyroid after an average of 46 ± 13 days, and 5 patients became euthyroid after an average of 112.8 ± 59 days. These 5 subjects have subsequently remained euthyroid with a follow-up of 3 to 32.5 months, as shown in the Figure.

DISCUSSION

Graves’ disease is the most common cause of hyperthyroidism in children, adolescents, and adults.11-13 In addition to Graves’ disease, the differential diagnosis of hyperthyroidism during childhood includes Htx, toxic adenoma, multinodular goiter, exogenous ingestion of thyroid hormone, McCune-Albright syndrome, struma ovarii, and subacute thyroiditis.11 It is essential that the correct cause be identified because the prognosis and appropriate therapy depend upon the underlying mechanism of hyperthyroidism.

Htx is a rare complication of autoimmune thyroiditis.10 Although it has long been recognized that some features may be shared between different types of autoimmune thyroid disease (such as positive anti-thyroid antibodies),14-15 differentiating between Graves’ disease and Hashimoto thyroiditis is usually straightforward. The finding of an elevated TSI titer is typically considered supportive evidence of Graves’ disease, as it is increased in the vast majority of children with this disorder.16 An additional test used to differentiate Htx from Graves’ is the 123I uptake scan, with a low to normal uptake suggesting Htx and a high uptake favoring Graves’ disease.11,17

In our study, 8 of 69 patients with Hashimoto thyroiditis (11.5%) initially presented with hyperthyroidism. Although a presumptive diagnosis of Htx was made, this was confirmed by spontaneous resolution of the hyperthyroidism.
with subsequent development of hypothyroidism or euthyroidism. Remarkably, 5 of the 8 patients had features that made the diagnosis confusing. In 3 patients, a positive TSI titer was found. In these 3 subjects, the TSI titer was not checked initially but was measured later in the clinical course when additional test results were inconsistent with hyperthyroidism. No obvious explanation exists for the elevated TSI when additional test results were inconsistent with hyperthyroidism. Alternatively, this could represent some degree of nonspecificity of the immune dysregulation characteristic of autoimmune thyroid disease. In the two cases in which an elevated 123I scan was observed in conjunction with a normal TSI titer, the possibility of TSI-negative Graves’ disease was entertained. However, these 2 patients subsequently developed euthyroidism without treatment. Some reports involving adults suggest that the thyroid scan in Hashimoto thyroiditis can mimic a wide range of thyroid disorders, including Graves’ disease.10,19-21 It is possible that some aspects of the dynamic evolution in thyroid function from hyperthyroidism to euthyroidism is responsible for this phenomenon.

In summary, this report characterized the frequency and natural history of Htx in children with autoimmune thyroiditis. One flaw of our study is that it consists solely of a clinic-referred sample. Therefore, the actual incidence of Htx may well be higher, especially because the majority of our patients were asymptomatic. Our results demonstrate the extreme clinical variability and potential diagnostic pitfalls that are sometimes encountered. Although transient, the hyperthyroid phase is of variable duration and may be followed by extended periods of euthyroidism in some cases. Factors predisposing to the development of Htx in patients with Hashimoto thyroiditis were not identified. These findings expand the clinical and biochemical spectrum of autoimmune thyroid disease in the pediatric population and provide valuable prognostic information for clinicians taking care of children with hyperthyroidism.

**REFERENCES**

50 Years Ago in *The Journal of Pediatrics*

**INFANT SPEECH DEVELOPMENT: A REPORT OF THE STUDY OF ONE CHILD BY MAGNETIC TAPE RECORDINGS**


Using magnetic tape recordings—a new technology for that period 50 years ago—and a captive subject in his own home, Parmelee recorded his daughter’s utterances and the sequences of speech development at monthly intervals during the first 2 years of her life. However, being the quintessential developmental pediatrician, Parmelee did not rely on the novel technological instrument alone. He also simultaneously recorded the social responsiveness, reciprocity, motor milestones, and feeding behaviors, all of which are intricately related in the development of speech. Through a correlation table, Parmelee illustrates that as his daughter’s oro-motor mechanisms became more versatile with acceptance of a greater variety of food textures and emerging control over her mouth and jaw, her vocalizations became increasingly more complex. With the acquisition of progressive motor skills, such as mobility, her vocal-social development enlarged concomitantly within the wider world around her. Parmelee also noted changes in the tone and inflection of her speech, which became more interactive and sophisticated with age. Ultimately, despite technology, the human ear is called on to discern these nuances.

In short, Parmelee single-handedly integrated the complex utterances, behaviors, and motor activity of his daughter, which can now be recorded simultaneously and with considerably less effort, by the video-digital cameras of today.

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MIDLINE DEFECTS IN FG SYNDROME: DOES TETHERED SPINAL CORD CONTRIBUTE TO THE PHENOTYPE?
RAYMOND WANG, MD, JEANNIE VISOOTSAK, MD, MOISE DANIELPOUR, MD, AND JOHN M. GRAHAM, JR, MD, ScD

Objectives FG syndrome is an X-linked recessive mental retardation syndrome with ano-rectal anomalies, constipation, and occasional urinary incontinence. Because tethered spinal cord syndrome (TCS) has similar symptoms, we evaluated imaging for TCS in patients with FG syndrome.

Study design Patients were recruited from the International FG Syndrome Support Group, and an FG Syndrome Consensus Group reviewed clinical histories, medical records, and photographs of each responding patient. Results of cranial and spinal imaging studies were available for 12 patients.

Results Of 12 boys with FG syndrome, 6 had hypoplasia of the corpus callosum, and 3 of these had TCS (all with constipation and urinary symptoms). The other 9 did not have urinary symptoms. After surgical untethering, bowel and bladder symptoms improved.

Conclusions Tethered spinal cord syndrome occurred in 25% of patients with FG syndrome associated with hypoplasia of the corpus callosum and causing bowel and bladder incontinence. A high index of suspicion is necessary for early diagnosis, and timely intervention results in significant improvement in symptomatology. (J Pediatr 2005;146:537-41)

FG syndrome is an X-linked recessive mental retardation syndrome with associated congenital anomalies that was initially described by Opitz and Kaveggia in 1974. Craniofacial features include macrocephaly, prominent forehead with frontal cowlick, ocular hypertelorism with downslanting palpebral fissures, and small, low-set, round, and/or prominent ears (Figure 1). Affected individuals also have complete or partial agenesis of the corpus callosum (ACC), broad thumbs and halluces, prominent fingertip pads, other digital anomalies, occasional cardiac defects, and anal anomalies. Friendly, loquacious, hyperactive behavior is characteristic of FG syndrome. Functional abnormalities in FG syndrome include congenital hypotonia with joint hyperlaxity that evolves into spasticity and joint contractures later in life, severe chronic constipation, occasional seizures, and hearing loss. To date, the gene(s) for FG syndrome have not been identified, but analysis has demonstrated four loci: Xq12-q21.31, Xq28, Xp22.3, and Xp11.4-p11.3. This suggests considerable clinical and genetic heterogeneity.

Tethered spinal cord syndrome (TCS) is characterized radiologically by a conus medullaris below the level of L2, ventral displacement of the conus while in the supine position, or inability of the conus to fall ventrally when a child is placed in prone position. Cutaneous signs of TCS tend to be congenital and include lumbosacral dimples, sinus tracts, hair tufts, hemangiomas, nevi, or other alterations in pigmentation. TCS is also associated with anterior placement of the anal orifice. Neuromuscular and orthopedic symptoms can manifest at any age, especially during the first decade of life when accelerated leg growth acutely creates traction on the spinal cord. Symptoms include distal lower extremity muscle weakness with decreased muscle bulk, patellar and heel hyporeflexia, abnormal Babinski reflex, toe-walking or other gait disturbance, sensory deficits of the perineal region and distal lower extremities, bowel retention, bladder incontinence, progressive kyphosis or scoliosis, leg length discrepancies, and/or equinovarus feet. Back and leg pain are rare presenting symptoms of TCS in children.
Since some features of FG syndrome, such as anteriorly placed anus, progressive hypertonia, toe-walking, and constipation, are also signs and symptoms of TCS, we attempted to determine if TCS was a contributing factor to the symptomatology of FG syndrome. Since TCS can be surgically corrected, with significant improvement or complete resolution of symptoms, we hypothesized that early treatment would improve function.\textsuperscript{12,13}

Figure 1. Patient 1 (see Table) at age 7 years demonstrates wide, broad forehead with relative macrocephaly, hypertelorism, prominent ears, and broad thumbs and halluces.
Table. Clinical features and results of MRI imaging of the 12 patients with FG evaluated in this study

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>Percentage</th>
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<tbody>
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<td>AGE</td>
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<td>21</td>
<td>15.25</td>
<td>11</td>
<td>4.25</td>
<td>9.67</td>
<td>2.5</td>
<td>7.33</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>50%</td>
</tr>
</tbody>
</table>

**Neurological**

- Hypoplasia of corp. callos. (MRI) + + + + + + + - - - - 50%
- Tethered cord (MRI) + + + - - - - - - - 25%
- Mental retardation + + + + + + + + + + + + 100%
- Congenital hypotonia + + + + + + + + + + + + 100%
- Characteristic personality + + + + + + + + - + + + 92%
- Seizure/abnormal EEG - - - - + - - - + - + 33%
- Sensorineural deafness - - - - + - - - + - + 17%

**Facial Dysmorphism**

- Relative macrocephaly + + + + + + + - + - + - 75%
- Tall, broad forehead + + + - + + + + + + + + 92%
- Frontal cowlick + - - + + + + + - + - 75%
- Ocular hypertelorism + + + - + + + + + + - + 67%
- Abnormal auricles + + + + + + + + + + + + 92%
- Downward palpebral slant + + + - + + + - + + + + 83%

**Visceral anomalies**

- Constipation + + + + + + + + + + + + 100%
- Anal anomalies - - + - - - - - + + + - 33%
- Pyloric stenosis - - - - - - - - - - - 8%
- Congenital heart defect - - + + - + - - + - + 33%

**Genital defects**

- Cryptorchidism + - - + + - - - + + - + 50%
- Hypospadias - + - - - - - + - - + + 33%
- Hernia - + - - - - - - - - - - 25%

**Limb/skeletal defects**

- Broad thumbs/halluces + + + + + + + + + + + + 100%
- Persistent fetal fingerpads + + + + + + - - - + - + 67%
- Single palmar crease + - + - + - - - + + - - 42%
- Pectus excavatum + + - + - + - - - + - 67%
- Scoliosis - - - + + - - - - - - - 17%

**METHODS**

**Subject Recruitment and Clinical Evaluation**

After approval of our study protocol by the Cedars-Sinai Medical Center Institutional Review Board, participation in this study was solicited through a letter that was circulated by the International FG Syndrome Support Group to its membership, searching for FG syndrome patients who had both cranial and lumbosacral MRI.

The participants of this study were personally examined by each of the physicians of the GF Syndrome Consensus Group at the 2002 FG parent support group meeting and are also followed closely by the geneticists of the consensus group. The group met to review clinical information on each responding family to confirm the diagnosis, based on clinical examination and medical records. All patients included in this study were diagnosed with FG syndrome by the consensus group.

**RESULTS**

**Case Report**

We describe an 8-year-old boy with Opitz FG syndrome (patient 2, Table). He was born at gestational age 41 weeks, 3 days, and was noted to have hypertelorism with downslanting palpebral fissures, micrognathia, hypotonia, right-sided aortic arch, hypospadias with chordee, and bilateral inguinal hernias. Cranial MRI showed a hypoplastic, thin corpus callosum with
aplasia of the rostrum (Figure 2). Despite several genetics evaluations, no specific syndromic diagnosis could be made until age 2½, when a diagnosis of Opitz GBBB syndrome was suggested. His diagnosis was revised to Opitz FG syndrome when he was evaluated by Dr John Opitz at age 3 years. He is currently enrolled in 100% special education classes at the 4th grade, reading and spelling above grade level, but doing poorly in mathematics. Other clinical features include friendly personality, relative macrocephaly (occipitofrontal circumference, 98%; height, 50%; weight, 75%), high forehead, prominent ears, velopharyngeal insufficiency, persistent finger pads, broad thumbs and toes, bifid second left toe, shawl scrotum, and severe constipation. He has required several hospitalizations for stool disimpaction, ascribed to functional stool retention. Tethered spinal cord was not suspected due to lack of lumbosacral cutaneous stigmata.

At age 7 years, he was noted to have new-onset daytime enuresis, urinary urgency, and frequency. He had no evidence of toe-walking, leg pain, “saddle distribution” anesthesia, or hyperreflexia. He had tight Achilles heel tendons despite muscular hypotonia. Workup for infection was negative; behavioral modification was unsuccessful. Tethered spinal cord was suspected, and he underwent urodynamics studies, which showed signs of neurogenic bladder with normal bladder capacity but diminished bladder compliance (rapid increase in pressure at 45% of total bladder capacity) and incomplete relaxation of the bladder neck. MRI of the spine showed a normal cervical and thoracic cord, but the conus medullaris was abnormally low at the level of L3, contained a small syrinx, and failed to fall forward in prone position (Figure 2). He underwent surgical correction without complications, and, after the procedure, symptoms of daytime enuresis, urgency, and frequency resolved. After surgery, orthopedic evaluation confirmed improved thoracic kyphosis. He was able to pass stool daily without laxatives for 2 weeks after the surgery, but constipation subsequently returned.

Results From the Other FG Patients Recruited for the Study

The Table shows the clinical characteristics of the patients enrolled in this study and results of brain and spinal MRI imaging studies. Of a total of 12 patients, 3 had TCS and had difficulties with urinary incontinence, constipation, and lower extremity spasticity. After surgical untethering, all 3 patients had improvement in urinary continence, with limited

Figure 2. Cranial MRI showed a hypoplastic corpus callosum with aplasia of the rostrum. Spinal MRI showed the conus medullaris was abnormally low at the level of L3, with a small syrinx, and the conus failed to fall forward in prone position. Digital anomalies included persistent finger pads, broad thumbs and toes, and a bifid second left toe.
improvement in constipation and spasticity. None of the patients without TCS had urinary symptoms. Interestingly, agenesis of the corpus callosum was present in the 3 patients with TCS; there were 3 patients with ACC who did not have TCS.

**DISCUSSION**

Since progressive lower extremity spasticity and constipation are features common to both FG syndrome and TCS, we reviewed spinal imaging on FG patients known to the FG parent support group. Twelve FG syndrome patients had MRI imaging: 3 (25%) had TCS accompanied by chronic constipation and ACC; 1 of the 3 had anal anomalies. After surgical untethering, all 3 patients had improvement in urinary continence with limited improvement in constipation and spasticity, suggesting that their tethered spinal cords were symptomatic. None of the patients without TCS had urinary symptoms. It is also important to note that in the index case, symptoms were limited to neurogenic bladder and constipation without the “classic” findings of cutaneous lumbar sacral stigmata, saddle-distribution anesthesia, and lower extremity hypertonia and hyperreflexia. Therefore, a high index of suspicion is required to make the diagnosis of TCS, especially in boys with FG syndrome. Constipation, spasticity, and anteriorly placed anus should not be attributed solely as inherent features of FG syndrome; rather, diagnostic studies for TCS should be pursued in all boys with FG syndrome.

Of the 6 FG patients with abnormalities of the corpus callosum, 3 (50%) had TCS. The percentage of patients with and without tethered cord having anal anomalies was identical (33%). Interesting conclusions can be drawn from these results, although the study population was small. Lumbosacral cutaneous stigmata were not diagnostic of underlying cord abnormalities, nor were anal placement anomalies. Tethered cord was only seen in the patients with hypoplasia of the corpus callosum; conversely, a high percentage of patients with callosal abnormalities had tethered cord. This observation further suggests abnormal development of midline central nervous system structures in FG syndrome.

Timely diagnosis of tethered cord in FG syndrome will result in prompt surgical correction, decreasing the potential morbidity. Further studies must be done on more FG patients to verify a true association and document its frequency. Until ACC can be verified as a risk factor for tethered cord, we recommend that all patients with FG syndrome be evaluated for TCS.

**REFERENCES**

Objective To determine if serum levels of CIT (a nonprotein amino acid synthesized by the intestine) correlate with total parenteral nutrition (PN)-independence in children with short bowel syndrome (SBS).

Study design We prospectively obtained serum amino acid profiles over a 24-month interval from all infants with SBS 3 weeks to 4 years of age. Remaining small intestine length was recorded at surgery, and percent enteral calories tolerated (enteral calories divided by enteral plus parenteral calories \( \times 100 \)) was determined in 24 infants with SBS and 21 age-matched controls (blood drawn for nongastrointestinal symptoms).

Results Mean CIT for controls was \( 31 \pm 2 \mu \text{mol/L} \). In patients with SBS (n = 24), serum CIT correlated linearly with percent enteral calories (R = 0.85; \( P < .001 \)) and with bowel length (R = 0.47; \( P \leq .03 \)). CIT level in patients with SBS weaned off PN was \( 30 \pm 2 \mu \text{mol/L} \); in those subsequently weaned off PN, \( 20 \pm 2 \mu \text{mol/L} \); and in those who would remain PN-dependent, \( 11 \pm 2 \mu \text{mol/L} \) (\( P \leq .01 \)). Serum CIT \( \geq 19 \mu \text{mol/L} \) had 94% sensitivity and 67% specificity for being off or coming off total PN.

Conclusions Serum CIT level >19 \( \mu \text{mol/L} \) in children with SBS is associated with development of enteral tolerance and may be a useful predictive test. (J Pediatr 2005;146:542-7)
renal arginine (ARG) production. The serum levels of CIT are not influenced by body mass index or creatinine clearance. Crenn et al. assessed plasma CIT in postabsorptive adults with SBS; CIT level correlated with fat absorption and, with a 20 μmol/L cutoff, CIT level had a sensitivity of 92% and specificity of 90% for intestinal adaptation. It is not clear if serum CIT will be a useful indicator of intestinal function in infants with SBS because children show much greater bowel adaptability than adults, as demonstrated by a high number of infants who develop SBS as a complication of prematurity and yet can be weaned from PN. We aimed to determine whether serum CIT concentration is a useful indicator of enteral tolerance in children with SBS.

**METHODS**

Children ≤4 years of age with SBS hospitalized or seen in gastroenterology clinic were followed prospectively for 6 to 12 months. Controls were well-nourished infants receiving elective endoscopy, bronchoscopy, or adenoidectomy with or without tonsillectomy, 3 weeks to 4 years of age. Infants with SBS had been on total parenteral nutrition (TPN) for at least 2 weeks and had >20 cm bowel resected.

Bowel length at initial surgery was recorded from the surgical records. No information on bowel length was available for 4 patients referred from other hospitals. At subsequent time of phlebotomy, the percentage of enteral and parenteral calories was calculated. We attempted to obtain follow-up amino acid levels at 3-month intervals; however, these measurements were made only in 4 cases because the infants were on home PN, and blood testing was often done far from our hospital. All infants were on amino acid-based formula (Neocate, SHS, Gaithersburg, Ma) by continuous infusion. Enteral tolerance was defined as ≤8 stools per day and no perianal excoriation in a patient with normal growth and serum electrolytes. Serum CIT and other amino acids were determined by high pressure liquid chromatography as described by Wu et al. Blood samples were not analyzed if the patient had septicemia or urinary tract infection; or if the child had been transfused within 1 week of phlebotomy; or if the child was not gaining weight.

Informal consent was obtained from all subjects. The study was approved by the Committee for the Protection of Human Rights (University of North Carolina, Chapel Hill) and Ochsner Clinic Foundation's Internal Review Board.

**Table I. Age, diagnosis, bowel length, and age weaned off PN (n = 24)**

<table>
<thead>
<tr>
<th>Age (mo) at entry</th>
<th>Diagnosis</th>
<th>Bowel length (cm) measured at original surgery</th>
<th>Age weaned off PN (mo)</th>
<th>Duration of follow-up</th>
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<tbody>
<tr>
<td>3</td>
<td>Volvulus</td>
<td>18</td>
<td>†</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>NEC</td>
<td>43</td>
<td>17</td>
<td>25</td>
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<tr>
<td>2</td>
<td>NEC</td>
<td>41</td>
<td>6</td>
<td>w</td>
</tr>
<tr>
<td>14</td>
<td>NEC</td>
<td>17</td>
<td>-</td>
<td>Deceased, 20 mo</td>
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<tr>
<td>16</td>
<td>NEC</td>
<td>70</td>
<td>4</td>
<td>w</td>
</tr>
<tr>
<td>4</td>
<td>Gastrochisis</td>
<td>*</td>
<td>5</td>
<td>w</td>
</tr>
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<td>8</td>
<td>Jejunal atresia</td>
<td>85</td>
<td>1</td>
<td>w</td>
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<td>Gastrochisis, multiple atresias</td>
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<td>2</td>
<td>w</td>
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<tr>
<td>4</td>
<td>Multiple jejunal atresias</td>
<td>34</td>
<td>†</td>
<td>24</td>
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<td>0.5</td>
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<td>w</td>
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<td>w</td>
</tr>
<tr>
<td>8</td>
<td>NEC</td>
<td>25</td>
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<td>Deceased, 12 mo</td>
</tr>
<tr>
<td>17</td>
<td>Midgut volvulus</td>
<td>70</td>
<td>†</td>
<td>17</td>
</tr>
</tbody>
</table>

NEC, necrotizing enterocolitis.
W indicates patient was weaned off PN.
* Surgery note from hospital records failed to indicate length of remaining bowel.
† Patient not weaned off PN.
**Table II. Serum amino acid levels in control infants and in infants with SBS**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Control (n = 21)</th>
<th>Off (n = 6)</th>
<th>On-Off (n = 10)</th>
<th>On (n = 8)</th>
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<tr>
<td>ALA</td>
<td>239.1 (70.5)</td>
<td>378.6 (77.3)</td>
<td>325.7 (32.6) *</td>
<td>356.1 (15.3) **</td>
</tr>
<tr>
<td>ARG</td>
<td>68.3 (3.3)</td>
<td><strong>122.9 (12.0)</strong> *</td>
<td>127.5 (15.2) *</td>
<td>135.3 (16.6) **</td>
</tr>
<tr>
<td>CIT</td>
<td>31.2 (1.6)</td>
<td>28.7 (1.9)</td>
<td><strong>18.1 (2.3)</strong> *</td>
<td>14.1 (2.9) *</td>
</tr>
<tr>
<td>GLN</td>
<td>550.4 (19.5)</td>
<td>723.0 (133.4)</td>
<td>503.0 (70.2)</td>
<td>433.1 (63.8)</td>
</tr>
<tr>
<td>GLU</td>
<td>81.9 (3.5)</td>
<td>164.0 (34.1)</td>
<td><strong>203.0 (34.5)</strong> *</td>
<td><strong>104.9 (7.6)</strong> *</td>
</tr>
<tr>
<td>GLY</td>
<td>218.4 (10.0)</td>
<td><strong>369.1 (54.7)</strong> *</td>
<td><strong>354.0 (48.9)</strong> *</td>
<td><strong>440.4 (67.4)</strong> *</td>
</tr>
<tr>
<td>HIS</td>
<td>67.0 (3.4)</td>
<td>97.8 (12.3)</td>
<td>82.3 (11.3)</td>
<td>92.9 (12.4)</td>
</tr>
<tr>
<td>ILE</td>
<td>46.3 (2.3)</td>
<td>69.3 (9.7)</td>
<td>72.3 (13.3)</td>
<td>93.5 (12.2) **</td>
</tr>
<tr>
<td>LEU</td>
<td>86.8 (4.1)</td>
<td>137.4 (8.8) *</td>
<td>148.1 (23.7) *</td>
<td>152.6 (15.7) **</td>
</tr>
<tr>
<td>LYS</td>
<td>106.9 (4.2)</td>
<td><strong>156.2 (17.7)</strong> *</td>
<td>181.9 (30.5) *</td>
<td>208.5 (26.6) *</td>
</tr>
<tr>
<td>MET</td>
<td>18.5 (0.7)</td>
<td>91.5 (57.6)</td>
<td>37.1 (4.8) *</td>
<td>56.5 (13.3) *</td>
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<td>ORN</td>
<td>55.4 (5.0)</td>
<td>121.4 (45.8)</td>
<td>105.0 (20.2) *</td>
<td>150.2 (36.7) *</td>
</tr>
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<td>PHE</td>
<td>42.8 (2.1)</td>
<td><strong>94.4 (6.5)</strong> *</td>
<td><strong>97.7 (9.4)</strong> *</td>
<td><strong>92.7 (7.5)</strong> *</td>
</tr>
<tr>
<td>PRO</td>
<td>201.2 (3.3)</td>
<td><strong>176.7 (1.3)</strong> *</td>
<td><strong>163.5 (4.3)</strong> *</td>
<td><strong>160.1 (5.1)</strong> *</td>
</tr>
<tr>
<td>SER</td>
<td>105.6 (4.0)</td>
<td>275.9 (77.5)</td>
<td><strong>193.5 (21.9)</strong> *</td>
<td><strong>258.2 (56.6)</strong> *</td>
</tr>
<tr>
<td>TAU</td>
<td>49.2 (2.0)</td>
<td><strong>128.8 (25.6)</strong> *</td>
<td><strong>126.2 (9.8)</strong> *</td>
<td><strong>85.3 (16.4)</strong> *</td>
</tr>
<tr>
<td>THR</td>
<td>91.8 (6.2)</td>
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<td><strong>236.5 (41.0)</strong> *</td>
<td><strong>236.2 (50.2)</strong> *</td>
</tr>
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<td>TRP</td>
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<td>66.5 (11.0)</td>
<td>66.9 (12.8)</td>
<td>71.8 (13.9)</td>
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<td>TYR</td>
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<td>101.9 (19.2)</td>
<td>60.7 (8.2)</td>
<td>57.8 (9.6)</td>
</tr>
<tr>
<td>VAL</td>
<td>162.1 (9.5)</td>
<td><strong>245.7 (31.5)</strong> *</td>
<td>230.2 (40.4)</td>
<td>233.4 (31.1)</td>
</tr>
<tr>
<td>β-ALA</td>
<td>98.1 (1.4)</td>
<td>28.8 (9.6)</td>
<td>11.7 (2.2)</td>
<td>14.2 (2.7)</td>
</tr>
</tbody>
</table>

Off, PN-independent; On, PN-dependent; On-Off, supported on PN at the time of measurement and subsequently came off PN.

Mean (SEM). Values are expressed as μmol/L.

**Amino acid**; ALA, alanine; ARG, arginine; GLN, glutamine; GLU, glucose; GLY, glycine; HIS, histidine; ILE, isoleucine; LEU, leucine; LYS, lysine; MET, methionine; ORN, ornithine; PHE, phenylalanine; PRO, proline; SER, serine; TAU, taurine; THR, threonine; TRP, tryptophan; TYR, tyrosine; VAL, valine.

Values significantly different from controls are shown in bold font.

*P < 0.05.

**P < 0.01 compared with controls.

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**Statistics**

Data were expressed as means ± SEM. Means and standard errors were computed for each amino acid and TPN group. The groups were compared via two-sided t-tests. Linear regressions were performed with serum CIT as the response and percent of enteral calories or bowel length as a predictor.

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**RESULTS**

**Citrulline Levels in Normal Infants and Children**

Twenty-one children had blood drawn in the hospital for clinical chemistry laboratory tests. Mean serum CIT level was 31.2 ± 1.6 μmol/L. For comparison, a normal value for serum CIT in normal adults is 40.0 ± 1.4 μmol/L.8

**CIT Levels in SBS Patients**

Clinical information for the 24 infants (20 from University of North Carolina and 4 from Ochsner Clinic) with SBS entered in the study is provided in Table I. The infants were ≤48 months of age; most were <1 year of age. The most common diagnosis was necrotizing enterocolitis (NEC); other diagnoses included small intestinal atresia(s), volvulus, and Hirschsprung’s disease. Six infants had already been weaned off PN. Ten infants with SBS came off PN during the follow-up period for this 2-year study. Ten remained PN-dependent. Clinical follow-up ranged from 3 to 25 months. Four infants died from complications of cholestasis and sepsis.

Comparing all the amino acids in the sera of control subjects and those with SBS, significant differences were found in serum level of many amino acids (Table II and Figure 1). Comparing infants with SBS with normal control infants, we observed a lower level of CIT and PRO in the infants with SBS (P < .01). To rule out the possibility that the CIT level was lower in infants with SBS because some of them might have been younger than controls (although the control group was age-matched), we reviewed data from our previous prospective cohort study of premature infant amino acid levels.9 In that study, we found that in normal, uninfected premature infants with no evidence of NEC, the serum CIT level at 28 days of life was 36 ± 8 μmol/L (n = 5). Mean postconceptual age of these infants was 31 weeks (at birth).

In the current study, there were significant differences compared with control infants in these infants remaining on PN in the levels of alanine (ALA), glutamate (GLU), glycine (GLY), leucine (LEU), lysine (LYS), ornithine (ORN), phenylalanine (PHE), serine (SER), ARG, taurine (TAU), and threonine (THR). However, in contrast to CIT and PRO, the level of each of these latter amino acids was significantly higher than in controls. Differences comparing the groups of...
infants with SBS who had come off PN or who came off during the study with controls were similar but less significant. For CIT there was a stepwise increase in level comparing SBS infants who were on PN, infants who were “on, then off” PN during the 1-year study period, and infants who were already weaned off PN (Figure 1). Infants who were on PN continuously, as well as those who were on PN at the time of CIT measurement and later came off PN, had a significantly lower level of CIT compared with those who had been weaned off PN ($P < .01$).

CIT level significantly increased in a linear relationship with the percentage of enteral calories tolerated ($R = 0.85; P < .001$) (Figure 2). All children who had come off PN when the measurement was made had CIT values ≥22 μmol/L (5 of these were off PN at initial determination) (Figure 1). All 13 infants with ≥70% enteral tolerance had CIT levels ≥16 μmol/L. Conversely, 5 of 6 infants with <30% enteral tolerance had CIT levels <17 μmol/L, and 4 of these infants had CIT levels ≤14 μmol/L.

When we included all values of CIT for the 19 infants with a single measurement, a CIT > 19 μmol/L had a 94% sensitivity (15/16) for coming off PN, and a CIT <19 μmol/L had a 67% specificity for staying on PN (8/12).

Bowel length measured at the time of surgery had a significant linear relationship with serum CIT level ($P < .03; R = 0.47$) (Figure 3). The serum CIT level in 6 of 7 infants with bowel length <50 cm was <16 μmol/L, was 6 to 21 μmol/L in the 4 infants with bowel length from 50 to 80 cm, and was ≥19 μmol/L in 5 infants with bowel length >80 cm. The greatest discrepancy between bowel length and CIT was for an infant with 24 cm of remaining bowel who showed marked bowel adaptation and came off PN at 23 months of age. Her CIT level, determined while she was still on PN, was surprisingly high at 26 μmol/L.

Infants with Sequential Determinations

For 5 infants, CIT was measured twice. Three infants came off PN during the 12-month investigation. One infant had an initial value of 6 μmol/L at 2 weeks of age, during the postoperative period for NEC, and a subsequent value of ≥33 μmol/L 6 months later, just after she had been weaned off PN. The second infant had a serum CIT level of 20 μmol/L while taking PN and 26 μmol/L, 6 months later, after coming off PN. The third had an initial serum CIT level of 16 μmol/L at 9 months of age and a follow-up value of ≥23 μmol/L 3 months later; 4 months later he came off PN. One infant with very short bowel, 17 cm in total, who had two determinations 3 months apart and made little progress in feeding showed very low CIT (3 and 7 μmol/L). This patient never came off PN and eventually died while waiting for an intestine-liver transplant.

A fifth infant had multiple jejunal atresias. During an episode of sepsis, she became hypotensive and lost an additional segment of bowel 3 months after her initial CIT determination. When her bowel measured 34 cm in length, the CIT level was 12 μmol/L; after her second surgery, when her bowel was 15 cm long, the CIT level had decreased to 6 μmol/L. Thus, in each infant with sequential determinations, the serum level paralleled the infant’s clinical progress.

**DISCUSSION**

Our study determined whether CIT is a useful serum marker reflecting absorptive bowel mass in children with SBS.
It is not entirely clear if our data reflect a linear or hyperbolic relationship between CIT and length of bowel because there may be a relative plateau in serum CIT in children with bowel length >100 cm, or about 40% of normal bowel length in newborn infants. We had a paucity of data for infants with “mild short bowel syndrome” because we only enrolled 3 patients with bowel length in the 100 to 200 cm range. Our data indicate a better correlation of serum CIT with enteral “tolerance,” an indirect measure of calorie absorption, than with measured bowel length.

Previous investigations by Crenn et al in adults with SBS have demonstrated a correlation between CIT level and bowel length and calorie absorption. The same group demonstrated a correlation between the severity of mucosal villus atrophy and serum CIT. Celiac patients with mucosal recovery on a gluten-free diet had normal CIT levels. This study also showed that patients with anorexia nervosa had normal CIT levels, ruling against malnutrition as a cause for low CIT level. Wasa et al also showed a significant (>50%) reduction in CIT level in infants and adults with SBS. Interestingly, ARG level also was reduced in these patients. Collectively, these investigations support the concept that serum CIT is low in infants with SBS because of reduced gut mass. However, an alternative explanation is that children with SBS have reduced substrate(s) for intestinal CIT synthesis. Levels of PRO, a substrate for CIT synthesis, were reduced in all three groups of infants with SBS. Either PRO utilization is increased in the extrahepatic tissues of patients with SBS or their dietary PRO intake is reduced. The former seems more likely.

A low concentration of circulating GLN could limit substrate availability for CIT biosynthesis. Although we did not directly quantify GLN intake, most of the infants were receiving the same formula, which contains GLN, and our PN solutions contain no GLN; one may assume that the GLN intake was proportional to percent enteral calories. Thus, low GLN intake could correlate with low CIT synthesis in the bowel. However, we do not have evidence suggesting that serum GLN is limiting the synthesis of CIT in patients with SBS. Chen et al determined the impact of GLN-supplemented PN on intestinal production of CIT in rats. They found that intravenous GLN did not increase CIT production in rats with SBS.

We found a significant linear relationship between percent of calories tolerated enterally and serum CIT. This relationship suggests that our clinical protocol for advancing feeding volume to a maximal level based on stool number and consistency results in a feeding volume that reflects maximal absorption. However, significant exceptions, such as an infant on 40% calories who had a serum CIT = 20 μmol/L, suggest complex mechanisms for the regulation of intestinal CIT synthesis and plasma CIT homeostasis in infants. Our data may have been more precise if we had quantified the actual number of calories absorbed. Such studies would require quantitative fecal collection.

Serum CIT level also may be a useful marker for intestinal injury, as well as for intestinal mass. Pappas et al showed that serum CIT is a useful marker of acute cellular rejection in patients with intestinal transplantation. In individual patients, serum CIT increased progressively during each 2-week interval after transplantation (from 12.5 to 28.2 μmol/L). Thus, the mean serum CIT in patients recently receiving transplants without rejection was ~30 μmol/L, whereas mean serum CIT was progressively less at each level of rejection. The lowest levels were found in patients with grade 4 rejection. Serum CIT as a possible marker of intestinal disease is also supported by data from our previously published prospective cohort study of premature infants, some of whom developed NEC. We found that CIT level was normal at the onset of NEC and dropped below normal subsequently. For several of the other amino acids, particularly GLN and ARG, levels were lower than normal beginning 1 week before the onset of NEC. This finding suggested to us that CIT was a marker of intestinal “health” as well as mass in neonates.

In the current study, we also found elevated serum levels of GLU, GLY, LEU, LYS, ORN, PHE, PRO, SER, ARG, TAU, and THR in the infants with SBS, particularly in the group remaining PN-dependent throughout the study. It is now recognized that the small intestine plays an important role in catabolizing both essential and nonessential amino acids. Thus, the higher serum levels of these amino acids in the infants with short gut may result from a decrease in their intestinal degradation. For example, plasma levels of multiple amino acids are elevated in neonatal pigs maintained on TPN solution compared with enteral feeding, likely resulting from reduced amino acid catabolism by the gut. Additionally, when serum amino acid levels are plotted as a function of age in normal infants and children, many are 50% to 200% higher in the first 6 months of life than later during years 2 to 8 of life, including six of the amino acids that were elevated in our infants with SBS: ARG, methionine (MET), ORN, PHE, THR, and ARG. Given the magnitude of developmental changes in intestinal absorption and digestive enzyme activity that have been measured early in life, decreased catabolism would seem a reasonable explanation for the increase in certain serum amino acid levels in infants with SBS.

Many amino acids (GLU, GLY, LEU, LYS, ORN, PHE, PRO, SER, TAU, and THR) are quite stable in plasma, making breakdown during serum handling an unlikely explanation for lower levels in the PN-fed children. Intestinal CIT synthesis is highly compartmentalized. Thus, because ORN was not diminished in concentration, we do not believe that CIT synthesis was limited by serum concentration of ORN. In support of this view, dietary supplementation of ORN is not effective to increase plasma CIT concentrations in humans and animals.

Shortcomings of our study were that it was cross-sectional in nature, with a relatively small number of patients. Additionally, we did not define the optimal timing of assessment of serum CIT. For example, one infant had serum CIT level of 6 μmol/L at 2 weeks of age, in the immediate postoperative period, and subsequently 33 μmol/L 6 months later. More frequent blood sampling may provide more precise information on changes in serum CIT. Data in 3 of our
patients with rising CIT levels during adaptation suggest that sequential determination may be more helpful than cross-sectional determination to ascertain if bowel adaptation is taking place.

Despite these limitations, the finding that all infants who had discontinued PN had serum CIT levels >19 μmol/L may be of clinical importance. The test is routinely available in all tertiary pediatric referral centers to allow the diagnosis and follow the treatment of amino acidopathies in children. Given that a serum CIT ≥19 μmol/L had a 93% sensitivity for predicting PN-independence, we suggest that a serum CIT level, with longitudinal determination, warrants further testing in larger prospective studies of infants with SBS as a measure of bowel adaptation.

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CREATINE KINASE MB (CK-MB) IN BENIGN PAROXYSMAL VERTIGO OF CHILDHOOD: A NEW DIAGNOSTIC MARKER

PEO RÖDOÖ, MD, AND DAN HELBERG, MD, PHD

Objectives To evaluate the relation between creatine kinase-MB (CK-MB) and benign paroxysmal vertigo in childhood (BPV).

Study design We prospectively evaluated and followed serum CK-MB in 22 children with BPV diagnosed between 1998 and 2003.

Results The average age of debut for BPV was 1.7 years, and follow-up time was 2.8 years. The CK-MB values were elevated in all children. CK-MB values were persistently increased (mean, 6.0 μg/L) during the study period and were not related to duration of BPV, time since last attack, or frequency of attacks. CK-MB became normal in 7 children who recovered during the study period. After the initial increased CK-MB value, CK, aspartate aminotransferase, and cardiac troponin I (in 16 children) were measured as markers of muscular disease. CK was slightly increased in 7 (31.8%) and aspartate aminotransferase in 14 (63.6%) of the children. Cardiac troponin I was normal in all children.

Conclusions In this study, serum CK-MB levels were associated with BPV. These findings indicate a possible muscular involvement in BPV. Further studies will be needed to determine if CK-MB is useful as a diagnostic test for BPV. (J Pediatr 2005;146:548-51)

METHODS

From December 1998 to March 2003, 22 children were diagnosed with BPV at the Department of Pediatrics, Falun Hospital. All children were referred to and followed up by

| BPV | Benign paroxysmal vertigo |
| CK  | Creatine kinase |
| cTnI | Cardiac troponin I |
CK-MB was also analyzed in 198 children attending the Department of Pediatrics, mainly at the Emergency Room, with other diagnoses. The responsible physician ordered the analysis, and priority was given to children with conditions in which CK-MB could be expected to be increased, such as myocarditis/myositis, other infections, suspected heart problems, and in two cases of β-oxidation defects and one case with a burn injury.

Distributions and comparisons were calculated on the JMP 3.1 statistical program (SAS Institute, Cary, NC). For crude comparisons of continuous variables, t tests and F tests were used as appropriate. Powerpoint (Microsoft) was used for graphics. The study was approved by the Ethics Committee, Medical Faculty of Uppsala University. Informed consent was obtained from the parents.

RESULTS

Of the 22 children who were diagnosed with BPV from December 1998 to March 2003, 15 (68.2%) were girls and 7 (31.8%) were boys. Average age when BPV was diagnosed was 2.1 (SD, 0.8) years, and history of debut age was 1.7 years (SD, 0.9). No child had an onset after 4 years of age. Average follow-up time was 2.8 years (SD, 1.4; range, 12 months to 5 years). The number of episodes per month at peak was 1 in 4 children, 2 in 5 children, 3 to 19 in 6 children, and more than 24 episodes per month in 7 children. There were only small differences by history of onset and month or season.

The first 4, and in case of more observations also the latest, CK-MB values are given in the Table, based on 85 observations. Nineteen (86.4%) of the 22 children in the study population had an increased CK-MB above or equal to 3 μg/L when BPV was first diagnosed clinically. The remaining 3 children had an increased CK-MB value within some weeks.

There was a tendency (P = .04) for lower values by duration since the last BPV attack (Figure). The mean CK-MB value was 8.4 μg/L when sampled during the attack but was still 5.0 μg/L when more than 90 days had elapsed since latest attack. There was no correlation between the first serum CK-MB value and time elapsed since history of onset of BPV. Five children were observed with CK-MB every third hour for 24 hours. There were only small and nonsignificant differences
DISCUSSION

There are no known markers in serum for the diagnosis of BPV. The elevation of CK-MB levels in children with BPV was first discovered by chance in the first patient in this series. This finding led to the current study. Our results show that CK-MB was increased during BPV and in a large proportion of patients at each observation. In no case was CK-MB normal when the measure was repeated at the first two observations after diagnosis of BPV. A prospective, population-based study would be required to determine sensitivity and specificity. The CK-MB increase appeared to be reproducible when the measure was repeated and followed over time. There was no variation by 24-hour measurements or by season of debut. Finally, CK-MB decreased to normal when the children recovered from BPV.

BPV is probably a common but misunderstood and unrecognized condition in small children.1-2,4-11 In our experience, awareness of the condition has increased our detection rate of BPV during the past 6 years. The diagnostic criteria have been purely clinical, and BPV has commonly been a diagnosis of exclusion. This has necessitated expensive and time-consuming investigations for the inexperienced clinician.

The investigations of children with BPV have uniformly showed a normal neurologic and laryngeo-otologic status and no positional vertigo in a healthy child. Audiography, electroencephalography, radiology, and CT have invariably been normal. Spontaneous nystagmus has sometimes been observed but is dependent on observation of the child during an attack of vertigo. BPV was thought to be caused by a destructive process or vestibular neuronitis central to the cochlea and labyrinth but peripheral to the brain stem and cerebellum.2,5 However, caloric tests have in general been normal.7-9

The mechanism for increased CK-MB in BPV remains unknown. We have shown that cTnI levels are normal in BPV, thus excluding a cardiac origin of the CK-MB increase. In this study population, a moderate increase of CK and AST were seen in many cases, indicating a muscular origin of CK-MB. Clinically, BPV is not associated with muscular involvement between attacks, in contrast to the finding of an increased CK-MB value in inflammatory or hypoxic muscular diseases. Twitching and muscle tension during BPV attacks are mild and not comparable with those of epileptic convulsions. No previously known condition is characterized by a permanent CK-MB elevation during the course of the disease or in symptom-free periods.

Epilepsy remains the major differential diagnosis for BPV. In this study, all 9 patients with established epilepsy had normal CK-MB values except for one 6-month old girl, whose primary diagnosis was atypical hypsarrhythmia, and 2 children with suspected epilepsy but with normal electroencephalograms, who had elevated levels. Another study found an increase in CK-MB during status epilepticus in 2 patients, which was probably caused by strong muscular activity.12,13

The recurrent episodes without impaired consciousness and with no signs or symptoms between BPV attacks has also raised speculations whether BPV is a migraine precursor or equivalent.8-10,11 There is sometimes also a family history of migraine. CK-MB has never been measured in patients with migraine, although there were 4 children with migraines who had normal CK-MB in our comparison group.

The relatively large number of cases of BPV presented here is probably not due to increased frequency of BPV in our area but to our awareness of the condition. Much will be gained if there was a simple diagnostic tool for BPV. False diagnoses and costly examinations could be avoided.

The results of our study suggest that serum CK-MB might be used as a marker of BPV. It is also possible that this may be a clue to help establish the cause of BPV. Future studies will be needed to evaluate these possibilities. An initial step must be to establish the origin of CK-MB found in these patients.

The authors express gratitude to Professor Orvar Eeg-Olofsson for reviewing the patient records.

REFERENCES


In the article by Jacobziner, accidents were identified as the leading cause of deaths from ages 1 to 34. This was appropriately noted as a major health issue since the incidence of other causes of death in children and young people were dropping precipitously, while accident deaths were increasing. Unfortunately, this trend has continued over the last 50 years; mortality rates from medical causes has declined, but in mortality and morbidity from accidental injuries has increased.

It is interesting to note how causes of injury deaths in children from birth through age 6 years have changed compared with the causes listed in this article. Using current data from the Centers for Disease Control (CDC) WISQARS web site, striking differences are noted from the data presented in Jacobziner’s paper. He found falls to be the most significant cause of injury deaths, followed by poisoning, suffocation, and burns. Recent CDC data puts falls behind motor vehicle accidents, suffocations, drowning, and burns. One wonders if the excessive number of deaths from falls noted in the paper from 1954 included many children who died from unrecognized abuse. Of course, Jacobziner’s data were collected in one large city, whereas the CDC data are nationwide and use different methods to obtain data and categorize death.

Jacobziner notes suffocation as the leading cause of injury deaths in children younger than 1 year of age. He discounts this, however, by suggesting that sudden infant death is most likely caused by “undiscovered illness.” The recent CDC data, however, rank unintentional suffocation at the top of the modern list of causes of injury deaths, emphasizing the need for further parent education on “safe sleep” environments and choking hazards. The data on nonfatal injuries is much more in keeping with our current experience. Jacobziner found falls to be the most common nonfatal injury, which is what we find currently as well.

In some ways, this article suggests a current approach to improving the lives of children. Jacobziner’s analysis is similar to the output of many state and regional fatality review groups convened to study the epidemiology of child deaths and to promote injury prevention.

The author’s “solution” to the problem of injuries is as relevant today as it was 50 years ago. The study of accidents, the assessment of prevention strategies, and the importance of physicians in educating families continue to be important avenues to promote prevention.
FINANCIAL COMPENSATION TO ADOLESCENTS FOR PARTICIPATION IN BIOMEDICAL RESEARCH: ADOLESCENT AND PARENT PERSPECTIVES IN SEVEN STUDIES

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Objective  To examine the impact of financial compensation on pediatric asthma research participation decision-making and determine whether perceptions of fair compensation differed for parents and adolescents, lower and higher income participants, and compensation-informed and uninformed participants in minimal and above minimal risk research.

Study design  Adolescents (n = 36) with asthma and their parents reviewed 7 pediatric asthma research protocols, decided whether they would choose to participate, and provided estimates of “fair” compensation for their participation. Chi-square, analysis of variance, and analysis of covariance were used to determine the affects of compensation on participation and whether various respondents differed in the perceptions of fair compensation.

Results  Financial compensation did not affect participation decisions. Estimates of fair compensation were lower for adolescents, lower income respondents, and participants who were naïve about potential compensation. Fair compensation estimates were higher than actual compensation for minimal risk studies and lower for above minimal risk studies.

Conclusions  Financial compensation may be a minor consideration in pediatric research participation decision-making. Still, differences in how pediatric researchers and their prospective participants judge fair compensation create the potential for undue influence. Pediatric researchers should use caution when determining a reasonable financial compensation for research participation. (J Pediatr 2005;146:552-8)

Research ethicists have questioned the influence of financial compensation for research volunteers. Some have contemplated whether people with fewer economic resources may be exploited, whether financial compensation may lead prospective participants to overlook potential risks in favor of financial gain, and whether financial compensation undermines altruistic motives. Others assert that research participation imposes costs on participants, that monetary payments are offers that expand options, not threats that coerce, and that research participation is analogous to unskilled labor, consequently compensation should approximate minimum wage.

Financial compensation to children and adolescents for research participation is even more problematic. Children and adolescents may be more susceptible to the allure of any monetary gain for research participation, and even minimum wage-based payments may result in large sums of money being offered to children and adolescents for compensation. Small amounts of money may be sufficient to induce children and adolescents to overlook research risks, and the promise of monetary gain may deter children and adolescents from withdrawing from participation when they believe they are compelled to continue to receive their “pay.” Finally, child and adolescent research participation requires surrogate decision-making by parents, which may lead to conflict and undue influence to assent or consent when parents and children or adolescents disagree about research participation.

Although researchers doubt that financial compensation is a compelling factor in research participation decision-making, this has not been empirically tested. In this report, we present findings from a larger study of adolescent assent, in which we examined the

TSDS  Transformed standardized difference scores

From the College of Education, University of New Mexico, Albuquerque, New Mexico; the Center for Family and Adolescent Research, Oregon Research Institute, Eugene, Oregon; University of New Mexico Health Sciences Center, Albuquerque, New Mexico; the University of New Mexico, Albuquerque, New Mexico; the Medical College of Wisconsin, Milwaukee, Wisconsin; and Informatix Laboratories Corporation.

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perspectives of adolescents with asthma and their parents about what constitutes “fair” compensation for participation in a variety of pediatric asthma research studies. We hypothesized that knowing financial compensation will be provided would affect participation decisions and that people who would choose to participate may seek more or less compensation than people who would not. We also hypothesized that adolescents would deem fair compensation to be less than parents, that low-income families would estimate fair compensation to be less than higher income families, that respondents naïve about research compensation would provide estimates of fair compensation less than respondents who were told what to expect, and that estimates of fair compensation in above minimal risk studies would be higher than in minimal risk studies.

METHODS

This study, including its assent and consent procedures, was reviewed and approved by the University of New Mexico Health Sciences Center Human Research Review Committee. Child and parent participants were recruited from a children’s hospital pediatric pulmonary outpatient clinic that serves as the statewide referral center for children with asthma. For those agreeing to participate, a separate appointment was made to conduct the research at an office located outside the medical clinic. Two families indicating early interest later declined to participate in the study.

Development of Stimulus Materials

Nine pediatric asthma protocols were selected from a sample of 37 consent forms obtained from pediatric asthma researchers in the United States and England and used in publicly and privately funded studies conducted during the 1990s and 2000. Studies were identified via a Medline literature review, and consent forms were requested from those for whom contact information was available and by contacting prominent asthma researchers known to the authors. Specific attempts were made to obtain consent forms from studies that involved varied designs and procedural elements.

A representative sample of the protocols for this study was selected by a panel of 8 physicians, clinical pharmacists, and psychologists recognized as having expertise in ethics, pediatric asthma research, or both. The panel unanimously rated 5 of the 9 studies as minimal-risk research and 4 as above minimal risk. Although institutional review boards vary in how minimal and above minimal risk is defined, the value of our panel’s distinctions lies in its having distinguished between those studies that involve higher risk from those that involve less risk. Similarly, both parent and adolescent respondents rated the minimal risk studies as having less risk than the above minimal risk studies. Data were collected from all 9 studies, but 2 studies were dropped from the analyses reported here because they lacked any financial compensation. The remaining studies involved the use of typical asthma research procedures, including medication trials comparing standard medications with a placebo control (Table 1).

Vignettes were developed from each of the selected protocols and were presented to adolescents and parents in a standardized written format. An informative study title was followed by a brief statement of the reason for the study, study procedures in bullet format, and a description of study incentives. Study procedures were described in detail in a separate part of our study and included explanation of the potential risks involved in undergoing a procedure. Medication trials included a description of the medications and any known risks or adverse effects. Participation requirements were summarized after a presentation of all information in the research vignette. The vignettes remained faithful to the original research protocol, although information provided was formatted differently, and in some cases, procedure descriptions were more detailed than in the original consent form.

Measures

Measures included a 15-item Demographic Questionnaire and a 33-item Asthma History Questionnaire developed from the Guidelines for the Diagnosis and Management of Asthma and included items pertaining to current asthma medications, experiences with various asthma-related procedures, and prior participation in asthma research. The Asthma Research Procedures Questionnaire assessed the participant’s evaluation of the risks and benefits associated with 11 asthma research procedures, the results of which are presented elsewhere. A 12-item Asthma Vignette Questionnaire evaluated participant responses to each of the 7 study vignettes. Ten Likert scaled questions assessed participants’ perceptions of study risks, benefits, discomforts, and burdens, their willingness to participate, and to what extent a parent or child’s opinion would influence their decision. One yes/no question asked participants to decide whether they would choose to participate in the study. Finally, each participant indicated what they believed to be fair compensation for participating in the hypothetical study (“In your opinion, what amount of money is fair compensation for a person participating in this study?”). This paper reports on the subset of this data about participants’ estimates of fair compensation.

Procedures

Adolescents and parents met together with a research assistant to review and sign informed assent and consent documents. Parents completed the demographic form, and the parent and adolescent together completed the Asthma History Questionnaire. Parents and adolescents were then separated and presented with the study vignettes. Presentation orders of the vignettes were altered with a standard Latin square design. Half of the participants were told the actual amount of money offered for participation in each study (“told” condition); the other half were informed only that they would be “fairly compensated” for their time, effort, and expenses incurred (“not told” condition). After hearing each vignette, responses were obtained for the Asthma Vignette Questionnaire. After completing the study, each parent and adolescent participant received $25.
Chi-square analyses were used to determine whether participants who were told the amount of financial compensation would choose to participate more or less often than those who were kept naïve. The alpha level was set at .007 via a Bonferoni adjustment to minimize the potential for type I error. Analysis of variance was used to determine whether respondents who would decline participation in a study estimated fair compensation differently than those who indicated they would participate. Again, the alpha level was adjusted to minimize the potential for type I error.

Several steps were required to transform the estimates of fair compensation into a dependent variable that could be used to examine the differences among study participants and across the 7 research protocols. For each participant, a difference score was calculated, subtracting the actual amount of compensation from the expected amount of compensation (Table II). To ensure consistent comparisons across vignettes, standardized difference scores were calculated by dividing respondents’ difference scores for each vignette by the SD for difference scores associated with that vignette. These standardized difference scores were then weighted by 2 and transformed into base 10 logarithms to correct distorting skew in the data caused by statistical outlying responses, yielding transformed standardized difference scores (TSDS) used as the dependent variable in subsequent analyses.

A mixed effect analysis of covariance with an alpha level of .05 was used to evaluate our 4 independent hypotheses. Interaction effects were included in our statistical model to control for any statistical influence they may have on main effects, but in the absence of hypotheses on interaction effects, we did not use these tests of significance.

### Table I. Summary description of research protocols and procedures

<table>
<thead>
<tr>
<th>Protocol descriptions</th>
<th>Required procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Minimal-risk studies</strong></td>
<td></td>
</tr>
<tr>
<td>Can some medicine make it harder to learn?</td>
<td>Random assignment to 1 of 2 “over-the-counter” medicines (diphenhydramine or loratadine) or placebo; allergy skin testing; psychological testing</td>
</tr>
<tr>
<td>Three weekends of school-like participation in 1 month to evaluate impact of medication on learning ability</td>
<td></td>
</tr>
<tr>
<td>What are the characteristics of adolescents with mild to severe asthma?</td>
<td>Eight clinic visits including physical examination, spirometry, and asthma symptom/treatment questionnaires at each visit; 1 blood test</td>
</tr>
<tr>
<td>Three years participation to evaluate the characteristics of adolescents with mild to severe asthma</td>
<td></td>
</tr>
<tr>
<td>Can a HRCT help in studying asthma?</td>
<td>Spirometry test; HRCT x-ray</td>
</tr>
<tr>
<td>One day participation to examine usefulness of HRCT in studying asthma</td>
<td></td>
</tr>
<tr>
<td>How much cortisol and nitric oxide do I have in my body?</td>
<td>Twenty-four hour urine collection; 1 spirometry test; 3 peak flow measures; nitric oxide levels measured every 4 hours</td>
</tr>
<tr>
<td>Thirty-six hour hospital observation to evaluate levels of cortisol and nitric oxide</td>
<td></td>
</tr>
</tbody>
</table>

### Above minimal risk studies

<table>
<thead>
<tr>
<th>Protocol descriptions</th>
<th>Required procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Which of these medicines works better?</td>
<td>Medication change every 4 weeks; 3 overnight hospital stays; 8 spirometry tests; 8 Nitric Oxide measures; 6 —24hr urine collections</td>
</tr>
<tr>
<td>Fifteen-week clinical trial comparing 2 FDA-approved medications with a double-blind, double-dummy placebo crossover design</td>
<td></td>
</tr>
<tr>
<td>How often should asthma medicine be taken?</td>
<td>Random assignment to treatment; medical history; 12 physical examinations; 12 spirometry tests; 7 methacholine challenge tests; 1 allergy skin test; 1 electrocardiogram; 1 quality of life questionnaire; 7 urine pregnancy tests</td>
</tr>
<tr>
<td>Twenty-six week trial to examine fixed versus as-needed dosing schedule for established asthma medication</td>
<td></td>
</tr>
<tr>
<td>How effective are these treatments for asthma over time?</td>
<td>Random assignment to treatment; 6 comprehensive physical examinations; 2 allergy skin tests; 6 Tanner Staging examinations; 6 spirometry tests; 2 blood draws; 5 psychological tests; 6 methacholine challenge tests; 2 neuropsychological tests; 6 bone density measurements</td>
</tr>
<tr>
<td>Five-year clinical trial comparing 2 investigational medications versus placebo</td>
<td></td>
</tr>
</tbody>
</table>

HRCT=High Resolution Computer Tomography; FDA=US Food and Drug Administration.
RESULTS

Participants

Participants in this study included 36 adolescents with asthma and their parents or guardians; 68 (94%) of the adolescents’ parents or guardians were parents, the remainder were identified as grandparents. Most parents were mothers (65%); the remainder were identified as fathers. The 4 grandparents were equally split between men and women. The mean age of parents was 43.2 years (SD, 7.04; range, 30-60 years). Twenty-two (61%) of the 36 adolescents were male. The adolescent mean age was 13.2 years (SD, 1.75; range, 11-17 years). The ethnic constitution of our sample mirrored the population in the Southwest United States and consisted of whites (43.4%), Hispanics (40.3%), Blacks (2.8%), Asians (5.6%), and other/mixed ethnicity (8.3%). One adolescent participant responded to only 2 of the 7 protocols and was dropped from subsequent analyses. One parent participant declined to provide estimates of fair compensation and consequently was dropped from the subsequent analyses. Two adolescents provided 3 facetious estimates of fair compensation ($20,000, $25,000, and $30,000) for 2 of the above minimal risk studies. These 3 responses were also dropped from further analyses.

Participation Analyses

Our chi-square analyses revealed that a difference in willingness to participate on the basis of knowing the compensation amount existed in only 1 vignette (the minimal risk, Can some medicines make it harder to learn? vignette; χ² = 10.3; P = .002). In this vignette, respondents who were not told the financial compensation were more likely to indicate they would decline participation in this study, whereas the participants who were told what to expect were more inclined to participate. There were no significant findings for the analysis of variance that tested whether those who declined to participate estimated fair compensation differently than those inclined to participate.

Financial Compensation Analysis

Because respondents with prior research experience might have a better ability to estimate actual compensation than those with no prior experience, we examined the effect of previous research experience on the TSDS. Respondents were split into dichotomous groups, those with and those without previous research experience (66.7% of adolescents and 61.1% of parents had no previous experience). Adolescents with previous research experience expected more compensation for their participation (P <.045). Because an adolescent’s prior research experience had a significant effect on standardized compensation scores, we opted to include this variable as a covariate in all subsequent analyses (Table III).

Adolescents in our sample designated fair compensation values (mean TSDS, .236; SE, .016) that were significantly lower than those designated by parents (mean TSDS, .301; SE, .015). Families with an annual income of $40,000 or less per year also provided estimates of fair compensation (mean TSDS, .227; SE, .019) that were significantly less than families with an annual income >$40,000 per year (mean TSDS, .318; SE, .019). Respondents who were not told what they would receive for financial compensation estimated fair compensation (mean TSDS, .331; SE, .020) significantly lower than those respondents who were told what to expect (mean TSDS, .214; SE, .020). Within-subject analyses revealed that, contrary to our hypothesis, the TSDS for minimal-risk studies (mean TSDS, .310; SE, .015) were greater than the TSDS for above-minimal risk studies (mean TSDS, .236; SE, .016). That is, estimates of fair compensation exceeded the actual compensation more often in minimal-risk studies than in above minimal risk studies.

Table II. Means and standard deviations for difference between actual compensation and estimates of “fair” compensation in dollars

<table>
<thead>
<tr>
<th>Vignette title (actual compensation)</th>
<th>Fair compensation in dollars minus actual compensation in dollars (Means/SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adolescents</td>
</tr>
<tr>
<td>Minimal risk studies</td>
<td></td>
</tr>
<tr>
<td>Learn ($550)</td>
<td>–205/224.9</td>
</tr>
<tr>
<td>Characteristics ($105)</td>
<td>116/229.8</td>
</tr>
<tr>
<td>HRCT ($35)</td>
<td>6/29.3</td>
</tr>
<tr>
<td>Cortisol and NO ($100)</td>
<td>11/63.0</td>
</tr>
<tr>
<td>Above minimal risk studies</td>
<td></td>
</tr>
<tr>
<td>Medicines ($300)</td>
<td>–23/205.7</td>
</tr>
<tr>
<td>How often ($1000)</td>
<td>–453/415.7</td>
</tr>
<tr>
<td>How effective ($400)</td>
<td>502/1723.8</td>
</tr>
</tbody>
</table>

NO = nitric oxide.
United States and Canadian regulations reflect concern about undue influence, but permit financial compensation for participation in research studies. In contrast, the European Union prohibits providing children and adolescents with financial compensation for research participation. Practices on financial compensation for pediatric asthma research differ considerably. The 37 consent forms reviewed for this study used no compensation (7-15 studies), a small gift certificate (1 study), or financial compensation ranging from $20 to $2400 (21 studies). Most of the consent forms solicited for this study did not indicate the time commitment required for completion of the study, making it difficult to calculate financial compensation on an hourly basis. However, interpolating from the studies that did offer an expectation of time involvement, we calculated “best-guess” estimates of hourly compensation and found widely varied hourly rates of compensation (all studies: mean, $32.05/hour; range, $2.65-$100.00/hour; 7 studies used in these analyses: mean, $18.76/hour; range, $2.78-$50.00/hour).

Financial compensation is only one consideration among a complex variety of factors that adolescents and their parents use to decide about research participation. For example, human immunodeficiency virus-positive and -negative adolescent research participants revealed that financial compensation was a relatively minor factor in their decisions to participate in research, compared with the importance of their relationship with research personnel. This may also be true in most studies on pediatric asthma. In only 1 study of the 7 we analyzed did knowing the amount of financial compensation make a difference in choosing to participate. Nor did we detect any differences in the estimates of fair compensation between participants who would enroll in a study versus those who would not. Our study was not designed to detect the extent to which financial compensation may constrain autonomous decision-making, but it appears that the actual compensation in our selected studies was not perceived to be irresistible or coercive in nature. However, this issue continues to warrant special attention for studies that offer large sums of financial compensation. Our results suggest that when concerns exist that financial compensation may unduly influence participation decisions, pediatric researchers could opt to inform potential participants that financial compensation will be provided, but choose to not indicate the exact amount, without discouraging enrollment.

The pediatric asthma studies we sampled revealed important differences in what prospective participants estimate to be fair compensation. Adolescents’ estimates were significantly less than those of parents. Lower income respondents estimated fair compensation as significantly lower than respondents from higher incomes families. The compensation-naive participants in our study indicated fair compensation values that were significantly lower than the amounts stated by participants who were told what to expect.

**DISCUSSION**

**Table III. Transformed standardized difference scores within and between subjects using analysis of covariance**

<table>
<thead>
<tr>
<th></th>
<th>Sum of square</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>P value</th>
<th>Eta²</th>
<th>Observed power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within subjects effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk</td>
<td>.136</td>
<td>1</td>
<td>.136</td>
<td>29.975</td>
<td>.000</td>
<td>.307</td>
<td>.999</td>
</tr>
<tr>
<td>risk x previous research (adol)</td>
<td>.001</td>
<td>1</td>
<td>.001</td>
<td>.233</td>
<td>.631</td>
<td>.004</td>
<td>.076</td>
</tr>
<tr>
<td>risk x pa/adol</td>
<td>.008</td>
<td>1</td>
<td>.008</td>
<td>1.661</td>
<td>.202</td>
<td>.027</td>
<td>.245</td>
</tr>
<tr>
<td>risk x t/nt</td>
<td>.075</td>
<td>1</td>
<td>.075</td>
<td>14.969</td>
<td>.000</td>
<td>.197</td>
<td>.968</td>
</tr>
<tr>
<td>risk x income</td>
<td>.002</td>
<td>1</td>
<td>.002</td>
<td>.387</td>
<td>.536</td>
<td>.006</td>
<td>.094</td>
</tr>
<tr>
<td>risk x pa/adol x t/nt</td>
<td>.006</td>
<td>1</td>
<td>.006</td>
<td>1.147</td>
<td>.288</td>
<td>.018</td>
<td>.184</td>
</tr>
<tr>
<td>risk x pa/adol x income</td>
<td>.004</td>
<td>1</td>
<td>.004</td>
<td>.784</td>
<td>.379</td>
<td>.013</td>
<td>.141</td>
</tr>
<tr>
<td>risk x t/nt x income</td>
<td>.000</td>
<td>1</td>
<td>.000</td>
<td>.005</td>
<td>.944</td>
<td>.000</td>
<td>.051</td>
</tr>
<tr>
<td>Risk x pa/adol x t/nt x income</td>
<td>.000</td>
<td>1</td>
<td>.000</td>
<td>.998</td>
<td>.000</td>
<td>.050</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>.307</td>
<td>61</td>
<td>.005</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between subects effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>5.495</td>
<td>1</td>
<td>5.495</td>
<td>199.742</td>
<td>.000</td>
<td>.766</td>
<td>1.000</td>
</tr>
<tr>
<td>Previous research (adol)</td>
<td>.141</td>
<td>1</td>
<td>.141</td>
<td>5.117</td>
<td>.027</td>
<td>.077</td>
<td>.605</td>
</tr>
<tr>
<td>pa/adol</td>
<td>.111</td>
<td>1</td>
<td>.111</td>
<td>4.038</td>
<td>.049</td>
<td>.062</td>
<td>.507</td>
</tr>
<tr>
<td>t/nt</td>
<td>.439</td>
<td>1</td>
<td>.439</td>
<td>15.970</td>
<td>.000</td>
<td>.207</td>
<td>.976</td>
</tr>
<tr>
<td>Income</td>
<td>.282</td>
<td>1</td>
<td>.282</td>
<td>10.268</td>
<td>.002</td>
<td>.144</td>
<td>.884</td>
</tr>
<tr>
<td>pa/adol x t/nt</td>
<td>.025</td>
<td>1</td>
<td>.025</td>
<td>.895</td>
<td>.348</td>
<td>.014</td>
<td>.154</td>
</tr>
<tr>
<td>pa/adol x income</td>
<td>.000</td>
<td>1</td>
<td>.000</td>
<td>.018</td>
<td>.894</td>
<td>.000</td>
<td>.052</td>
</tr>
<tr>
<td>t/nt x income</td>
<td>.200</td>
<td>1</td>
<td>.200</td>
<td>7.267</td>
<td>.009</td>
<td>.106</td>
<td>.756</td>
</tr>
<tr>
<td>pa/adol x t/nt x income</td>
<td>.057</td>
<td>1</td>
<td>.057</td>
<td>2.086</td>
<td>.154</td>
<td>.033</td>
<td>.296</td>
</tr>
<tr>
<td>Error</td>
<td>1.678</td>
<td>61</td>
<td>.028</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pa=parent; adol=adolescent; t=told; nt=not told.
Also, our participants responded differently to minimal risk studies versus above minimal risk studies. When financial compensation exceeds the value prospective participants place on their involvement, the potential exists that financial compensation will become a more salient and complicating factor in their participation deliberations and may inhibit altruistic and intrinsic motivations that enhance enrollment and retention. This may not be particularly troublesome for minimal risk studies in which the amount of financial compensation is fairly modest and the risks of participation are negligible, but may be problematic in above minimal risk studies that tend to offer substantial compensation for research participation.

Several cautions must accompany the interpretation of these data. Although our sample was ethnically diverse, it may not be representative of research participants in other parts of the United States. The adolescents in our sample were young. Older adolescents may place an entirely different value on research participation. Prospective participants might respond differently to actual research participation decisions than they did to our hypothetical research decisions. Although our effect sizes were in some cases quite robust (told versus not told, lower versus higher income), in other cases the effect sizes were modest (parent versus adolescent). Also, the participants in this study received a small stipend for their participation, and it’s possible this affected our “told” versus “not told” analysis. Further research is needed to clarify the ability to generalize these findings to pediatric research.

In the meantime, the most central questions we continue to face are clarifying the circumstances under which offering financial compensation is ethically appropriate and determining how to calculate financial compensation in a variety of contexts that is both respectful of participants and facilitates research participation without compromising authentic decision-making. Cognitive psychologists assert that people use different heuristics for determining value.29 In some circumstances, value rises as the scope of what is being evaluated increases. Providing more money for longer periods of effort is an example of this type of “valuation by calculation” that theoretically has no upper limit. However, people often use “valuation by feeling” or affective valuation, particularly in situations that evoke strong emotional responses, which is relatively unaffected by scope and results in an upper limit on the value placed on a stimulus. The differences found in how our respondents perceived “fair” compensation may be caused by using these different heuristics. For example, pediatric researchers may be more inclined to assign a financial compensation amount on the basis of calculation and scope of what participants are being asked to do, whereas many prospective participants may estimate appropriate financial compensation on the basis of emotion. These different formulas used for determining the value placed on research participation may result in researchers “calculating” a financial compensation figure that may exceed what participants “feel” is fair.

Pediatric researchers must engage in a thoughtful analysis of how financial compensation may affect potential research participants and use prudence in determining that amount. Because of the variability in pediatric research and the current lack of consensus on appropriate compensation amounts, institutional review boards may need to empirically evaluate community standards on appropriate compensation for studies involving children and adolescents. Creating a more uniform and empirically based process for determining financial compensation will minimize the potential for coercion in the decision-making process and strengthen the ethical grounding of pediatric research.

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50 Years Ago in *The Journal of Pediatrics*

**MORQUIO’S DISEASE**


In 1955, clinical findings and radiographs were the only methods used for classifying skeletal dysplasias. Consensus was usually reached with classical radiographs. Unfortunately, then, as today, too many children with dysplasias had subtle or atypical findings that delayed or obscured a diagnosis. Such was the dilemma faced by Dr Lipschutz at Hahnemann Hospital in Philadelphia when confronted with a 15-month-old boy and his 7-year-old sister. Both children had with marked kyphoscoliosis, generalized hypotonia, severe developmental delays, and normal eyes during their second year of life. Both had abnormal radiograph findings, with flattened and wedge-shaped thoracic and lumbar vertebrae and flattened acetabular cavities. Their long bones were less severely involved. Lipschutz relentlessly pursued a diagnosis. He showed the radiographs to 5 prominent pediatric radiologists, 3 of whom felt that the features were diagnostic of Morquio’s disease. The other 2 had entirely different opinions.

Today, the diagnosis of Morquio’s disease, also know as mucopolysaccharidosis type IV (MPS IV types A and B), and of other lysosomal enzyme disorders is usually more straightforward. Symptoms in children affected with MPS IV usually present in the second year of life, with mild coarse facial features, hearing loss, mild hepatomegaly, inguinal hernias, and normal intellect. Characteristic radiographic findings include platyspondyly, kyphoscoliosis, coxa valgus, odontoid hypoplasia, and cervical subluxation that can cause cervical myelopathy. Increased urinary excretion of 2 glycosaminoglycans (keratin and chondroitin 6-sulfate) and deficient enzyme activity of either N-acetylgalactosamine-6-sulfatase in type A and of Beta-galactosidase in type B confirm the diagnosis. Mutational analysis is available. Although lysosomal replacement enzyme therapy is not yet available for MPS IV, multispecialty management improves the quality of life and minimizes complications.

Why do we, as Lipschutz did in 1955, try so hard to confirm a diagnosis? It is not only of academic interest. It is critical for parents to pursue the best and most experienced care for their child with a rare disorder, to consider their reproductive options, and to network with other families. Because both of these children were profoundly delayed, had normal eyes, and neither had evidence of cervical subluxation, I doubt that they had Morquio’s disease. Although Lipschutz may have never achieved a final diagnosis, it was his pursuit of a diagnosis that was remarkable and, I suspect, most appreciated by his colleagues and fellow caregivers.

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YMPD1373
A 16-year-old girl weighing 264 kg (559 lbs) presented to the emergency room with the chief complaint of redness, warmth, and tenderness of the skin over her abdomen. She had been in her usual state of health until 3 days previously, when she noted redness over the suprapubic area, which subsequently extended to the umbilicus. There was no history of trauma or previous infections.

The parents reported that she had always been tall and heavy for age, that weight gain accelerated after age 10 years, and that there was a 50 kg gain over the past year. Previous growth data were not available, and she had not seen a primary care physician for more than 5 years. Because of her weight, she had difficulty getting out of bed or walking without aid. She had therefore stopped attending school and had been home schooled for 2 years. She ate almost hourly and believed that she might die if not for frequent food intake. She reported no headache, vomiting, visual disturbances, polyuria, polydipsia, joint pain, neck swelling, abnormal hair or skin, hirsutism, easy bruising, or symptoms relieved by eating. Until mobility became problematic, she obtained her own food; then her parents served her meals. She had never sought medical help for obesity and had not attempted any weight loss program.

The medical history was otherwise unremarkable. Menses were regular. The patient was an only child living with her parents, both of whom were of average weight.

On examination, the girl was alert, cooperative, and appeared anxious. The weight was 254 kg (559 lb) and the height was 169 cm (66.5 inches; 75th to 90th percentile), for a body mass index (BMI) of 88.9 (markedly above the 95th percentile). Pulse was 80 per minute; respirations, 20 per minute; and temperature, 37.5°C. The blood pressure was repeatedly 135 to 150/80 to 85 mm Hg with a large cuff. Acanthosis nigricans was present. The abdomen had striae and a pannus. Redness, warmth, and superficial tenderness were present, extending from the suprapubic skin fold to the umbilicus. Erythematous macerated skin was present beneath the pannus and on the medial thighs. There was no lymphadenopathy. There was no abdominal guarding, rigidity, or tenderness. Breasts and pubic hair were Tanner stage V. With aid, the patient could be brought to a standing position. The rest of the examination was unremarkable.

The patient was assessed as having cellulitis, superimposed on marked obesity. The cellulitis was treated with antibiotics and resolved. A psychiatry consultation indicated anxiety and depression. Prozac (fluoxetine hydrochloride; 20 mg per day) and family therapy were advised and begun. The following tests related to causes and sequelae of obesity and/or depression were normal: oral glucose tolerance test (peak glucose, 141 mg/dL), thyroid function, overnight dexamethasone suppression test, lipid profile, liver enzymes, leptin, and chromosomes.

The abnormal weight gain was assessed as due to excessive food intake and inadequate activity and was not considered to reflect a primary endocrine or genetic disorder. Obesity-related morbidity included infection, elevated blood pressure, and psychological dysfunction. Therapeutic options discussed included caloric modification, physiotherapy and graded increases in activity, residential facility, appetite suppressants, and bariatric surgery. There was disagreement among consultants about the potential role of intensive treatments (pharmacotherapy and/or bariatric surgery) in this patient, centering on the place (if any) of bariatric surgery in youth, the degree of obesity or comorbidity warranting intensive approaches, the absence of previous attempts at weight control and the need for psychologic therapy in this patient, and the roles of the patient and parents in decisions.
ISSUES RAISED BY THE CASE

Although the current epidemic of obesity generally involves children with less severe degrees of overweight and dysfunction, this case raises several general questions about the role of intensive obesity treatments (pharmacotherapy and bariatric surgery) in pediatrics. Decisions about the use of intensive treatments for pediatric obesity will have profound impact on US healthcare. These decisions are particularly problematic because of linked clinical and ethical questions. In the Discussion, we examine these issues to assess the role of intensive obesity treatments in youth and conclude by applying the discussion to the case presented.

DISCUSSION

The current epidemic of obesity has been widely reported in the medical and lay press.1,2 Although obesity at all ages is a concern, it is particularly alarming in pediatric populations because of the expectation that it will result in an escalation of morbidity and mortality as these children mature. Depending on the definition used, 15% to 30% of US 6- to 17-year-olds (ie, 7 to 14 million children) are overweight or obese, with disproportionately high prevalence in ethnic minorities.1-4 The proportion of US children who are obese has risen 3-fold over the past 30 years.5 It has been suggested that the growing problem of childhood obesity should be addressed through population-based preventive measures.6 However, confronted with a rising population of obese youth, together with lack of broad-based preventive measures proven to be effective,6 the treatment of individual obese children and adolescents (as illustrated by this case) is also crucial.

The growing problem of childhood obesity, the evidence for it as a harbinger of adult disease, and the frustration of many practitioners and families with available behavior/diet programs7 are now raising questions about the role of pharmacotherapy and bariatric surgery in pediatric populations. These intensive approaches are expanding rapidly in adults, are reportedly effective in adults,8-10 and are increasingly considered for pediatric populations in the public media and medical journals.11-13

Should Pediatric Obesity Be Considered a Disease? Does Pediatric Obesity Resolve Without Intervention?

An issue underlying approaches to obesity management has been historic ambivalence about the extent to which obesity represents a disease state.14 The concept that obesity is self-induced and defined by cultural or statistical norms has led some to question whether it should be considered “within the biomedical paradigm” and whether attempts at treatment are warranted.15-16

DEFINITIONS AND UNDERLYING CAUSES OF PEDIATRIC OBESITY. The Centers for Disease Control and Prevention defines obesity based on the BMI (kg/m²),1 with BMIs between the 85th and 95th percentile considered “at risk for overweight” and BMIs over the 95th percentile considered “overweight.” These respective levels were previously termed overweight and obese, or obese and severely obese. Changes in terminology create apparent inconsistencies among reports. In this paper, we use the term obesity in considering overall issues pertaining to excessive weight gain and the Centers for Disease Control and Prevention–based definitions when referring to potential guidelines.

There have been major advances in understanding the physiologic and genetic bases of appetite and weight control. Familial factors clearly influence susceptibility to obesity,17 and some molecular bases for obesity have been identified. However, most childhood obesity is believed to be caused primarily by an imbalance between energy consumption and expenditure.3

CHILDHOOD OBESITY AND MORBIDITY. There is persuasive evidence that obese youth are at increased risk for several medical problems3,18—including type 2 diabetes mellitus3,18 and hypertension19—that may improve with weight loss. Elevated cholesterol levels in youth track into adulthood. Of obese children and adolescents, 40% may have development of steatohepatitis and 32% sleep apnea.3 Asthma, ovarian hyperandrogenism, pseudotumor cerebri, slipped capital femoral epiphysis, and cholelithiasis are exacerbated by or caused by childhood obesity.3 Obese youth are also at significant risk for psychologic and economic sequelae.18,20 Long-term studies generally indicate that obese youth have increased cardiovascular morbidity and mortality.21,22 The evidence thus suggests that pediatric obesity causes profound morbidity and probably will increase mortality rates—particularly for older children and adolescents and those with severe degrees of obesity. The patient presented has obesity-related morbidity.

Together, these data support the concept that pediatric obesity should be considered a disease, since it not only represents a deviation from established norms but is also linked to increased morbidity and mortality rates. This is consistent with general definitions of disease23 and chronic illness of childhood.24

If pediatric obesity is likely to resolve without intervention, then treatments during childhood and/or adolescence would not be necessary. The evidence suggests, however, that pediatric obesity (together with parental obesity) is a strong risk factor for adult obesity.17 In fact, after age 10 years, childhood obesity becomes the dominant predictive factor for adult obesity, and approximately 80% of children 10 years or older with BMIs above the 95th percentile become obese adults.17 Obesity in adolescence is considered the best single predictor of adult obesity. Since the link between pediatric and adult obesity is age-dependent and particularly strong after age 10 years, some have cautioned against obesity treatments in very young children.17 By contrast, obese adolescents, like the patient presented, represent an appropriate age group for therapeutic intervention.
What Obesity Treatments Are Available? What Are Their Potential Applications to Youth?

Although prevention of obesity depends on maintaining a healthy lifestyle, treatments for obesity include two major therapeutic categories: lifestyle-based treatments (diet, exercise, and/or behavior therapy) and medical/surgical interventions that are often termed intensive treatments (pharmacotherapy and/or bariatric surgery). Many guidelines exist, and studies have been performed on adult obesity treatments; in comparison, guidelines and systematic controlled studies in youth are scant.

**OBESITY TREATMENT FOR ADULTS.** The National Institutes of Health (NIH) guidelines suggest lifestyle-based treatment for adults with BMI > 30 kg/m² and no additional risk factors and for those with BMI 25 to 29.9 kg/m² plus 2 or more risk factors for obesity-related diseases. Such programs can cause weight loss of 5% to 10% over a period of 4 to 6 months. This may reverse or prevent some comorbid conditions and result in cost savings due to reduction in long-term morbidity. However, for most adults, weight is regained.

The second category of “intensive” medical/surgical treatments (pharmacotherapy and surgical weight loss procedures) is recommended by professional organizations and included in NIH guidelines for certain adults. The NIH guidelines consider weight loss drugs approved by the Food and Drug Administration (FDA) to be useful adjuncts to lifestyle modification for adults with BMI >30 kg/m² and no concomitant risk factors and those with BMI > 27 kg/m² plus concomitant risk factors, if there is failure to lose 1 pound per week after 6 months of lifestyle intervention. Currently, drugs approved by the FDA for obesity management in adults include sibutramine and orlistat. Other drugs (eg, metformin) approved by the FDA for other disorders have been used for treating obesity as well.

Bariatric surgery has gained prominence in medical journals and the media. The NIH guidelines consider surgery an option for adults with severe obesity (BMI ≥ 40 kg/m² or BMI ≥ 35 kg/m² with comorbid conditions) when less invasive methods have failed and in association with an integrated weight loss program. In adults, surgery can result in substantial and sustained weight loss, improvement in comorbid conditions, reduced long-term mortality rates, and sustained improvement in health-related quality of life. The Cochrane group and the Agency for Health Care Research and Quality suggest that despite limited evidence, surgery is more effective than conventional weight management in morbid obesity. In adults, meta-analysis suggests operative mortality of <1.1%. Nutritional deficiencies may result from surgery, necessitating life-long supplements and follow-up. In addition to the NIH, other professional bodies emphasize the primacy of lifestyle intervention in overweight adults but also clearly indicate a role for pharmacotherapy and weight loss surgery.

**OBESITY TREATMENT FOR CHILDREN AND ADOLESCENTS.** In striking contrast to adults, treatment guidelines for pediatric obesity have focused almost exclusively on lifestyle interven-

tion. Evidence for the efficacy of such approaches includes well-controlled research studies in select populations (eg, Epstein et al). There are few studies of their effectiveness in non-research practice settings. The Maternal and Child Health Bureau and other professional bodies recommend that childhood obesity treatment programs include lifestyle modification, family education, and an emphasis on gradual long-term changes.

Few guidelines for obesity treatment in youth have discussed pharmacotherapy and bariatric surgery. Those that do vary in the approaches suggested. One article suggested that “children and adolescents with BMI above the 95th percentile for age and sex and who have a medical complication of obesity that may be remediable through weight reduction should be considered for intensive regimens.” The FDA has approved orlistat for the treatment of obesity in 12- to 16-year-olds, but specific guidelines are lacking for appropriate conditions under which it should be used. A recent article suggested bariatric surgery for highly selected groups of adolescents, although commentaries suggested alternative approaches.

The dearth of guidelines from professional organizations or government agencies regarding pharmacotherapy and bariatric surgery in youth may reflect limited data on these modalities in pediatrics. Yet, recent studies have provided some information. Orlistat for 3 months caused a 4% weight loss in adolescents. Sibutramine, in combination with a behavioral program, caused more weight loss than behavior therapy plus placebo. Metformin caused a modest but significant reduction in BMI in obese adolescents. In children with hypothalamic dysfunction, octreotide can cause weight loss.

There are several reports on bariatric surgery in adolescents. In one, surgery was followed by sustained weight loss in 84.8% of 33 adolescents, and obesity-related morbidity generally improved. Another, a gastric banding procedure resulted in a median loss of 35.6 kg or 59.3% of excess weight in 17 adolescents. Another reported that after mean follow-up of 5.5 years, 18 of 19 adolescents who had undergone bariatric surgery at ages 13 to 17 years had lost adequate weight and all had resolution of comorbid conditions. These reports include data on operative and long-term morbidity. There is increasing interest in bariatric surgery for pediatric populations, and information on available programs is widely available on the Internet.

**Pediatric Obesity: Should the Focus Be Prevention, Treatment, or Both?**

Since childhood obesity appears related primarily to the balance between intake and activity, efforts to address the current epidemic need a foundation of preventive measures. Prevention programs have been suggested at the population level in targeted communities such as schools, and for individuals. These approaches are based on the intuitive logic and expected cost-effectiveness of prevention.

Despite the clear merits of this approach, it probably is not practical or appropriate for the medical community to rely...
solely on prevention. First, effective preventive measures at population levels remain elusive. Second, clinicians face with a large population of youth who are already obese—like the patient presented—and who either have developed or are at significant risk for obesity-related maladies. These children are in need of intervention; prevention efforts will be too late for this cohort, and the fact that such conditions may be avoidable for other children in the future does not alter responsibilities toward those already affected.

These considerations suggest that strategies to combat pediatric obesity be based on a two-pronged approach that includes both treatment at the individual level and prevention through public health initiatives. Preventive approaches may be applied broadly to the population, whereas treatments (potentially including intensive treatments) can be targeted to specific individuals who are at greatest risk.

What Are Potential Roles for Pharmacotherapy and Bariatric Surgery in Treating Pediatric Obesity?

It is considered unethical to deny to children and adolescents, on the basis of age, those treatments that are beneficial for adults. In this sense, one might wish to apply the NIH guidelines for pharmacotherapy/bariatric surgery in adults directly to pediatric populations. However, treatments for children require specific evaluation of safety and efficacy. Together, these considerations suggest that since pharmacotherapy and bariatric surgery are included in recommendations for adults, (a) these therapies should also be strongly considered for the treatment of pediatric obesity, and (b) rigorous clinical trials are needed to examine their safety and efficacy in pediatric populations. Such trials need to address short- and long-term outcomes—including child-specific issues such as growth, puberty, and psychologic development. It is important that the trials include evaluation of both medical and surgical interventions.

If rigorous clinical research studies on the safety and efficacy of pharmacotherapy and bariatric surgery in youth are not undertaken, it is likely that families will request and practitioners will provide these treatments. Even if clinical trials are undertaken, practice may well evolve before results are known. These concerns suggest the need for development of clear statements by professional organizations and/or government agencies that wish to establish practice-based weight loss programs for youth, based on available evidence. The statements could address whether there is currently a place for intensive obesity treatments in pediatric populations; if so, they could address the weight change expected by youth through lifestyle interventions, which treatment is next if lifestyle fails, the minimal age and degree of obesity for intensive treatment, the role of comorbidities in decisions, consent and parental permission considerations, and recommended personnel/facilities for centers developing programs. Further, the statements should explicitly plan for regular updates. Such guidelines have been established for adult bariatric surgery programs.

Inconsistency and the possibility of misuse, potentially exposing children to significant harm.

Decision-Makers for Intensive Obesity Treatments in Pediatric Populations

Nonemergency medical decisions for older children are generally made by a triad of parents, health care provider, and the patient. The key medical decision-makers for the patient presented are no different than for other diseases. However, the nature of obesity brings added complexity to decision-making. This includes the semi-elective nature of treatment despite serious risk of long-term morbidity, the concept that individual behaviors are central to the condition, and the balancing of single decisions to undertake intensive treatment against ongoing repeated decisions to adhere to less invasive lifestyle changes. In addition, the role of adults in decisions regarding pediatric obesity is particularly important because children are not always in control of the food available to them.

Despite a legal standard that considers the 18th birthday as a threshold for competency in most states, an ethical framework for understanding decision-making is based on the gradual acquisition of decision making capacity. Most experts consider the age range of 8 to 14 years to be appropriate for assent as a general rule, with younger children incapable of meaningful participation in medical decisions and older individuals capable of providing authentic informed consent. At a minimum, the patient presented should provide assent for any proposed treatment.

Parents are expected to act in the best interests of their children and are generally given great decision-making authority. Rather than informed consent, the ethics literature suggests that the most accurate way to describe the role of parents is that they may provide permission on behalf of the child. Society allows wide discretion in parental decisions, resulting in the tolerance of significant variability. Decisions about obesity treatment may be influenced by the parent’s own experience, and it may be questioned whether parents who have been unable to successfully treat overweight youth by lifestyle changes are likely to be able to follow the steps needed for implementation of intensive treatments. For these reasons, clinicians must be careful to focus on the best interests of the obese child and help parents to make sound decisions on behalf of their child.

The approach to children with obesity should be consistent with that taken for other serious childhood conditions such as cancer, diabetes, or heart disease. The ethical considerations regarding developmental capacity to give assent and consent suggest that intensive treatments should focus on older children and adolescents and that all decision-makers (parent, child, and physician) must reach consensus before embarking on intensive treatment.

Which Pediatric Populations Should Be Eligible for Intensive Obesity Treatments?

If intensive treatments are considered for pediatrics, it will be necessary to delineate which subgroup(s) should be
offered these interventions and to assess the impact of candidate selection guidelines on US children. There is no single point on weight index scales above which children are at great risk and below which they are not at risk. Based on the prevalence of overweight and associated morbidities, together with census data, the number of US adolescents potentially eligible for intensive treatments can be assessed. If, as suggested (and consistent with NIH guidelines for adults), adolescents are eligible for intensive treatments if they have both a BMI over the 95th percentile and an associated comorbidity, the number of eligible candidates could range from approximately 141,000 to over 2 million—depending on which comorbid conditions were included in eligibility criteria. As most guidelines for intensive treatment would first require serious attempts at weight loss through lifestyle modification, these estimates would decline if lifestyle interventions were effective in some children.

These data indicate that large numbers of US youth are potentially affected by decisions about intensive obesity treatments. If candidates were to be selected on the basis of having the greatest risk if untreated, current data suggest that eligibility focus on adolescents with extremely high BMI and those with a complication that itself results in significant morbidity.

If Pediatric Obesity Is a Disease, What Are the Implications for Medical Rights and Societal Attitudes?

Central to the goals of the medical profession is the obligation to evaluate, and, when indicated, to treat individuals who have a disease. Yet, insurance coverage is often unavailable or inadequate for obesity services—even those that are noninvasive and recommended by professional and governmental organizations (eg, evaluation for medical disorders; nutritional counseling). This problem also raises questions of distributive justice, since hurdles in payment may aggravate existing sociodemographic disparities in obesity.

It is important that the medical problem of pediatric obesity be clearly distinguished from the cultural value placed on being thin. Otherwise, attempts to reduce obesity may be viewed as enhancement therapies rather than efforts to treat a disease. This concern also supports the adoption of conservative guidelines and the limitation of intensive treatment to those most severely affected.

SUMMARY

Assessment of basic questions regarding the treatment of pediatric obesity leads us to the following conclusions. Childhood obesity is a disease. Families, health care professionals, researchers, insurers, and policy-makers should treat it as they treat other diseases. Although preventive measures are necessary, they are not sufficient. Ethical and scientific assessments suggest that a two-pronged policy approach that simultaneously addresses treatment as well as prevention is needed; this approach is consistent with the way we approach other emerging diseases and public health threats. If medication or surgery are considered beneficial in adults, we should be open to their application to youth; they should be explicitly evaluated for pediatric populations through rigorous institutional review board–approved clinical studies, and funding should be available to enable such evaluation. At the same time, interim guidelines for intensive obesity treatments are needed from professional organizations and/or government agencies; explicitly designed for regular updates as data from trials emerge, they can set a standard to minimize risk to youth. Both scientific data and ethical analyses suggest that eligibility for intensive treatments should focus on adolescents with severe obesity (not those with modest obesity) and those with obesity–related comorbidity, since they are at greatest risk for sustained obesity to adulthood, are generally capable of informed assent or actual consent, and are at great risk for poor outcomes if not treated successfully.

APPLICATION OF DISCUSSION TO CASE

This patient was markedly overweight, with evidence of obesity–related morbidity. Obesity at her age is unlikely to resolve without intervention. The degree of obesity places her at significant risk for further morbidity. The BMI would qualify for intensive obesity treatment (including bariatric surgery) in an adult program or according to some suggestions for youth. Based on the discussion above, it was considered inappropriate to deny such treatment simply on the basis of her age. However, intensive obesity treatment was not advised at this time because the patient had not yet attempted any standard weight management program (a generally accepted prerequisite for bariatric surgery in adults) and identified psychosocial issues might interfere with successful adherence to comprehensive intensive management programs. Therefore, after discussion about the nature and risks of obesity, plans were made with the family to initially engage in a program of psychologic and family counseling with behavior modification, structured reduction in food intake, and physical rehabilitation. Intensive obesity treatments will be reconsidered in the future, pending assessment of progress. The family is interested in controlled trials that may be available at that time.

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Assisted reproductive technology (ART) has now become a cornerstone of treatment for involuntary infertility. Recent studies have raised concern regarding potential associations between ART and chromosomal aneuploidy, imprinting anomalies, and monochorionic placentation in dizygotic (DZ) twins. We report a case of DZ twins conceived by ART in which all three problems coexist. (J Pediatr 2005;146:565-7)

The birth of the first “test tube” baby just over 25 years ago brought hope to infertile couples worldwide and has spurred the development of a multi-billion dollar industry dedicated to overcoming infertility. Between 1% and 3% of live births per year occur in Western countries as a result of assisted reproductive technology (ART), which includes intra-cytoplasmic sperm injection (ICSI) and gamete and zygote intrafallopian transfer in addition to classic in vitro fertilization (IVF). Although most studies have suggested that the procedures are safe, there is growing evidence that ART may be associated with a wide variety of abnormalities in resultant offspring. These include chromosomal abnormalities, especially of the sex chromosomes, imprinting anomalies, multiple births, and low birth weight infants. A recent report documenting the first well-defined case of dizygous (DZ) monochorionic twins conceived following IVF suggested that IVF may be associated with alterations in the membrane relationships of DZ twins.

We present a second case of DZ monochorionic twins conceived by ART, of which one has both Klinefelter syndrome and Beckwith-Wiedemann syndrome (BWS). There are a number of potential health surveillance issues for children born following ART, of which pediatricians should be aware given the rapidly increasing use of ART in Western countries.

CASE HISTORY

The proband, twin A, was born at 33½ weeks gestation to a 34-year-old G1P0 mother and a 37-year-old father. Infertility was felt to be the result of low paternal sperm count. Fertilization was achieved through ICSI and pregnancy through classic IVF, following transfer of three embryos. There was bleeding at 2 months, and the progesterone dose was subsequently increased, otherwise the pregnancy was uncomplicated. Only two gestational sacs were ever visualized on repeated prenatal ultrasonography.

On physical exam, twin A had macroglossia, an omphalocele, and right-sided hemihypertrophy, which is consistent with a diagnosis of BWS. Birth weight was 2660 g, birth length was 47 cm, and head circumference was 30 cm. Twin A had an inguinal hernia, otherwise the rest of the exam, including the genitalia, was normal. Chromosomal analysis revealed a 47,XXY karyotype, consistent with Klinefelter’s syndrome. Molecular studies documented maternal origin of the extra X chromosome (data not shown). Microscopic evaluation of the placental membranes revealed a diamniotic, monochorionic membrane relationship (Figure 2; available online at www.us elsevierhealth.com/jpeds).
Chromosomal analysis of the co-twin was performed, as a similar karyotype was expected. Twin B, however, was 46,XY, and the physical exam was completely normal. Birth weight was 2221 g, birth length was 45.8 cm, and head circumference was 32 cm.

Zygosity studies revealed discordance at 11 loci including 3 (TH01, HBB, and D11S1981) within the BWS critical region on chromosome 11p15 (not shown). Hypomethylation at KCNQ1OT1/KvDMR1 and mosaic paternal uniparental disomy for 11p were not identified in peripheral blood, and the molecular basis for BWS in the proband remains unknown.

DISCUSSION

This is the second reported case of DZ monochorionic twins born following ART, and the first report of twins discordant for BWS and Klinefelter’s syndrome occurring in the same person. Although the mechanism by which these abnormalities occur is unknown, it is unlikely to represent mere coincidence.

The placental findings have broad implications with respect to the nature of birth defects seen in monozygotic (MZ) versus DZ twins, as well as the means through which zygosity is established for the purposes of genetic and epidemiologic twin studies in general. The fascinating case of DZ twins conceived by ART with a monochorionic placenta with vascular connections and blood chimerism presented by Souter and colleagues⁴ provides convincing evidence challenging the dogma that monochorionic twins are invariably identical. Although the role played by ART in this single case can only be hypothesized, fusion of preimplantation mammalian embryos has been induced in vitro, yielding chimeric blastocysts that contain a single inner cell mass of dual origin.⁵ Blastocysts of this type could potentially give rise to a twin monochorionic pregnancy, although the twins would be genetically distinct.⁵ It is possible that a similar series of events gave rise to the findings in our case. ART is associated with an increase in embryo splitting, causing MZ twinning, and blastocyst culture may further increase this risk.⁴ The concept that ART is associated with MZ twinning in humans may need to be reevaluated in light of these findings, as it is almost impossible to distinguish same-sex monochorionic DZ twins from monochorionic MZ twins in the absence of detailed zygosity studies. Tests of zygosity should be carried out using tissue other than peripheral blood in order to avoid the possibility of blood chimerism.⁴

Several studies have suggested that ART, particularly ICSI, is associated with an increased risk of de novo chromosomal abnormalities in the offspring.²,⁶ Although some authors insist this finding is more likely a reflection of the reason for the infertility rather than the procedure itself, the association is real.² Pediatricians should be aware of this issue and its implications when following patients born after ART.

Evidence suggests that ART is associated with genetic disorders as a result of imprinting anomalies or errors in regulation of gene expression from a specific parental allele.¹,³,⁷ BWS is a recognizable pattern of malformation characterized by neonatal hypoglycemia, abdominal wall defects, somatic overgrowth, hemihyperplasia, visceroomegaly, macroglossia, and ear abnormalities. Children with BWS also
have an increased risk of developing embryonal cancers, and they require routine tumor surveillance. Many studies have documented an association between ART and BWS in the offspring, and have found that the main mechanism underlying the development of BWS was an imprinting defect of the \( KCNQ1OT \) gene at 11p15.\textsuperscript{8,9,10} Weksberg and colleagues found a marked increase in the incidence of female MZ twins among patients with BWS. In their series of 10 MZ twin pairs discordant for BWS, all had an imprinting defect of the \( KCNQ1OT \) gene.\textsuperscript{11} Monozygosity was established by analysis of polymorphic microsatellite markers. They speculated that the discordance for BWS in their population of MZ twins might have been caused by differential maintenance of imprinting at the \( KCNQ1OT \) locus following unequal splitting of the inner cell mass during the twinning process. An alternate hypothesis they proposed is that an early imprinting defect that occurred during pre-implantation development also might have caused twinning. Whether ART predisposes to one of these potential mechanisms remains unclear. We were unable to obtain mutation status of the \( p57(KIP2) \) gene (not typically associated with ART) or maternal \( H19 \) methylation studies for our patient. Maternal methylation of the \( H19 \) locus would indirectly support an association between ART and imprinting defects. Genetic disorders as a result of other defects of imprinting have been reported in children born following ART, most notably Angelman’s syndrome.\textsuperscript{7,12} There is new evidence that suggests that paternal imprinting errors may be associated with abnormal spermatogenesis.\textsuperscript{13} ART procedures may then facilitate inadvertent transmission of these errors to future offspring.

Many investigators now are calling for heightened awareness of the possible complications of ART. Because many imprinted genes have been implicated in disorders of growth, increased risk of childhood tumors, and developmental delay,\textsuperscript{\textsuperscript{8}} it is particularly important for pediatricians to routinely ascertain the mode of conception for their patients and to be aware of the potential problems. This will facilitate appropriate diagnostic testing, counseling, and health surveillance measures for these children.

**REFERENCES**

We describe cholestasis as a result of bile duct abnormalities in 8 children with portal vein obstruction. In a clinical, biochemical and radiological investigation of 121 children with cavernous transformation of the portal vein seen between 1986 and 2000, 8 presented with jaundice, pruritus, and/or raised serum aminotransferases and/or gamma glutamyl transpeptidase (gamma GT) activities. Each displayed dilation and narrowing of intra- and/or extrahepatic bile ducts. Surgical decompression of the portal system (portal-systemic or Rex anastomosis) resulted in the regression of the signs of cholestasis in all children. We conclude that children with portal vein obstruction may exhibit clinically significant cholestasis as a result of external compression of the bile duct by the cavernoma. (J Pediatr 2005;146:568-73)

Portal vein obstruction is the second most frequent cause of portal hypertension in children, cirrhosis being the leading cause. Gastrointestinal bleeding can occur in up to 80% of cases; pulmonary hypertension and hepatopulmonary syndrome are less frequent. There is, however, an additional risk associated with portal vein obstruction that is linked to the development of hepatopetal collaterals, bypassing the obstructed portal vein, the so-called portal cavernoma. A portal cavernoma is made up of dilated pancreatico-duodenal and peribiliary veins, which normally are small but increase in size because of the raised pressure. Increase in the size and pressure may result in the compression of the adjacent bile ducts. Although it is commonly accepted that children with portal vein obstruction display normal liver tests and normal or near-normal liver histology, slight and unexplained increases in serum aminotransferases levels are occasionally observed. Clinically significant cholestasis has been reported in adults with portal vein thrombosis, and it has been suggested to be the consequence of the compression of the bile ducts by the cavernoma. Here we report 8 children with portal vein obstruction who presented with cholestasis.

METHODS

Between 1986 and 2000, 121 children were investigated for portal vein obstruction in this unit. Among them, 8 (5 boys) presented with clinical, biochemical, and/or imaging features that led to a diagnosis of cholestasis secondary to bile duct abnormalities. Neonatal omphalitis and umbilical vein catheterization had been present in 1 patient each. The diagnosis of portal vein obstruction was made at ages ranging from 5 months to 10 years (median: 4 years). Fortuitous finding of splenomegaly and gastrointestinal bleeding led to the diagnosis in 7 children and 1 child, respectively. Diagnosis of portal vein obstruction relied on abdominal ultrasonography; the diagnosis was confirmed in each case later by angiography performed before shunt surgery. Patients were followed on a yearly basis with clinical examination, total and direct serum bilirubin concentrations and serum aminotransferases and gamma glutamyl transpeptidase (gamma GT) activities, and color duplex abdominal ultrasonography looking for dilation of bile ducts. Further imaging studies of the bile ducts consisted of intravenous cholangiography (in the earliest 4 patients), endoscopic retrograde cholangiopancreatography in 2 patients, endoscopic ultrasonography in 1 patient, computed tomography in 2 patients, magnetic resonance imaging in 2 patients, and/or operative cholangiography in 1 patient (Table). Upper gastrointestinal endoscopy was carried out every 2 to 3 years or earlier in case of bleeding. Gastrointestinal bleeding occurred in 4 children at ages ranging from 9 months to 14 years. Two of these 4 patients were first treated elsewhere by endoscopic sclerotherapy. In the children who did not bleed, upper gastrointestinal endoscopy showed grade II or III esophageal varices in 3 patients and grade I varices with portal hypertensive gastropathy in 1 patient at the time of investigation.

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shunt surgery was decided upon. Liver histology was studied on surgical liver biopsies carried out at the time of shunt surgery in 7 patients.

RESULTS

The presenting symptoms of cholestasis; results of clinical, biochemical, and imaging studies at the time of diagnosis of bile ducts abnormalities; and the outcome after shunt surgery are shown in the Table. The first sign of cholestasis was recorded at ages ranging from 4 to 11 years and consisted of raised aminotransferases and gamma GT activities in 6 children, and of the fortuitous finding of liver enlargement or dilated bile ducts on ultrasonography in 1 patient each. Evolution of liver tests could be recorded in 6 children over a 2- to 10-year period: in 3 children alanine aminotransferase activity remained permanently elevated; in the other 3 children they fluctuated, being at times normal or close to normal. At the time of diagnosis of bile ducts abnormalities, jaundice was present in 3 children, pruritus in 3 children, and hepatomegaly in 2 children; 3 children did not display any clinical sign of cholestasis. Serum direct bilirubin concentrations were raised (from 2.8 to 8.2 times above the upper limit of normal (N); mean: 4.8 N) in 7 children; and serum gamma GT activity was raised (from 1.3 to 48 times above the upper limit of normal; median: 8 × N) in all children. Tests for hepatitis A, hepatitis B, hepatitis C, and α1 antitrypsin were negative or normal.

Dilation of the bile ducts was shown by imaging studies in all children. Color doppler sonography showed dilated bile ducts in 7 children (Figure 1). Ultrasonography without color doppler failed to show the dilation in patient 6. Dilation was not present at the time of the diagnosis of portal vein obstruction and appeared 3 months to 10 years later (mean: 4 years). This dilation concerned the intrahepatic ducts in all cases and the extrahepatic duct in 4 children. The size ranged from 2 to 15 mm on intrahepatic ducts (mean: 6 mm) and 3 to 11 mm on extrahepatic duct (mean: 4 mm). The gallbladder was large in 3 children, small in 1 child, and had thickened walls in 2 children. Two early patients were studied by intravenous cholangiography only that did not allow a complete study of the biliary tree: it showed dilation of the intrahepatic ducts. In the other 6 children, cholangiographic findings consisted of a short stricture of the common bile duct (3 patients), a short stricture of the hepatic duct (2 patients), and a long stricture near the hilum (1 patient) (Figure 2). Marked angulation of the extrahepatic duct in its suprapancreatic part was visible in 3 children. Parietal irregularities of the intra and extrahepatic bile ducts were present in 5 children. Angiography showed voluminous intra- and extrahepatic hepatopetal cavernoma in all children but one, always associated with porto-systemic collaterals. In the last child (patient 7), cavernoma consisted of two, small paraocholedocal veins. When compared with other patients without biliary complication, the cavernomas in this series had no specific pattern. Cross-sectional imaging methods (magnetic resonance imaging and computed tomography) showed the close relation between cavernoma and intrahepatic ducts with segmental narrowing and extrinsic deformity of the bile ducts against the peribiliary veins (Figure 2).

The decision to perform shunt surgery was based on gastrointestinal bleeding in 4 children (patients 2, 6, 7, and 8) and, in the other 3, on the combination of grade II or III esophageal varices, implying a high risk of bleeding,7 with jaundice or pruritus (patients 4 and 5) or signs of cirrhosis (patient 3). In the most recent patient (patient 1), shunt surgery was performed because of the dilation of the bile ducts only. Surgery consisted of mesocaual shunt with jugular vein interposition in 5 children, of an atypical jejuno-caval shunt with jugular vein interposition in a child in whom obstruction of the portal system also comprised the splenic and superior mesenteric veins, and of a Rex anastomosis8 in 2 children. All shunts were proven to be patent as shown by disappearance of varices on upper gastrointestinal endoscopy. Liver histology showed portal fibrosis in all 7 children studied and ductular proliferation in 6; in one child there were patent signs of biliary cirrhosis; periductular fibrosis akin to what is seen in sclerosing cholangitis was present in 3 patients.

Following surgery, serum aminotransferases and gamma GT activities returned to normal levels within 1 to 6 weeks in 5 children and have remained normal with follow-ups ranging from 5 to 15 years. In one child, aminotransferases and gamma GT were found to be normal 1.5 years after surgery and remained normal with a total postoperative follow-up of 6 years. In patient 2, normalization of transaminases was recorded 2 years postoperatively, and a regular decrease in gamma GT over a period of 8 years was recorded, gamma GT activity being 1.5 × N at the end of follow-up. In patient 1, liver function tests were still abnormal 2.5 years after surgery but were normal 2 years later. After follow-ups ranging from 4.5 to 15 years, all children are alive, leading normal lives and displaying no dilation of bile ducts on abdominal ultrasonography except patient 8, in whom regression of a major dilation of the intrahepatic bile ducts is partial 5 years after surgery.

DISCUSSION

The results reported here indicate that clinically significant cholestasis may be present in up to 6% of children with portal vein obstruction. Cholestasis is associated with ductular proliferation and portal fibrosis, and it may end in biliary cirrhosis. Surgical shunts, either portal-systemic or of the Rex anastomosis type, are associated with full and long lasting resolution of cholestasis.

When endoscopic retrograde cholangiography is systematically carried out in adults and children with portal vein obstruction, abnormalities in the morphology of the bile ducts are found in 80% to 100% of patients.9-11 The abnormalities consist of strictures of various lengths, displacements, caliber irregularities, extrinsic nodular narrowing, and dilation. These abnormalities are thought to result either from extrinsic compression of the bile ducts by the dilated veins of the
cavernoma that develop in the paracholedocal vein and the epicholedocal plexus or from ischemic lesions as a consequence of portal thrombosis. The resulting picture may mimic sclerosing cholangitis or cholangiocarcinoma. Indeed, differentiation from sclerosing cholangitis may be difficult, although typically sclerosing cholangitis present with multifocal short and concentric stenoses along the intra- and extrahepatic bile ducts, whereas extrinsic compressions by a cavernoma are central or extrahepatic and are not concentric and the dilation predominates on intrahepatic bile ducts. Although children and adolescents were included in these morphological studies, the clinical significance of these radiological findings has been so far mostly described in adults as obstructive jaundice, bacterial cholangitis, cholelithiasis, abdominal pain, or abnormal findings of liver tests. There has been a limited number of reports on this clinical condition in children and adolescents, the youngest patient being 11 years old. The results reported here indicate that cholestasis as a result of bile ducts abnormalities should be suspected in children with portal vein obstruction who display, even at a very young age, jaundice, pruritus, liver enlargement, or abnormal liver tests.

Color doppler ultrasonography of the abdomen is a useful first imaging modality as it will show dilation of the bile ducts in most instances. Further imaging studies such as endoscopic retrograde cholangiopancreatography and magnetic resonance imaging show simultaneously the cavernoma and the bile ducts with

### Table. Results of clinical, biochemical, imaging, and pathological investigations and outcome in 8 children with portal vein obstruction and cholestasis

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (y)</th>
<th>Type</th>
<th>Clinical signs</th>
<th>Liver tests</th>
<th>Imaging studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 4</td>
<td>ALT: 4xN None</td>
<td>ALT: 3.7xN</td>
<td>US: dilation of BD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 10</td>
<td>ALT: 5xN GGT: 20xN Jaundice and pruritus (age 14)</td>
<td>ALT: 6.5xN GGT: 48xN</td>
<td>US: dilation of BD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 10</td>
<td>Hepatomegaly Hepatomegaly</td>
<td>ALT: 2.8xN GGT: 5xN</td>
<td>US: dilated BD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 4</td>
<td>ALT: 1.5xN GGT: 2xN Pruritus and hepatomegaly (age 14)</td>
<td>ALT: 5.3xN GGT: 13xN</td>
<td>US: dilation of BD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 5</td>
<td>ALT: 4xN GGT: 9xN Jaundice and pruritus (age 9)</td>
<td>ALT: 3.1xN GGT: 1.3xN</td>
<td>US: dilated BD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 5</td>
<td>ALT: 8.2xN GGT: 8xN None</td>
<td>ALT: 8.2xN GGT: 8xN</td>
<td>US&quot;: no dilation of BD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 4</td>
<td>ALT: 4xN GGT: 6xN T bilirubin: 59 μM</td>
<td>ALT: 4xN GGT: 6xN</td>
<td>US: dilated BD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 11</td>
<td>Dilated bile ducts on US</td>
<td>ALT: 1.5xN GGT: 1.5xN</td>
<td>US: dilated IHBD (15 mm)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; BD, bile ducts; C bilirubin, conjugated bilirubin concentration; EHBD, extrahepatic bile ducts; ERCP, endoscopic retrograde cholangiopancreatography; FU, follow-up; GGT, gammaglutamyl transferase; IHBD, intrahepatic bile ducts; IVC, intravenous cholangiography; JV, jugular vein interposition; T bilirubin, total serum bilirubin concentration; THC, percutaneous transhepatic cholangiography; Uendo, endoscopic ultrasonography; US, abdominal ultrasonography.

*Ultrasonography without color doppler.
intravenous injection of gadolinium and may prove extremely useful and less risky if the results reported in adults also are found in children.20-23

The short- and long-term complications of cholestasis, including biliary cirrhosis, require treatment of these biliary abnormalities as soon as they become clinically significant. Endoscopic sclerotherapy or ligation of oesophageal and/or gastric varices, the often recommended treatment of children with portal vein obstruction and gastrointestinal bleeding, cannot have any beneficial effect; on the contrary, repeated endoscopic treatment may increase the risk of biliary complications as it suppresses portal systemic collaterals. Endoscopic treatment of strictures, although advocated by some, will not result in permanent cure of the problem and carries a risk of bleeding as well as of acute bacterial cholangitis;24,25; direct surgical approach of the bile duct is not indicated as it carries a risk of severe bleeding.12,26

If the bile duct abnormalities are secondary to extrinsic compression by the dilated veins of the portal cavernoma, it is reasonable to expect the improvement of these lesions after portal decompression. This was shown to be the case in an adult patient by Choudhuri14 and further supported in a few other adults and one adolescent.12,19 The results of the surgical shunts in the children reported here clearly indicate that a successful surgical portal decompression results in resolution of the biliary abnormalities. The decision to perform such surgery in these patients is easy to make when biliary abnormalities are associated with a previous gastrointestinal bleeding because of varices or when major varices are present; in such cases shunt surgery will both cure the bile duct

<table>
<thead>
<tr>
<th>Surgical shunt</th>
<th>Liver histology</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meso-caval with JVI</td>
<td>Portal fibrosis</td>
<td>FU: 4.5 y</td>
</tr>
<tr>
<td></td>
<td>Ductular proliferation</td>
<td>US: no dilation of BD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal ALT and GGT</td>
</tr>
<tr>
<td>Jejuno-caval with JVI</td>
<td>Portal fibrosis</td>
<td>FU: 8 y</td>
</tr>
<tr>
<td></td>
<td>Ductular proliferation</td>
<td>US: no dilation of BD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal ALT; GGT: 1.5xN</td>
</tr>
<tr>
<td>Meso-caval with JVI</td>
<td>Cirrhosis</td>
<td>FU: 15 y</td>
</tr>
<tr>
<td></td>
<td>Ductular proliferation</td>
<td>US: no dilation of BD</td>
</tr>
<tr>
<td></td>
<td>Periductular fibrosis</td>
<td>Normal ALT and GGT</td>
</tr>
<tr>
<td>Meso-caval with JVI</td>
<td>Portal and septal fibrosis</td>
<td>FU: 6.5 years</td>
</tr>
<tr>
<td></td>
<td>Ductular proliferation</td>
<td>US: no dilation of BD</td>
</tr>
<tr>
<td></td>
<td>Periductular fibrosis</td>
<td>Normal ALT and GGT</td>
</tr>
<tr>
<td>Spleno-Rex with JVI</td>
<td>Portal and septal fibrosis</td>
<td>FU: 5 y</td>
</tr>
<tr>
<td></td>
<td>Ductular proliferation</td>
<td>US: no dilation of BD</td>
</tr>
<tr>
<td></td>
<td>Periductular fibrosis</td>
<td>Normal ALT and GGT</td>
</tr>
<tr>
<td>Meso-caval with JVI</td>
<td>Slight portal fibrosis</td>
<td>FU: 15 y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US: no dilation of BD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal ALT</td>
</tr>
<tr>
<td>Meso-Rex with JVI</td>
<td>Portal fibrosis</td>
<td>FU: 5 y</td>
</tr>
<tr>
<td></td>
<td>Ductular proliferation</td>
<td>US: no dilation of BD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal ALT and GGT</td>
</tr>
<tr>
<td>Meso-caval with JVI</td>
<td>Not done</td>
<td>FU: 5 y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US: IHBD 6 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal ALT and GGT</td>
</tr>
</tbody>
</table>
abnormalities and definitively eliminate the risk of bleeding because of varices. Because of the risk of specific complications, symptomatic bile duct abnormalities in a child with portal vein obstruction also are likely to be an indication for shunt surgery even if the risk of bleeding is not high as shown by grade I varices only.

There have been, however, a few adult patients for whom portal shunt surgery has failed to completely cure the biliary problem; such patients had biliary strictures that did not regress after shunt surgery.12 These surgeries may have failed because of ischemic lesions of the bile duct or a longer duration of the compression of the bile ducts resulting in

Figure 1. Color doppler sonography of the liver hilum in patient 4 showing anechoigenic biliary tree dilation (between crosses) contrasting with the colored blood vessels of the cavernoma.

Figure 2. Patient 2: (A) portography shows large para-choledocal veins (●) replacing the portal vein from its origin with hepatofugal flow in the splenic, the inferior mesenteric, and the posterior gastric veins (●). (B) Percutaneous transhepatic cholangiography shows the important dilation of intra- and extrahepatic ducts above a mild stricture of the common bile duct. There is an important angulation of the bile duct at the level of the stricture (●). (C) Computed tomography with intravenous injection of contrast media at the level of the pancreatic body shows the close relation between the para-choledocal varices that are enhanced (●) and the bile duct (●), with an aspect of narrowing of the common bile duct by the extrinsic compression of the cavernoma.
permanent damage or to an insufficient lowering of the portal pressure as a result of an only partially effective surgical shunt. This could be used as another argument to perform shunt surgery early.

We thank Dr Fournier-Favre for follow-up information on patient 6.

REFERENCES

ORBITAL VARICES DIAGNOSED AS EPISCLERITIS IN A CHILD WITH JUVENILE IDIOPATHIC ARTHRITIS

A 10-year-old girl with juvenile idiopathic arthritis (JIA) was followed in the ophthalmology clinic for 2 years with a diagnosis of episcleritis after recurrent episodes of a unilateral painful eye with left lateral scleral injection. She denied having diplopia, photophobia, headache, or visual loss. Examination revealed a normal fundus and no signs of uveitis. She was treated repeatedly with topical steroids, and each attack settled within a few days. She then presented with a proptosed, red, painful left eye associated with diplopia. Examination revealed temporal scleral injection, proptosis of 7 mm with no signs of uveitis, and a normal fundus. After conservative treatment, her symptoms resolved by day 3.

Full blood count, vasculitic screen, and serum ACE were normal. The MRI scan revealed a serpiginous soft tissue structure medial to the left lateral rectus muscle (Figure). A diagnosis of left orbital varices was made.

Episcleritis and scleritis are exceptionally rare in definite cases of JIA. Their appearance should initiate a reexamination of the original diagnosis to one more likely to be associated with episcleritis. Arthritis associated with systemic vasculitis, sarcoidosis, or inflammatory bowel disease\(^1\) may resemble polyarticular JIA with extrarticular features developing several years after the onset of arthritis. These conditions may present with episcleritis or scleritis.

Orbital varices usually are seen in children as a dark blue swelling in the superomedial part of the orbit, orbital hemorrhage, proptosis,\(^2\) or intermittent exophthalmos that can be brought on by bending or by the Valsalva maneuver.\(^3\) Our patient appeared to have intermittent dilation of the episcleral varix in the absence of noticeable proptosis or exacerbation on the Valsalva maneuver for a few years before symptomatic proptosis occurred. Delays in diagnosis were also compounded by the rare coincidence of this orbital tumor with polyarticular JIA. The difficulties of imaging of low-flow lesions are also exemplified by this case, in which the acute CT scan showed an indistinct soft tissue mass. Despite initial MRI findings, MRI 3 months after acute proptosis did not show varices on venography. Color Doppler ultrasound and orbital venography, however, have been useful.\(^4\)

Conservative treatment is the rule, except when complications have occurred,\(^4\) as surgery can be complicated and hazardous.

It is important to consider the more unusual and occasionally more serious differential diagnoses of painful red eyes in children with systemic disease. The atypical presentation in our case may have delayed diagnosis. On clinical suspicion of a retrobulbar mass, ophthalmologic review and imaging should be obtained immediately.

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REFERENCES
CONGENITAL MALALIGNMENT OF THE GREAT TOENAILS MIMICKING ONYCHOMYCOSIS

A healthy 2-year-old girl was referred for toenail thickening and yellow discoloration; she was otherwise asymptomatic. The patient had no history of diabetes, infection, or trauma to her toenails. Physical examination revealed both great toenails to have marked thickening of the nail plate with closely spaced transverse superficial ridging, lack of luster, and dark tan-yellowish discoloration (Figure 1). There was no subungual debris. There was mild separation of the nail plate from the underlying nail bed (onycholysis) and subungual hemorrhage on her right great toenail. Micronized toenail clippings failed to grow dermatophytes or nondermatophyte yeasts or molds.

The bilateral presentation, persistence, absence of a history of trauma, and negative fungal culture confirm the diagnosis of congenital malalignment of the great toenails. This condition, which is often misdiagnosed and treated as onychomycosis, may occur in 1% to 2% of children (personal communication: R. Baran. E-mail, June 24, 2003). Congenital malalignment of the great toenail describes the condition in which the longitudinal axis of the nail is laterally deviated from birth1 (Figure 2). A malaligned nail matrix causes angular lateral nail plate growth. The toenails develop transverse ridges, appear thickened, and acquire gray, green, or brown discoloration.1 The ridges occur at regular intervals and appear to result from repeated microtrauma to the nail matrix. Hemorrhage and infection cause discoloration.2 In addition, they may be tender to palpation. One or both great toenails may be involved, and other toenails are occasionally also affected. Associated paronychia frequently develops.

Properly fitting footwear reduces microtrauma that causes transverse ridging. Treatment may involve surgical realignment of the nail matrix.2 Spontaneous improvement by 5 and 10 years of age, however, has also been reported.3 Although toenail disorders in children are rare, recognition of congenital malalignment of the great toenail can reduce the cost in time, money, and adverse treatment effects spent on children mistakenly treated for onychomycosis.

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REFERENCES

Figure 1. Photograph of affected toenails shows yellowish-tan discoloration, horizontal ridging, and lateral deviation.

Figure 2. Diagram shows lateral deviation of the nail matrix relative to axis of distal bony phalanx and resulting direction of nail growth.
Treatment of adolescents with gynecomastia

This letter represents the author’s views and does not constitute an official position of the Department of Health and Human Services.1

To the Editor:

The potential beneficial outcomes attributed to raloxifene or tamoxifen for the treatment of pubertal gynecomas-tia,1 in the absence of any data showing the evolution of the control group, appear to be overreaching, particularly considering that this condition is self-limited.2,3 Although the title indicates beneficial effects when using these medications, the authors properly acknowledged the shortcomings of the study and the imperfect value of its retrospective design. The main limitation of the paper, however, is not this methodological constrain but the lack of depiction of outcome data in the 13 control subjects. This outcome, however, is listed as 50% total resolution—by self reports—in the Discussion section. Results of the objectively assessed changes in breast size in this group, by the same medical team, could have provided a clearer picture of the natural evolution of the gynecomastia and would have allowed for comparison of these changes to the changes in breast size experienced by the boys receiving either tamoxifen or raloxifene, thereby giving a true estimate of the actions of all different interventions.

In summary, after more than 35 years of tamoxifen availability and more than 20 for raloxifene, we still do not know whether any of these medications could be of use for the treatment of pubertal gynecomastia. I agree with the authors and the editorial that underscore the need for well-designed studies.

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10.1016/j.jpeds.2004.10.041

REFERENCES


To the Editor:

I read with interest the paper by Lawrence et al on the use of selective estrogen receptor modulators (SERMS) in the treatment of adolescents with gynecomastia.1 Although this was an uncontrolled, retrospective chart review, the implications of the data are interesting. I am, however, concerned that there is no mention of proper obtaining of written informed consent. The authors state that, “The choice of therapy was based on current clinical practice in the clinic with increasing trend over time toward treatment with tamoxifen and in later years, with raloxifene.” To my knowledge, neither drug is presently approved in the United States or in Canada for the use in males with gynecomastia, hence the systematic treatment of male youth with SERMS for research purposes certainly requires informed consent. If none was obtained, I think the authors should at the very least state whether their IRB exempted them from written informed consent.

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YMPD1282
10.1016/j.jpeds.2004.10.048

REFERENCES


Reply

To the Editor:

Dr. Malozowski makes a very important point about the natural history of spontaneous regression of pubertal gyneco-mastia. Although the longer duration of gynecomastia in the treated groups reduces the chance of spontaneous resolution in these patients, we cannot quantify treatment effect. This underscores the need for an untreated control group in future studies.

Dr. Mauras’ comments highlight the issue of off-label use of medications, which is common in pediatrics as many drugs are not approved for use in children. Our objective at the time the medications were administered was purely clinical based on reports in the literature that tamoxifen is safe and effective in the treatment of gynecomastia in teens and adults. As such, written consent was not required. Informed consent
is certainly necessary in the use of innovative therapies, and verbal consent was clearly documented in the chart, including information on the absence of long-term or controlled trials. We performed a chart audit to further evaluate our clinical impression of the efficacy and safety of the treatment, leading to the retrospective chart review and reporting of our experience. Canada is regulated by its own research ethics guidelines that are philosophically similar, but not identical to, the Code of Federal Regulations, which are used in the United States. Research Ethics Board approval wasn’t required for retrospective chart reviews in our center at the time the study was conducted. They now fall under the purview of the Research Ethics Board under an expedited review stream, but written informed consent is generally not required.

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YMPD1283
10.1016/j.jpeds.2004.10.049

“Globesity” and units of measurements

To the Editor:

The short report by Towbin et al1 was interesting and serves as a reminder that we in “developed” countries must be cognizant of disease states that our international colleagues might recognize more easily. Perhaps more accurately, given our current global epidemic of obesity (globesity), the United States is an “over-developed” country. We also must be cognizant of the units of measurements for laboratory results. There seems to be an error in their Table comparing laboratory values of the three cases of beriberi: “Free T4” is denoted in the Table but the reference range and the units of measurement (5.6–11.7 μg/dL) do not correspond to free T4. This reference range and the units reflect “total” and not “free” T4; serum free T4 concentrations typically range about 0.8 to 2.3 ng/dL in older children and teens, when measured in metric mass, non-SI units. Furthermore, if one presumes that the total T4 was indeed measured, then Case 2 shows evidence of central hypothyroidism, given low total T4 and non-elevated thyrotropin. I am concluding that Case 1 had total T4 assessed but free T4 indeed was measured in Case 2.

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Department of Pediatrics
Division of Endocrinology
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YMPD1276
10.1016/j.jpeds.2004.10.042

REFERENCE


Reply

To the Editor:

Dr Schwartz’s conclusion is correct; none of the patients was hypothyroid, and we appreciate his correction of this error.

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Children’s Hospital Medical Center
Cincinnati, OH 45229

YMPD1277
10.1016/j.jpeds.2004.10.043

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General Policies and Guide for Authors

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account/Auhtutorial.pdf. Manuscripts must adhere to the standard layout and length guidelines.

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**Format.** Original articles should not exceed 6 published pages; do not exceed 18 manuscript pages, including the title page, references, and tables. Figures are calculated at 3 per printed page. Failure to comply with length restrictions may result in a delay in the processing of your paper.

**Potential Reviewers.** To assist with a prompt, fair review process, authors should provide in the submission letter the names, complete addresses, fax numbers, and e-mail addresses of 5 to 7 potential reviewers with the appropriate expertise to evaluate the manuscript. Failure to provide 5 to 7 potential reviewers may result in delays in the processing of your paper.

**Letter of Submission.** A Letter of Submission must accompany the paper and provide the following information in accordance with the “Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication” available at http://www.icmje.org:

- Disclosure of any prior publications or submissions with any overlapping information; a copy of the work(s) must be provided;
- A statement that the work is not and will not be submitted to any other journal while under consideration by The Journal of Pediatrics;
- A statement of any potential conflict of interest, real or perceived; this must also appear on the title page;
- A statement that all the authors listed on the manuscript take full responsibility for the manuscript; if more than 6 authors, an explanation of the contributions of each author must be provided.

**Title Page.** The title page should include authors’ full names and academic degrees; departmental and institutional affiliations of each author; and sources of financial assistance or potential conflicts of interest, if any. Listed authors should include only those individuals who have made a significant, creative contribution to the manuscript; a list of more than 6 authors must be justified to the Editors in the Letter of Submission. One author must be designated as the correspondent, with complete address, business telephone number, fax number, and e-mail address. Include a list of key words not in the title and a short, running title. Proofs and order forms for reprints will be sent to the corresponding author, if the paper is published. The corresponding author is responsible for communicating with the Editorial Office and all other co-authors.

**Abstract.** Full-length papers for the Original Articles section of The Journal of Pediatrics must include a structured abstract of 200 words or less, to appear after the title page, in the general outline described by the Ad Hoc Working Group for Critical Appraisal of the Medical Literature (Ann Intern Med 1987; 106:598-604 and 1990; 113:69-76). The abstract must contain the following headings: Objective(s), Study design, Results, and Conclusion(s).

The objective(s) reflects the purpose of the study, that is, the hypothesis that is being tested. The study design should include the type of study, the setting for the study, the subjects (number and type), the treatment or intervention, principal outcomes measured, and the type of statistical analysis. The results section should include the outcome of the study and statistical significance if appropriate. The conclusion(s) states the significance of the results.

In lieu of the structured abstract, Clinical and Laboratory Observations manuscripts should include a brief summation of 50 words, without headings.

**Laboratory Values.** Laboratory values should be described in metric mass units. The International System of Units (SI units) can be provided in parentheses immediately after metric units. Conversion tables are available (see JAMA 1986; 255:2329-39 or Ann Intern Med 1987; 106:114-29).

**Drug Nomenclature.** Drugs should be described in both the United States Adopted Name (USAN) and International Nonproprietary Name (INN) nomenclature. At first usage cite the USAN with the INN in parentheses; subsequent appearances should use the USAN only.

**References.** References must be numbered according to order of appearance in the text and use superscript or parenthesized numbers in the text. For reference style, follow the format set forth in “Uniform Requirements for Manuscripts Submitted to Biomedical Journals” (http://www.icmje.org/), with journal abbreviations according to Cumulated Index Medicus. If reference is to an abstract, letter, or editorial, place the appropriate term in brackets after the title.

**Examples of references.** (if 6 or fewer authors or editors, list all; if 7 or more, list first 6 and add et al):

**For Journal articles:**


**For books:**


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**Invited Commentaries**

Commentaries are generally invited only. Authors who wish to propose a commentary can submit a proposal letter and outline to the Editors for approval before submitting the full paper.

**Clinical and Laboratory Observations**

Papers in this format should fill 3 journal pages or less, the text 1000 words or less with a brief abstract of 50 words or less. A combined total of 2 illustrations and tables, and approximately 10 references, is recommended.

**Insights**

Submissions to the Insights section of The Journal should succinctly illuminate clinical problems or solutions of interest to readers and must fit on one published page. Captioned photographs, brief anecdotes or analyses, or even cartoons are welcome; however, a fresh, useful clinical insight must be offered. All material must be original. Text must not exceed 300 words and is subject to shortening if the text and figure(s) do not fit on one published page. Figure(s) and references may be placed in the online only version of The Journal if the piece exceeds one published page. Photograph(s) must be original glossy prints, and artwork must be in the original form (see “Illustrations” above). Original, signed, written permission from the patient, or parent or guardian of a minor child, is required for publication of recognizable photographs in all forms and media. (See “Permissions” above.) Contributors will be required to sign a standard copyright transfer agreement; therefore, all submissions must have a title. Submissions will undergo review by the Editors, and their decision to accept or reject will be final (figures from rejected contributions will be returned).

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**Books for Review**

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  - List of abbreviations (double-spaced)
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April 2005


May 2005


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