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The stringent requirements for an 80-hour work week and only 30 hours “on call” has led to much soul-searching by the faculty of pediatric departments, including educators and program directors. The impact of these strict time limits has caused the American Academy of Pediatrics, the Association of Pediatric Program Directors, and the Association of Medical School Pediatric Department Chairs to both focus on and attempt to define the core competencies required to appropriately educate the contemporary pediatric resident. The central question is whether academic pediatric centers can train residents to meet the child health needs of today and the future.

The prediction that most children will never be admitted to a hospital for an acute illness during their childhood further complicates this issue. In addition, most inpatients in pediatric hospitals will have complex chronic diseases that involve many different groups of pediatric medical and surgical subspecialists. To a major extent, the health care needs of children in this century will occur in an outpatient setting and will be aimed at the prevention of both childhood and adult diseases. Many of these predictions are coming true and are having an appreciable impact on child health professionals of today.

This commentary focuses on a different, but still relevant issue. The illness patterns of children today are so radically different from those seen in my childhood years (1940s and 1950s) and training years (1965 to 1975) that any predictions we make today may not reflect the magnitude of the changes that actually will occur. Had we been effective “crystal ball gazers” in 1970, we might have not only anticipated these changes, but also defined a more effective educational process to match the necessary core competencies in child health care in the first decade of the twenty-first century.

To give some perspective to this claim, compare the prevalent diseases during my childhood and my years of medical school and residency with those seen today (Table). Not only was the diagnosis and treatment of these disorders a component of daily pediatric practice, but many of the “common childhood disorders” had consequences or side effects that were clinical challenges as well. For example, mumps orchitis, rubella-induced arthralgias, postvaricella cerebellar ataxia, and measles-induced pneumonia were frequently encountered then. At that time we could not have anticipated that children today would suffer accidents from 4-wheel all-terrain vehicles and skateboarding off walls and ramps, nor could we have predicted the growing antibiotic resistance of common organisms, the rising incidence of autism, the high rates of suicide and murder in children and adolescents, or the widespread obesity and type II diabetes mellitus.

It is remarkable to compare the widespread malnutrition in the rural south in the 1940s to 1950s, identified by Robert F. Kennedy, to the all-pervasive obesity seen today. Acanthosis was a rare finding in the past, but is now extremely common. This list goes on, but the point remains the same: Our discipline is now concerned with radically different disorders than those seen in the lifetime of a pediatrician now in his early 60s.

Ideally, as the health issues of children change, the training of pediatric residents should change accordingly. As a nation, we are fortunate that the process of residency education and its programmatic requirements are constantly reviewed, with changes recommended by the Residency Review Committee of the Accreditation Council for Graduate Medical Education at 5-year intervals. These reviews should permit a dispassionate inquiry into the actual health conditions of childhood and a consideration of relevant core competencies. A number of groups have identified numerous important deficiencies in residency education, and several of these deserve comment. The perceived needs include (1) a deeper understanding of the care of the special needs child; (2) better care of chronic, debilitating, complex medical conditions, such as chronic asthma, type II diabetes mellitus/insulin resistance/obesity (the so-called “metabolic syndrome”); and (3) realization of the impact of violence directed against
children on medical and psychosocial morbidity and mortality. A fourth need is to improve training in the neurosciences and associated conditions, such as attention deficit hyperactivity disorder, learning disorders, and psychiatric disorders.

Although my thesis is that we cannot truly imagine the changes in child health needs over the coming decades of the twenty-first century, we can anticipate that genetic issues will emerge. The definition of the human genome has led to an understanding of the number of genes and their function and to a fuller appreciation of common polymorphisms of many genes, or allelic variation. These polymorphisms can define differences in enzyme action, receptor binding, and drug metabolism. Just as DNA analysis has affected crime detection and death row events, genetics may be used to predict cardiovascular disease and certain types of cancer. The contemporary pediatrician will need to have more genetic information at hand, coupled with a far keener appreciation of the fields of probability and biostatistics.

Ideally, an education in genetics and probability should begin in high school. Waiting until pediatric residency to learn genetic principles is too late. Indeed, the 36 months of residency are far too crowded with other information necessary for practice, and the resident must enter with fundamental genetic knowledge.

I sincerely hope that our discipline can embrace a pattern of education based on “core competencies.” A component of these competencies, along with communication and professionalism, must be to acquire a knowledge base sufficient to deal with contemporary health problems and relevant diseases. Now is the perfect time to focus on these issues.

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In the United States, 65% of adult citizens are overweight, and almost one third are severely obese. This number is double the number from 20 years ago. Fifteen percent of children ages 6 to 19 years are overweight. This is 3 times higher than the number in 1980. If the United States population continues to gain weight at this remarkable rate, some experts predict that nearly every person in North America could be overweight by the year 2030. The obesity problem appears to be magnified in the US. This fact is most likely due to the country’s overall level of development of an obesogenic environment.

Other industrialized countries are also experiencing a tremendous increase in obesity, particularly in children. Childhood obesity has been identified in all social economic groups in industrially developed countries. This suggests that obesity is due mainly to poor lifestyle behaviors such as the consumption of fats and sugared products, which are commonly combined with an environment that has a marked reduction in physical activity. There is an urgent need, then, for a collective approach to the prevention, treatment, and management of obesity, particularly with respect to the problems this condition presents during the growing years.

The cause of this epidemic is multifactorial. In its most basic terms, it is the result of decreased energy expenditure and increased energy intake. In the last 2 decades, there has been a dramatic decrease in physical education that is offered in our school systems. The opportunities for spontaneous physical activity in the daily lives of our children are also decreasing. Many modern conveniences are available that compound this problem. A remote control for a television set is a blatant example. There are too many opportunities to increase the intake of calorie-dense foods offered in a convenient package (fast foods). Consumption of sweetened drinks and other snack foods is increasing. This is especially true in our schools.

One behavior that increases the amount of sedentary activity is screen time. The most common source of this screen time is television, but it also includes the use of computers and video games. Children are not active when they are engaged in these activities, and, in fact, they may also increase their caloric intake with junk food.

According to recent data, the average child or adolescent watches an average of nearly 3 hours of television per day. This figure does not include time spent watching videotapes or playing video games. There appears to be at least 3 distinct household factors that affect the access to television by youth. Viewing appears to be independently associated with having a television in the bedroom, lack of parental limit-setting on television time, and having few or no dinners involving sitting down as a family. One study has demonstrated that almost 40% of children have a TV set in their bedroom. These children are more likely to be overweight and spend more time watching TV or videos than children without a television in their bedroom. The long-term effects of this television viewing are dramatic. Children who watched the most television during childhood had the greatest incidence of increased body fat over time. In this issue of The Journal, Davison et al have demonstrated that girls watched significantly more TV when their parents were high-volume TV viewers or when their parents relied on TV as a recreational activity and failed to limit their access to TV. The authors encourage parents to limit their own TV viewing, reduce family TV viewing, and limit their children’s access to TV. This study demonstrated various “risk factors” that increased the likelihood that these girls would continue to watch TV and use TV as a form of entertainment as they grew up. They are, in a sense, learning that TV is not only a normal part of life, but it is their chief form of recreation.

The results of this study are not surprising. Previous studies have demonstrated that parents who consumed fewer fruits and vegetables tended to have daughters who consumed fewer fruits and vegetables. Other studies have demonstrated that there is a relationship between a mother’s and a daughter’s weight and their dietary disinhibitions and eating habits. Similarly, studies have shown that girls engaged in significantly higher levels of physical activity when at least 1 parent reported high levels of overall support for their daughter’s continued activity. These studies indicate that there is a positive contribution that parents can have on the

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See related articles, p 436, and p 429.
The diagnosis of UTI is confirmed when an appropriately obtained specimen of urine is documented to have both significant bacteriuria and pyuria. Definitions of significant bacteriuria are based, in part, on the method used to collect the urine specimen. When urine is collected by suprapubic aspiration, a method that bypasses the urethra, any colony count is considered to represent significant bacteriuria. All other methods of urine collection, (mid-stream clean catch, catheterization, and bag collections) require passage of urine through the urethra. Although obtaining a urine specimen by bag is easy and noninvasive, transurethral collections of urine will invariably be contaminated with both bacteria and white blood cells (WBCs) that originate from outside of the urinary tract. Accordingly, urine specimens obtained by bag are never recommended for culture.

Because the results of urine cultures are not available for at least 24 hours, there has been considerable interest in evaluating tests that may predict the results of the urine culture so that appropriate therapy can be initiated at the first encounter with the symptomatic patient. The tests that have received the most attention are urine microscopy for leukocytes and bacteria, and biochemical analyses for leukocyte esterase and nitrite that can be assessed rapidly by dipstick.

Recent studies to evaluate the best predictors of UTI have shown conflicting results. In 1999, Gorelick and Shaw concluded that both the presence of any bacteria on a Gram stain of an uncentrifuged urine specimen and activity and dietary habits of their children. The obesity epidemic is a “perfect storm” caused by a variety of obesogenic factors. It will take an organized effort of prevention to lessen the impact of this storm. Certainly, the decreased consumption of fast foods, sweetened drinks, and other high-calorie–dense foods will help. Children also need to be more physically active, and, as a society, we must decrease the opportunities for children to be sedentary. Television viewing is one of the most common forms of sedentary activity practiced by our children. This practice has led the Academy of Pediatrics to recommend that no more than 1 to 2 hours of quality television programming be viewed per day. This study, in this month’s Journal, supports the recommendation that parents need to limit and control the amount of television that they and their children view. This appropriately transfers some of this responsibility to the parents because most television that is viewed occurs in the home. Certainly the limitation of television viewing in and of itself will not solve the obesity epidemic. This is true of any other risk factor that is contributing to this perfect storm. For example, the total elimination of fast foods in the diet also will not solve this issue, but it will help.

Society, as a whole, must realize that to effectively control and prevent this obesity epidemic, all risk factors must simultaneously be reduced. If television viewing is going to be effectively decreased, parents must assume an appropriate role and set an example.

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**TO BAG OR NOT TO BAG**
I n this issue of The Journal, te Braake et al report the results of a randomized open-label trial evaluating the short-term safety and efficacy of amino acid administration to premature infants initiated immediately after birth. Very low birth weight infants (n = 135) were randomized to 2 different parenteral amino acid regimens. Infants in the intervention group received 2.4 g/kg/day of amino acids within 2 hours of birth; this level of amino acid intake was then maintained for the first 4 days of life. Infants in the control group received glucose alone on the first day of life, with a stepwise increase in amino acid intake thereafter (1.2 g/kg on day 2 and 2.4 g/kg on days 3 and 4). The investigators demonstrated positive nitrogen balance at 2 and 4 days of life in the group of infants who received early amino acids without any major adverse effects, whereas infants in the control group were in negative nitrogen balance on day 2.

The findings of this study are in agreement with previous studies that overwhelmingly demonstrated that negative nitrogen balance can be reversed in early postnatal life with amino acid intake of 1.1 to 2.5 g/kg/day and energy intake as low as 30 kcal/kg/day. The acute reversal of protein catabolism in response to varying amino acid intake is a consistent finding in all of these studies, despite differences in the composition of amino acid solutions used. Recently, Thureen et al prospectively evaluated the effect of a higher level of amino acid intake (3 g/kg) in extremely low birth weight infants to more closely duplicate fetal amino acid delivery rates. This study demonstrated the efficacy of 3 g/kg/day versus 1 g/kg/day in improving protein balance and increasing protein accretion, primarily through increased protein synthesis. Other studies using stable isotope techniques have also demonstrated that net protein accretion is accomplished by an increase in protein synthesis, rather than a reduction in proteolysis in premature neonates.

Recent studies of early amino acid administration, including the current study, have not demonstrated any short-term adverse metabolic effects. In addition, the study design used by te Braake et al demonstrates that the common practice of a stepwise increase in amino acid intake is unnecessary. Although an elevated blood urea nitrogen level is often cited as a reason to decrease amino acid administration, a recent study found no correlation between blood urea nitrogen level and amino acid intake in parenterally fed preterm neonates. Furthermore, elevated blood urea nitrogen may in fact reflect higher rates of amino acid oxidation, which more closely resembles the in utero situation whereby the fetus uses amino acids as a significant source of energy.

In the current study, concentrations of most plasma amino acids from infants in the intervention group were found to be normalized compared with those from healthy breast-fed term infants. Other investigators have evaluated amino acid concentrations in preterm infants receiving parenteral nutrition and have used other reference standards, such as concentrations obtained from fetal cordocentesis, cord blood, and plasma from premature infants receiving parenteral nutrition. The choice of an appropriate reference standard has important research implications as the compositions of amino acid solutions are further refined to better meet the needs of preterm neonates.

Postnatal growth failure is nearly universal and is associated with an increased risk of poor neurodevelopmental outcome in extremely low birth weight infants. Wilson et al, in a randomized clinical trial of aggressive nutritional support, demonstrated that early parenteral nutrition (combined with early enteral feeding) resulted in better growth in the early neonatal period and at hospital discharge. Observational studies and related research have established amino acids as a significant source of energy.
studies also support changes in short-term growth outcomes in response to early amino acid and protein intake.\textsuperscript{14,15} There is also an emerging body of observational data suggesting that early amino acid intake has longer-term benefits on neurodevelopmental outcomes.\textsuperscript{16}

Despite accumulating evidence of the efficacy of parenteral amino acids, many clinicians delay administration of parenteral amino acids in this population of infants. Reluctance may be related to concerns of safety, logistics, or lack of conclusive efficacy data. In addition, even with the best of intentions, actual intake often varies considerably from that prescribed in the parenteral nutrition order because of interruptions for medications, blood transfusions, or alterations in glucose or electrolytes prompting discontinuation of parenteral nutrition.

During the NICHD Neonatal Research Network randomized clinical trial of parenteral glutamine supplementation conducted between 1999 and 2001, extremely low birth weight infants did not achieve an intake of 3 g/kg/day of amino acids until the tenth day of life on average.\textsuperscript{17} Significant differences were observed among the 15 participating centers, with 5 of the centers providing early amino acids to a greater proportion of their infants. Considerable site variation related to nutritional practices has also been reported by other investigators.\textsuperscript{18,19}

From a practical standpoint, the goal of providing early amino acids can be accomplished in a relatively easy and efficient manner if the hospital pharmacy has a “stock” amino acid and dextrose solution readily available for use 24 hours a day. A solution consisting of 7.5% dextrose and 5% neonatal amino acids can be administered at a rate of 60 ml/kg/day to provide 3 g/kg/day of amino acids. In addition to ready availability, another advantage of this approach is that parenteral nutrition does not need to be interrupted if any changes in glucose concentration and/or electrolytes are needed. Additional fluids can be confused or “Y’d” in to the intravenous line as necessary to maintain glucose homeostasis and fluid and electrolyte balance.

Although the short-term efficacy of amino acids in reversing protein loss in extremely preterm neonates is clear, it is important to point out that few prospective studies have evaluated the longer-term effects of nutritional interventions in this population. Although there is a growing body of evidence supporting the long-term benefits of early nutrition on growth, neurodevelopment, and even adult-onset disease processes, future studies are needed to prospectively evaluate optimal nutritional support for the most preterm neonates including long-term growth and neurodevelopmental outcome measures. Nonetheless, providing extremely low birth weight infants with a minimum of 2.5 to 3 g/kg/day of parenteral amino acids as soon as possible after birth seems to be a reasonable recommendation based on the currently available evidence, which indicates that this is safe, effective, and feasible.

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Any clinical syndromes assembled in the Online Mendelian Inheritance in Man database have been associated with short stature. Mutations in single genes have been identified for some of these syndromes; examples include genes associated with bone development and skeletal dysplasia and genes of the growth hormone pathway.\(^1\)\(^-\)\(^4\) Quite a number of growth-controlling genes encode transcription factors, and many of these belong to the homeodomain-containing family.\(^5\)\(^,\)\(^6\) The sequencing of a variety of candidate genes in individuals with idiopathic short stature (ISS) has also greatly added to our understanding of genes directly responsible for short stature.\(^7\)

Complex traits such as stature have a multifactorial background, because of the genetic variation within the population and the number of qualitative trait loci involved. Three major approaches have been used to identify genes underlying complex traits: linkage analysis, association studies, and candidate gene analysis. Genome-wide linkage analysis have identified several genomic regions that affect growth, including 3p26, 5q31-36, 7pter, 9pter, 12p11-q14, 13q32-33, Xq25, and Xp22.\(^8\)\(^-\)\(^11\) Furthermore, association studies between gene variants and short stature are complementary and have led to a number of associations.\(^12\) Gene variations in the known growth-controlling genes may also turn out to be candidates for modulating growth and adult height in the general population (Table; available online at www.jpeds.com). Some of these genes have also been associated with the related trait of pubertal timing, which is also a highly heritable complex trait influencing adult height.\(^2\)

One rather well-studied gene is the short stature homeobox gene (SHOX), thought to be responsible for the height deficit associated with Turner syndrome\(^13\) and the short stature and skeletal anomalies associated with Leri-Weill dyschondrosteosis (LWD) and Langer dyschondrosteosis.\(^14\)\(^,\)\(^15\) The distal end of Xp22.3 and Yp11.3, where the SHOX gene resides, is composed of identical 2.6-Mb DNA sequences on the X and Y chromosomes.\(^16\) Distal Xp and Yp, termed the short-arm pseudoautosomal region (PAR1), is where the X and Y chromosome recombine during meiosis. Because similarly small Xp and Yp terminal deletions both lead to short stature, it has been suggested that a growth gene escaping X-inactivation resides in PAR1, and that haploinsufficiency of this growth gene, inherited as a dominant phenotype, causes short stature in both sexes.\(^17\) However, SHOX haploinsufficiency (ie, 1 copy instead of 2 copies) causes not only short stature, but also in some cases characteristic skeletal features, including Madelung deformity; partial dislocation of ulna at the wrist, elbow, or both; curvature of radius and ulna/tibia; short fourth metacarpals; cubitus valgus; and a high arched palate (Figure).

Expression analysis in human embryos has shown that SHOX is expressed in the developing skeletal tissue, especially in the distal limbs and in the derivatives of the first and second pharyngeal arches, coincident with the skeletal and craniofacial abnormalities seen in Turner syndrome.\(^18\) The endogenous SHOX protein is also found in fetal and pubertal growth plate chondrocytes, particularly in the terminally differentiated hypertrophic chondrocytes.\(^19\)\(^,\)\(^20\) Extensive clinical and molecular studies have demonstrated that SHOX haploinsufficiency is implicated in approximately 3% of individuals with ISS\(^21\) and that it represents the predominant factor responsible for the Turner skeletal features and LWD.

Homozygosity for the SHOX deficiency causes the much more severe phenotype of Langer mesomelic dysplasia, a severe form of limb shortening marked by markedly hypoplastic fibulae with short legs and dwarfism.\(^22\) Conversely, SHOX overdosage, as seen in sex chromosome polyploidy, including 47,XXX, 48,XXXX, and 47,XYY, is associated with tall stature at pubertal age.\(^23\) Triple SHOX dosage would also explain the relatively tall stature in persons with Klinefelter syndrome (47,XXX), which is almost totally the result of increased leg length. The genes that control SHOX expression are still as elusive as the genes that are controlled by SHOX.

Ross et al,\(^24\) in this issue of The Journal, further refine the phenotypical characteristics of SHOX deficiency by contrasting the findings in children with LWD and Turner syndrome. Their study illustrates the unique power of a truly global pharmacoepidemiologic observational study that exceeds the capacity of any investigator-driven research project. We now know from this international survey that short stature is common in both LWD and Turner syndrome, with a mean height deficit of -2.3 to -2.7 standard deviation score in girls with LWD and -2.4 to -2.7 standard deviation score in girls with Turner syndrome. These data suggest that SHOX haploinsufficiency is responsible for most or all of the height deficit observed in Turner syndrome in childhood. The characteristic skeletal features, including increased carrying angle, scoliosis, and Madelung deformity of the wrist, are much more common in girls with LWD than in those with Turner syndrome. Ross et al
discuss that females with Turner syndrome may be protected from developing Madelung deformity by their sex steroid deficiency, whereas females with LWD generally have normal ovarian function and might not have such protection. But in fact, these authors actually favor a different explanation, because dyschondrosteosis is usually not evident at birth despite the high circulating estrogen levels occurring during pregnancy. In addition, Madelung deformity does not develop in girls with Turner syndrome treated with estrogens. Thus, Ross et al consider it less likely that estrogen promotes the skeletal features in patients with LWD, and speculate on the modifying effects of other genes on the X chromosome. There is no evidence as yet that growth hormone therapy, an integral part of clinical management of Turner syndrome, worsens skeletal anomalies by accelerating distorted skeletal growth resulting from imbalanced premature fusion. Indeed, beneficial effects of growth hormone therapy have been described in patients with SHOX haploinsufficiency, whereas females with LWD generally have normal ovarian function and might not have such protection. But in fact, these authors actually favor a different explanation, because dyschondrosteosis is usually not evident at birth despite the high circulating estrogen levels occurring during pregnancy. In addition, Madelung deformity does not develop in girls with Turner syndrome treated with estrogens. Thus, Ross et al consider it less likely that estrogen promotes the skeletal features in patients with LWD, and speculate on the modifying effects of other genes on the X chromosome.

When should the clinician initiate a search for SHOX deletions/mutations? What are the clinical benefits for identifying SHOX deficiency? We now know that children with short stature, short forearms or lower legs, tibial bowing, wrist changes suggestive of Madelung deformity, short fourth metacarpals, increased carrying angle, high arched palate, scoliosis, and muscular hypertrophy are the ones that may have SHOX deficiency. Although Madelung deformity may be difficult to diagnose before puberty, Ross et al state that radial lucenties predate Madelung deformity in many children. The identification of defects in the SHOX gene can promote accurate genetic counseling and appropriate orthopedic and therapeutic intervention. Future research will focus on genes upstream and downstream to SHOX expression that potentially may also aid in devising alternative strategies for therapy.

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REFERENCES
More and more congenital heart defects are detected prenatally. We are gradually appreciating that such defects as severe aortic stenosis can progress in utero in unexpected ways. Fetuses with echocardiographic signs of aortic stenosis in the second trimester may be born with classic hypoplastic left heart syndrome instead of simple aortic stenosis. With this understanding, a few centers have begun to attempt fetal cardiac intervention not only to treat severely ill fetuses, but also to attempt to change the course of development of the heart in fetuses thriving in utero. In this issue of The Journal, Marshall et al. describe their methodology for balloon dilation of the fetal stenotic aortic valve. This is the world’s largest series with 26 attempted procedures. It is exciting that the methodology has progressed to the point where an experienced team can reliably place a catheter into the heart and dilate a valve in an unborn child. Now comes the hard part. Various significant procedural, ethical, and outcomes issues need to be assessed and reassessed as we cross the threshold into this new world of fetal cardiac catheter intervention.

Procedural issues are probably the easiest to examine objectively. The field of fetal cardiac catheter intervention undoubtedly has been advanced significantly with the work, detailed in this issue. Marshall et al describe the development of methods that have improved their technical success rate from 25% of their first 4 patients to 90% of their last 10 patients. The authors emphasize several major methodological points. The factor with the greatest impact on technical success appears to be recognition of the critical importance of attaining an optimal fetal position for inserting the needle and the dilation balloon directly toward the aortic valve. In the earliest attempts at fetal catheter intervention, the teams either had hoped to manage difficult catheter manipulations or had tried to change the fetal orientation through external uterine massage. The needle for introducing the dilation catheter into the heart was inserted percutaneously through the mother’s abdominal wall and thence directly into the fetal heart. External manipulation of fetal position has definite limitations, however, and thus Marshall and colleagues took the bold step of offering a laparotomy to expose the uterus if noninvasive manipulations were unsuccessful. They found that exposing the uterus in this manner greatly improved both fetal positioning and the echocardiographic imaging required for the procedure. This is clearly beneficial to the technical success of the procedure, but significantly increases the gravity of the procedure for the mother. Laparotomy was necessary in 12 of the authors’ last 22 cases. The authors also found that they could be surprisingly aggressive with the size of the dilation balloons. They gradually increased the balloon-to-annulus ratio (ie, diameter of the dilating balloon compared with the diameter of the valve annulus) after finding that the ratio conventionally used in neonates had a variable clinical effect and caused no more than mild aortic regurgitation (AR). As expected, they immediately began to see more AR in the fetuses with relatively larger balloons, but

AORTIC VALVULOPLASTY IN THE FETUS: TECHNICALLY POSSIBLE BUT IS IT READY FOR PRIME TIME?

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0022-3476/$ - see front matter

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10.1016/j.jpeds.2005.09.026
The last decade has seen remarkable advances in genetics, but in many ways the advances show us what we do not know. The sequencing of the human genome led to recognition that we need to understand how proteins work, and that has led to a recognition that there is a lot that goes on in the cell that is not just the consequence of the sequence of the DNA. The term that has been used to describe heritable states that do not depend on the DNA sequence is “epigenetics.” The concept includes inheritable and reversible modifications of chromosomes and DNA, these modifications modulate chromosome structure and gene function and thus affect the phenotype—the structural and functional features of the organism. These modifications are of interest because they are a window into the control and regulation of gene expression and thus are very relevant to tissue specificity and the timing of expression. Furthermore, epigenetic changes have become of interest because when they do not function properly at a cellular level, cancer and other bad things can happen. The “epigenome” is the combination of all the sites in the genome (ie, the DNA sequences) in which there is this kind of control exerted by modification of the DNA sequence (both chemical and physical). An epimutation is a mutation that interferes with normal epigenetic (control) function.

Historically, Lamark (1744–1829) suggested that there might be inheritance of acquired characteristics or traits. He became a laughing stock in his lifetime because he suggested that acquired characteristics (such as when a giraffe stretched its neck) could be transmitted to offspring (the next generation of giraffes would have longer necks). The first time the term “epigenetics” was used is probably during the 1940s by Waddington. He used it to describe the control of genes during development that gives rise to the phenotypes that are observed. McClintock’s work on “jumping genes” in corn is also relevant because the transposable elements can lead to changes in gene and epigenetic expression.

Tissue variation in terms of the specific genes that are expressed is produced by epigenetic control, because the same genes (DNA sequences) are present in every cell, but tissue phenotype variation can be dramatically different, as we all know. Genomic imprinting has been recognized as the phenomenon by which there is expression of an allele (one of the pair of genes) inherited from one parent but not from the allele inherited of the other parent. It is also a form of epigenetics. X-inactivation uses RNA transcripts to silence much of the genetic material on one of the X chromosomes in mammalian females, and this too is a form of epigenetics.

To understand how epigenetic control works, it is important to realize that DNA is folded in a very complex way within the cell, and that complex packaging structures have something to do with control of gene expression as well. Each nucleated cell has about 2 yards of DNA that has to be folded and condensed so that it can fit into the nucleus of the cell. That folding and condensing is done by wrapping DNA around proteins called histones. What has been recognized in the last few years is that secondary chemical changes occur to both the DNA and the histones and these have a lot to do with whether or not a gene will be expressed. In the case of DNA, the modification involves methylation (attaching a carbon group to certain cytosines). In the case of histones, it involves methylation, acetylation, phosphorylation, and ubiquination, all of which are potentially reversible interactions. These DNA and histone modifications are mediated by enzymes, some of which are tissue specific. In DNA, the modified sites (CpG sequences) also seem to be prone to mutation.

In the human genome, there are DNA sequences called CpG islands. A CpG island is made up of length of DNA more than 500 base pairs long that has a high GC (guanine/cytosine) content. These CpG islands are usually outside genes but seem to have a lot to do with gene transcription or silencing. These sites can be identified using a molecular technique that determines whether the DNA is methylated. Consequently, it is possible to determine in a specific tissue (at a specific time) whether a gene is actively expressing.

Studies of the X-chromosome have led to the recognition that RNA antisense transcripts (RNA that uses the complementary base pairs, but “reads” in the opposite direction) hybridize, because of their complementary double-helix structure, to the DNA and through this mechanism turn the DNA off (permanently) for all subsequent cell divisions. Most recently, it has been recognized that if enough RNA is made to create double strands of RNA, it is a signal to the cells to cut up the RNA, and that then creates single antistrands of RNA that can turn the active gene off. The cells’ use of RNA to control gene expression seems to be a complex feedback system with multiple levels of

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control. But, in addition, there are many very small RNAs in the cell that interfere with DNA expression and tie things up all the time. Again, this is called “epigenetic” because it does not change the basic DNA sequence of the cells.

The “take home” message about epigenetics is that the mechanisms by which cells “turn on and off” gene expression are beginning to be unraveled, but it is very complex! DNA methylation, histone modification, and RNA-associated silencing of DNA are important actors.

All of this sounds terribly technical, but the reason it is so exciting and important is that by understanding the way it works, it may be possible to create forms of therapy in which small RNAs are introduced into the cell or methylation of the DNA is enhanced in such a way to accomplish the expression of the genes that are desirable or to turn off those genes that are undesirable.6

Pediatricians know that folic acid is important in the prevention of birth defects. It is not at all clear how folic acid does this, but it may have something to do with turning on and off genes at critical points in development because folic acid plays a major role in methylation.

Interestingly, epigenetic effects are very time and tissue specific in developmental processes. For instance, when a primordial germ cell begins to develop, the methylation of genes that had been present is lost, but as the germ cell matures the DNA become more and more methylated in a parent of origin way (ie, sperm and ova are different). As soon as fertilization occurs, there is loss of DNA methylation again that is then reestablished during tissue-specific development. This in turn must happen properly for genomic imprinting (an epigenetic phenomenon) to be established normally, without which a variety of abnormal phenotypes result. The exact control of all of this is not understood yet either, except that there are known to be very specific and different DNA methylation enzymes at different times in development.8

There are animal models that are relevant to the human situation. For instance, the Agouti mouse is a strain of mice in which there is variable color. Agouti mice that are fully yellow are prone to diabetes, obesity, and cancer. The yellow color was believed to be a maternally inherited trait in that the more yellow the mother was, the more yellow babies she had. In fact, if the grandmother was yellow, there would be even more yellow baby mice—all more prone to disease. There seems to be no male contribution to this trait. The Agouti yellow coat color correlated with the methylation of a transposable element upstream of the parakin protein gene. Interestingly, if Agouti mice are put on high–folic acid diets, they completely lose their yellow color and the secondary diseases. Methyl groups provided by the folic acid attach to the transposon and silence it, leading to normal expression of the parakin gene.9,10 Maybe that is the sort of thing that happens in human beings!

The mechanisms by which gene expression is controlled are key to understanding normal development and health, as well as using molecular mechanisms to prevent and treat disease and disability. Modifications of the secondary structure of DNA seem to be fundamental and, hence, worthy of investigation and understanding. It does seem as if epigenetics is going to be a very important concept for pediatricians!

REFERENCES
TELEVISION VIEWING IN EARLY CHILDHOOD PREDICTS ADULT BODY MASS INDEX

RUSSELL M. VINER, MB, PhD, AND TIM J. COLE, PhD

Objectives   To examine the effects of duration, timing and type of television (TV) viewing at age 5 years on body mass index (BMI) in adult life.

Study design and methods   1970 British Birth Cohort, followed up at 5 (N = 13,135), 10 (N = 14,875), and 30 years (N = 11,261).

Outcome measures   Weekday and weekend TV viewing at 5 years, type of programs, and maternal attitudes toward TV at age 5 years. BMI z-score at 10 and 30 years.

Results   Mean daily hours of TV viewed at weekends predicted higher BMI z-score at 30 years (coefficient = 0.03, 95% CI: 0.01, 0.05, \( P = .01 \)) when adjusted for TV viewing and activity level at 10 years, sex, socioeconomic status, parental BMIs, and birth weight. Each additional hour of TV watched on weekends at 5 years increased risk of adult obesity (BMI \( \geq 30 \) kg/m\(^2\)) by 7% (OR = 1.07, 95% CI 1.01, 1.13, \( P = .02 \)). Weekday viewing, type of program and maternal attitudes to TV at 5 years were not independently associated with adult BMI z-score.

Conclusions   Weekend TV viewing in early childhood continues to influence BMI in adulthood. Interventions to influence obesity by reducing sedentary behaviors must begin in early childhood. Interventions focusing on weekend TV viewing may be particularly effective. (J Pediatr 2005;147:429-35)

Obesity is the major public health challenge of the current day. A typical child in the USA watches television (TV) for 2.5 hours each day. Higher levels of TV viewing in early childhood and later childhood and adolescence have been associated with higher body mass index (BMI) in cross-sectional studies and in longitudinal studies confined to childhood and adolescence. Viewing 4 or more hours of TV per day has been suggested to be particularly associated with childhood obesity. As a result, public health interventions to prevent childhood obesity frequently include efforts to reduce duration of TV viewing. Only 1 study has addressed the long-term effects of childhood TV viewing on adult BMI, reporting that mean weeknight TV viewing between 5 and 15 years significantly predicted higher BMI at 26 years.

TV viewing may increase BMI through displacing physical activity or through unhealthy food choices related to eating while watching or to food advertising. These effects may vary depending on timing and content of viewing and level of parental supervision. However, no studies have examined whether the timing of TV viewing or the type of programs viewed may differentially affect current or later BMI. We used data from the 1970 British Birth Cohort to investigate the hypotheses that higher early childhood TV watching during weekdays and weekends and more favorable parental attitudes toward TV was associated with increased BMI in adulthood.

METHODS

The 1970 British Cohort Study is a continuing, multidisciplinary longitudinal study that takes as its subjects all those living in Great Britain who were born in the week of April.
5-11, 1970. A total of 16,567 babies born in England, Scotland, and Wales were enrolled,15 and subjects have been followed up at 5, 10, 16, 26 and 29-30 years of age. The sample was representative of the UK population in childhood, and attempts are ongoing to maintain the representativeness of the cohort by recruiting additional subjects immigrating to the UK who were born in the source week in 1970. At 30 years of age, 96.3% of the cohort identified themselves as white, with 0.6% black, 1.8% from South Asian ethnicities, 0.8% Chinese or other Asian, and 0.6% of mixed ethnicity. Data were obtained electronically from the UK Data Archive, University of Essex, UK, and SPSS code for cleaning the databases and deriving summary variables were obtained from the Centre for Longitudinal Studies, Institute of Education, London.16-19 Ethical review board approval was obtained by the original investigators but was not required for these analyses of anonymized data.

At 10 years of age (1980), 15,995 cohort members were traced and invited to participate, and data were obtained on 14,875 subjects. Response bias compared with the Birth survey showed no significant social class differences (gain of 1.7% in those with father in manual employment).20 In 2000, when subjects were 29-30 years of age, 14,087 of an estimated 16,695 cohort members were traced and invited to participate, of whom 11,261 (68%) underwent interview.16 Marked efforts were made to recruit difficult-to-reach subjects, and loss of those from lower social classes between the Birth and 30-year surveys was minor (3.9% loss from manual employment).16

### Table I. Daily TV viewing habits at 5 and 10 years

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Sample N</th>
<th>% (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5 years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Television viewing Monday to Friday</td>
<td>Hours per day</td>
<td>8158</td>
<td>&lt;2 hours 59 (5082)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2-3.9 hours 35 (2987)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>≥4 hours 6 (520)</td>
</tr>
<tr>
<td></td>
<td>Days watched after 6 PM</td>
<td>8104</td>
<td>0 60 (4834)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 11 (847)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 5 (405)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 3 (274)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 4 (315)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 18 (1429)</td>
</tr>
<tr>
<td>Television viewing Saturday and Sunday</td>
<td>Hours per day</td>
<td>8158</td>
<td>&lt;2 hours 58 (4757)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2-3.9 hours 32 (2612)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>≥4 hours 10 (856)</td>
</tr>
<tr>
<td></td>
<td>Days watched after 6 PM</td>
<td>8518</td>
<td>Neither 64 (5111)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 19 (1522)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 17 (1336)</td>
</tr>
<tr>
<td>Programs viewed</td>
<td>Children’s programming (excluding cartoons)</td>
<td>8158</td>
<td>Yes 94 (7673)</td>
</tr>
<tr>
<td></td>
<td>Cartoons</td>
<td></td>
<td>Yes 93 (7569)</td>
</tr>
<tr>
<td></td>
<td>Adult programming</td>
<td></td>
<td>Yes 83 (6771)</td>
</tr>
<tr>
<td><strong>10 years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Television viewing habit</td>
<td>Frequency</td>
<td>7350</td>
<td>Rarely or never 1 (63)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sometimes 20 (1461)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Often 79 (5826)</td>
</tr>
</tbody>
</table>

### Childhood Data

At 5 years, mothers provided data on the average number of hours per day that their child watched TV during the week (Monday to Friday) and weekends (Saturday and Sunday). Parents also reported the average number of days per week that their child watched TV after 6 PM during the week and weekend, and the type of TV programs they usually watched (categories being children’s programs [excluding cartoons], cartoons or adult programming [including drama, comedy, quiz programs, sport, news or documentary programs]). Mothers were also asked to indicate agreement on a 5-point Likert scale for questions regarding their beliefs about children and TV as part of a wider 43 item set of questions on maternal beliefs about child rearing. Questions included the following: (1) “Young children who never see TV miss a lot that is of value,” (2) “TV is a useful way of keeping the children amused,” (3) “Young children pick up a lot of bad habits from TV,” and (4) “Children under 5 should never be allowed to watch adult TV.” Factor analysis of the 43 items by the investigators suggested that the 4 questions constituted a separate dimension indicating beliefs regarding the effects of TV on young children; weighted scores for this dimension converted to a z-score in which a higher score indicated
stronger maternal beliefs that TV was harmful for young children.

At 10 years, mothers were asked to rate their child’s usual frequency of watching TV as either “Rarely or never,” “Sometimes,” or “Often.” Parents were also asked to report the frequency that their child played sport in their spare time, rated as “Rarely or never,” “Sometimes,” or “Often.”

Height was measured at 5 years by a trained nurse visitor, and height and weight were measured at 10 years of age by school medical staff using standardized techniques. Height z-scores at 5 and 10 years and BMI z-score at 10 years were calculated using the revised UK 1990 growth reference. Obesity at 10 years was defined using the contemporary definition of BMI ≥ 95th percentile on this growth reference. Height and weight of parents at 10 years of age were measured or self-reported at parental interview, and BMI z-scores for parents were calculated from cohort internal mean and standard deviation. Birth weight was recorded in the Birth survey. Socioeconomic status in childhood was defined by social class (paternal occupation) and maternal educational status at 5 years.

**Adult Data**

Height and weight were obtained by self-report at 30 years. Those pregnant at time of interview were asked to report their pre-pregnancy weight. BMI z-score at 30 years were calculated separately for each sex from cohort internal mean and standard deviation. Obesity was defined as BMI ≥ 30kg/m². Social class was obtained at interview.

**Analysis**

Data were cleaned and derived using SPSS and analyzed using Stata 8. Linear regression was used to estimate the associations of TV watching habits at 5 and 10 years with BMI z-score firstly at 10 years and then at 30 years. Associations were first examined for each factor adjusted for sex, social class, maternal educational achievement, birth weight, and BMI z-score of both parents. Associations were also adjusted for height at 5 and 10 years because BMI z-score is not entirely independent of height. Multivariable regression models were then derived adjusting for all other factors including sex, social class, and height. We then further adjusted the multivariable model at 30 years for BMI z-score at 10 years, because childhood obesity may be on a causal pathway between early childhood TV and adult (30 years) BMI.

**RESULTS**

Data on TV viewing in childhood and BMI at 10 and 30 years was available in 8158 subjects (68% of cohort at 30 years), who form the subject of these analyses. The distribution of data on TV viewing at 5 and 10 years is shown in Table I. Mean hours of TV watched at 5 years was 1.42 (SD 1.30) on weekdays and 1.57 (SD 1.54) on weekends. Data on maternal attitude toward TV z-score at 5 years was available in 13,135 subjects (mean z-score −0.03, SD 1.04). Mean BMI z-score at 10 years was −0.10 (SD 1.0) in the entire cohort and −0.09 (SD 1.0) in the group with data available on TV watching. Mean BMI z-score at 10 years was not significantly different between those who did and did not participate at 30 years. Obesity was found in 399 (4.3%) at 10 years and 931 (11.4%) at 30 years. There was minor additional loss to follow-up of those with higher daily TV viewing (those who watched ≥ 4 hours per day on weekends made up 12% of the cohort at 5 years compared with 11% of those who participated at 30 years).

The associations of TV viewing at 5 years and later BMI z-score are shown in the Figure. Higher duration of TV watching during weekdays and at weekends were both significantly associated with higher BMI z-scores at 10 and 30 years.

**Figure.** Mean hours of TV watched per day at 5 years and later mean zBMI.
obesity by 10% (OR = 1.10, 95% CI 1.03, 1.18, P = .003), when adjusted for maternal attitudes toward TV, TV viewing, and physical activity at 10 years, sex, socioeconomic status, birth weight, parental BMI z-scores, and height.

Table IV shows coefficients for the regression of mean hours of TV watching at 5 to 10 years, maternal attitudes toward TV, TV viewing, and physical activity at 10 years, sex, socioeconomic status, birth weight, parental BMI z-scores, and height.

DISCUSSION

In this large population-based sample, we found weekend but not weekday TV viewing in early childhood...
Television Viewing In Early Childhood Predicts Adult Body Mass Index

Television Viewing In Early Childhood Predicts Adult Body Mass Index

Strengths and Weaknesses of this Study

This study used longitudinal data from a large representative national birth cohort which provided detailed data on frequency and timing of TV watching, type of programs watched and maternal attitudes toward TV in early childhood. BMI data at age 30 were available on 66% of those with data on TV viewing at age 5. The lack of comparability between data on TV viewing and 5 and 10 years, and minor loss to follow-up of heavier TV viewers between 5 and 30 years. Overestimation of height and underestimation of weight may lead to underestimation of BMI. However, self-reports have been shown to be highly correlated with measured weight and height in adults in previous studies, and are accepted to be useful in epidemiologic studies of risk factors for obesity and overweight. It is likely that the direction of bias from self-report is toward obscuring a relationship between childhood sedentary behavior and later increased BMI. Higher loss to follow-up among those who watched higher levels of TV in early childhood and those of lower childhood social class may also bias our findings in a similar fashion. Weight was not measured at 5 years, and we were therefore unable to control analyses for BMI at this age. However, we controlled analyses for height at 5 years as a proxy for childhood growth and for BMI z-score at 10 years. The lack of comparability between data on TV viewing at 5 and 10 years was inherent in the initial study design. However, we believe this is unlikely to have affected as findings as our hypotheses concerned viewing at 5 years, and data at 10 years were only used to control analyses for later viewing.

Our findings did not confirm the report from the single previous longitudinal study between childhood and early adulthood that higher weekday TV viewing predicted higher later BMI. Hancox et al reported that in 1000 subjects followed from 5 to 26 years of age, average weekend viewing between 5 and 15 years was associated with higher BMI at 26 years when adjusted for childhood BMI, parental BMI, childhood exercise, and child and adult socioeconomic status. In contrast, we found that although weekday viewing was associated with higher BMI at the partially adjusted stage, this association disappeared when weekend viewing at 5 years was included in the model. The effects of weekday versus weekend viewing in childhood have not been previously examined. Our findings suggest that weekend differences in factors related to TV watching may be important mediators of the effects of TV on BMI, such as level of parental supervision, fat consumption

Table IV. Associations between television watching at 5 and 10 years and BMI z-score at 30 years

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Adjusted regression coefficient (95% CI)</th>
<th>P</th>
<th>Multivariate regression coefficient (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean daily hours of television at 5 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monday to Friday</td>
<td>6666</td>
<td>0.03 (0.01, 0.05)</td>
<td>.001</td>
<td>-0.01 (-0.03, 0.02)</td>
<td>.7</td>
</tr>
<tr>
<td>Weekend</td>
<td>6387</td>
<td>0.04 (0.03, 0.06)</td>
<td>&lt;.0001</td>
<td>0.03 (0.01, 0.05)</td>
<td>0.01</td>
</tr>
<tr>
<td>Maternal belief that television is harmful to young children (z-score)</td>
<td>6897</td>
<td>-0.01 (-0.03, 0.01)</td>
<td>.3</td>
<td>-0.01 (-0.03, 0.02)</td>
<td>0.5</td>
</tr>
<tr>
<td>Frequency of television watching at 10 years:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Often compared with Sometimes or Never</td>
<td>6840</td>
<td>0.03 (-0.03, 0.08)</td>
<td>.3</td>
<td>0.02 (-0.04, 0.08)</td>
<td>0.5</td>
</tr>
<tr>
<td>Frequency of playing sport in spare time at 10 years:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Often compared with Sometimes or Never</td>
<td>6833</td>
<td>0.00 (-0.04, 0.05)</td>
<td>.9</td>
<td>0.03 (-0.01, 0.08)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table shows coefficients for regression of TV watching at 5 and 10 years, attitudes to TV, and frequency of playing sport at 10 years on BMI z-score at 30 years. Coefficients are shown first adjusted for sex, birth weight, maternal and paternal BMI z-score, social class, and maternal educational status in childhood and adulthood and height at 5, 10 and 30 years, and then additionally adjusted for all other factors. Sample size (N) are shown for adjusted analyses. Sample size (N) for multivariate model = 6387 (78% of 8158 subjects); this reduction in model N is due to incomplete data on confounding factors in some subjects.
while viewing.\textsuperscript{33} displacement of physical activity and content of advertising.

Most longitudinal studies in this area have been limited to childhood and adolescence. Dietz and Gortmaker\textsuperscript{9} reported that the prevalence of obesity (as measured by triceps skin-fold thickness) increased by 2% for each additional hour of TV watched at baseline when adjusted for socioeconomic status and baseline BMI. However, this association was of low significance and was not adjusted for parental BMI. Kaur et al\textsuperscript{11} reported that daily hours of TV watched at baseline significantly predicted BMI z-score 3 years later, when adjusted for prior BMI z-score and ethnicity but not for other measures of socioeconomic status or parental BMI. Gortmaker et al\textsuperscript{8} reported that higher baseline TV watching was associated with an increased incidence and decreased remission of overweight at follow-up, when adjusted for baseline overweight, maternal BMI and socioeconomic status. In the only longitudinal study to examine TV viewing in early childhood, Proctor et al\textsuperscript{34} found that higher levels of TV viewing in early childhood predicted higher later BMI, after adjustment for parental BMI and socioeconomic status.

The observational nature of this study does not allow us to conclude that TV viewing in early childhood directly caused the observed increase in BMI. Further work is needed to establish potential mechanisms. The means by which early TV viewing influences later BMI remains unclear. TV viewing has been suggested to affect energy balance and thereby BMI through either displacement of physical activity and increased calorie consumption while viewing,\textsuperscript{37, 35, 36} through depression of metabolic rate while viewing,\textsuperscript{38} or through wider changes in diet choices caused by food advertisements.\textsuperscript{14, 36} Because we found that more frequent TV viewing at 5 years predicted more frequent viewing at 10 years, it is likely that early high levels of TV viewing sets viewing habits through childhood and into adult life, influencing later BMI through long-term eating behavior, displacement of physical activity, or other associated lifestyle factors.

There is evidence from observational studies that even brief exposure to televised food commercials can influence preschool children's food preferences.\textsuperscript{38} However, our findings relate to early childhood TV viewing habits in the United Kingdom in 1975, when only 3 TV channels were available in the country, 2 of which were public broadcasting, and only one of which carried advertising. Cable and satellite TV were unknown in the UK at the time, and TV broadcasting was limited to approximately noon to midnight. Advertising on the single commercial channel was relatively unsophisticated and limited.\textsuperscript{39} This suggests that the impact of TV viewing on later obesity seen in this UK cohort may relate more to displacement of physical exercise rather than effects caused by food advertisement but does not exclude an additional role for advertisement in the association between TV and obesity in other contexts.

REFERENCES


Objective  This longitudinal study examines links between parents’ television (TV)-related parenting practices and their daughter’s daily TV viewing hours.

Study design  Participants included 173 non-Hispanic white girls and their parents who were examined when girls were age 9 and 11 years. Girls’ daily TV viewing hours, mothers’ and fathers’ daily TV viewing hours, parents’ use of TV as a recreational activity, family TV co-viewing, and parents’ restriction of girls’ access to TV were assessed.

Results  Approximately 40% of girls exceeded the TV-viewing recommendations (ie, ≤2 hours/day). Girls watched significantly more TV when their parents were high-volume TV viewers, relied heavily on TV as a recreational activity, watched TV with them, and failed to limit their access to TV. A parenting risk score was calculated by collapsing information across all parenting variables. In comparison with girls exposed to 1 or fewer parenting risk factors at age 9, girls exposed to 2 or more parenting risk factors were 5 to 10 times more likely to exceed TV viewing recommendations at age 9 and 11.

Conclusions  Efforts to reduce TV viewing among children should encourage parents to limit their own TV viewing, reduce family TV viewing time, and limit their children’s access to TV. (J Pediatr 2005;147:436-42)

U.S. children and adolescents generally watch 2 to 3 hours of television (TV) per day;1 38% watch more than 3 hours per day,2 and 40% of children under age 5 years have a TV in their bedroom.3 Excessive TV viewing among children is of public health concern because it is associated with poor psychosocial and physical health.4-6 A recent study of individuals followed from birth showed that excessive TV viewing between age 5 and 15 years was associated with higher body mass index (BMI), lower fitness, increased incidence of cigarette smoking, and higher serum cholesterol level at age 26, after controlling for childhood BMI, parent BMI, and parent smoking.7

In response to evidence implicating the negative health effects of excessive TV viewing, the American Academy of Pediatrics (AAP) released pediatric guidelines for TV viewing in 20018 stating that children older than 2 years should watch no more than 2 hours of quality programming per day, children under age 2 should not watch TV, and TVs should be removed from children’s bedrooms. Despite these recommendations, however, there is no evidence that TV viewing has declined among U.S. youth.2 Consequently, the identification of effective targets and methods of intervention to reduce children’s TV viewing is a clear research priority.

In contrast with other health-related behaviors, such as alcohol intake, physical activity, and sexual behaviors, TV viewing occurs almost exclusively at home, often in the context of the family. Because parents serve as both models and gatekeepers for children’s TV viewing, the family is a key point of intervention in modifying children’s TV viewing behaviors. Research suggests that children watch more TV when their parents are high-volume TV viewers9-11 and fail to place limits on their total TV viewing time.12 Thus parents may play an important role in shaping children’s TV viewing behaviors. This conclusion is tentative, however, given the absence of longitudinal research.

AAP American Academy of Pediatrics  OR Odds ratio
BMI Body mass index  TV Television
CI Confidence interval  See related article, p 429, and editorial, p 417.

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Supported by National Institutes of Health grants HD 32973, HD 46567-01, and M01 RR10732.

Submitted for publication Nov 4, 2004; last revision received Mar 1, 2005; accepted May 5, 2005.

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0022-3476/$ - see front matter

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10.1016/j.jpeds.2005.05.002
To our knowledge, to date no longitudinal studies have examined links between parents’ TV viewing behaviors and parenting practices and the time that their children spend watching TV. As a result, it is not known whether TV-related parenting practices change across time and whether parenting at one point in time influences children’s TV viewing at a later point in time. Furthermore, it is not known whether TV-related parenting practices co-occur in families and cumulatively place children at risk of exceeding TV viewing recommendations across time. The current study was designed to answer these questions by examining parents’ TV-related parenting practices and their daughters’ TV viewing over a 2-year period.

METHODS

Participants

Families were recruited for participation in the study using flyers and newspaper advertisements. In addition, families with age-eligible female children within a 5-county radius received mailings and follow-up phone calls (MetroMail Inc.). Study participants included 187 non-Hispanic white girls and their mothers and fathers from central Pennsylvania who were part of a longitudinal study examining the health and development of young girls. Of the 187 families who completed data collection when their girls were age 9 years (median age, 9.34, ± 0.3 years), 173 were reassessed when the girls were age 11 (median age, 11.34 ± 0.3 years), representing a 93% retention rate. Only families who participated at both times of assessment were included in the analyses. No significant differences in family income, parents’ education, and girls’ and parents’ weekly TV viewing hours were identified between families who remained in the study and those who dropped out. Mothers and fathers were generally well educated, with means of 14.6 (± 2.2) and 14.7 (± 2.6) years of education, respectively. The median family income was > $50,000/year.

Procedures

Families visited the laboratory during summer when the girls were age 9 and again when they were age 11. The girls were individually interviewed by trained interviewers, and the parents completed a series of self-report questionnaires. Trained research assistants measured girls’ height and weight. The institutional review board of the associated university approved all study procedures, and parents provided consent for their family’s participation before the study began. Girls also provided informed assent.

Measures

GIRLS’ TV VIEWING. When the girls were 9 and 11 years old, mothers were asked the following question: “How many hours per day does your daughter spend watching TV/videos?” Mothers responded to this question with reference to an average school day and an average nonschool day (ie, weekends or in the summer months). Average hours per day spent watching TV at each age was calculated as follows: (5 × weekday hours + 2 × weekend hours)/7 days.

PARENTS’ TV VIEWING BEHAVIORS AND PARENTING PRACTICES. Five dimensions of TV-related parenting were assessed: mothers’ daily TV viewing time, fathers’ daily TV viewing time, parents’ reliance on TV as a recreational activity, family co-viewing practices, and restrictions placed on girls’ access to TV. Mothers and fathers completed questions assessing each of these dimensions. With the exception of mothers’ and fathers’ daily TV viewing time, scores for mothers and fathers were averaged for each construct to provide a single parent or family score without reference to a particular parent (eg, parent use of TV as a recreational activity; family co-viewing, parent restriction of access). Collapsing information for mothers and fathers provided a more reliable “family” score that incorporated multiple variables and reduced the number of parent variables used in analyses. All measures except restriction of girls’ access to TV were assessed when girls were age 9 and 11 years; restriction was assessed at age 11 only.

Mothers’ and fathers’ daily TV viewing hours were assessed using the following question: “On an average day, how many hours do you watch/limit TV/videos?” Parents’ dependence on TV as a recreational activity was assessed using the following question: “What percentage of your free time do you spend watching TV/videos?” Response options included 1, relatively little (0 to 25%); 2, less than half (25% to 50%); 3, more than half (50% to 75%); 4, almost all (75% to 100%). Family co-viewing practices were assessed using 2 questions; mothers and fathers were asked to rate, using a 4-point scale (from 1 [rarely] to 4 [regularly]), how often they watched TV/videos with their daughter and as a family.

Finally, parents’ restriction of girls’ access to TV was assessed using questions completed by parents and girls. First, mothers and fathers indicated the extent to which they limited the amount of television their daughter watched. The response options included 1, do not limit; 2, rarely limit; 3, moderately limit; 4, my daughter is only permitted to watch a few select programs or no television at all. Second, the girls indicated whether or not they had a TV in their bedroom. To maintain a 4-point scale for the restriction items, this variable was coded as 1 for yes and 4 for no. With the exception of mothers’ and fathers’ TV viewing, a single score ranging from 1 to 4 was created for each parenting construct by taking the average for all items addressing the construct, including items for mothers and fathers as mentioned earlier. Mothers’ and fathers’ TV viewing was a continuous variable; scores ranged from 0 to 5-1/4 hours per day.

GIRLS’ WEIGHT STATUS. At age 9 and 11 years, girls’ height and weight were measured in triplicate, and average height and weight were used to calculate BMI. Girls’ BMI values were converted to age- and sex-specific BMI percentiles using the 2000 Centers for Disease Control growth charts. Girls were classified as at risk for overweight if their BMI percentile
was ≥85 and <95 and overweight if their BMI percentile was ≥95. The categories of “at risk of overweight” and “overweight” were collapsed for the analyses (ie, ≥85th BMI percentile).

Statistical Analyses

All analyses were performed using SAS version 8.01 (SAS Inc., Cary, NC). Previous research has identified significant associations between demographic characteristics, such as income and education, and children’s and adults’ TV viewing. Thus family income and mothers’ education were entered as covariates in all analyses. Paired t-tests were used to examine whether mean scores for girls’ TV viewing and each measure of parenting changed significantly across time. Multiple logistic regression analysis was used to assess whether exceeding the AAP TV viewing recommendations predicted the likelihood of girls’ being overweight or at risk of overweight (ie, BMI percentile ≥85); cross-sectional and longitudinal analyses were performed. Spearman’s rank correlation analysis was used to assess covariation in TV-related parenting behaviors and parenting practices within families (Table I). Spearman’s rank correlation analysis was also used to examine cross-sectional and longitudinal associations between each parenting variable and the girls’ TV viewing (Table II). The final analysis examined the cumulative impact of parenting risks to which the girls were exposed at age 9 on the likelihood that girls exceeded TV viewing recommendations at age 9 and 11. To perform this analysis, each parent variable at age 9 was dichotomized as high (ie, 1) or low (ie, 0) based on a mean split. A family risk score ranging from 0 to 4 was created by summing these scores. Family risk scores of 0 and 1 were collapsed to increase the sample size of the referent group. Each family risk score was then entered as a categorical dummy-coded variable into a logistic regression analysis to predict the likelihood of girls exceeding TV viewing recommendations at age 9 and 11, controlling for family income and mothers’ education (Table III). Analyses were rerun including girls’ weight status at age 9 (BMI percentile < or ≥85th percentile) as an additional covariate, to control for the possibility that parents may have been responding to their child’s weight status.

RESULTS

Girls’ TV Viewing

Girls watched slightly less than 2 hours of TV per day at age 9 (1.92 ± .90) and 11 (1.91 ± .91) years, with no significant change across time (P = .98). A high degree of stability was noted in girls’ TV viewing across time (r = .73); that is, girls who watched high levels of TV relative to the sample at age 9 also did so at age 11. The percentage of girls (and parents) at age 9 and 11 who spent <1, 1 to 2, and >2 hours per day watching TV is shown in Figure. The proportions of girls in each viewing category did not change substantially across time. The percentage of girls exceeding the AAP TV viewing recommendations of 2 hours per day was 41% at age 9 and 38% at age 11; 35% of girls exceeded TV recommendations at both ages. Few girls (6% to 8%) watched TV <1 hour per day.

Exceeding the AAP TV viewing recommendations at age 9 was not associated with the likelihood of being overweight or at risk of overweight at age 9 (odds ratio [OR] = 1.28; 95% confidence interval [CI] = .65 to 2.5). However, girls who exceeded the recommendations at age 9 were marginally more likely to be overweight or at risk of overweight at age 11, controlling for whether or not they exceeded TV viewing recommendations at age 11 (OR = 2.3; 95% CI = .96 to 5.15) and girls who exceeded the TV viewing recommendations at age 11 were more likely to be overweight at age 11 (OR = 2.61; 95% CI = 1.3 to 5.3).

Parents’ TV Viewing and TV-Related Parenting

Mothers reported watching approximately 1.5 hours of TV per day when girls were age 9 (1.57 ± .99) and 11 (1.68 ± .97) years, with a significant increase of approximately 7 minutes across time (P < .05). Fathers watched between 1.5 and 2 hours of TV per day (1.63 ± 1.07 at age 9; 1.87 ± 1.09 at age 11), with a significant increase of about 15 minutes across

### Table I. Spearman rank correlations between parents’ TV viewing behaviors and parenting practices at both times of assessment

<table>
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<tbody>
<tr>
<td>1. Mothers’ TV viewing (9 years)</td>
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<td>2. Fathers’ TV viewing (9)</td>
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<td>.45***</td>
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<td>3. Parents’ use of TV as recreational activity (9)</td>
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<td></td>
<td>.68***</td>
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<td>4. Family co-viewing (9)</td>
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<td>.41***</td>
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<td>5. Mothers’ TV viewing (11)</td>
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<td>.74***</td>
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<td>6. Fathers’ TV viewing (11)</td>
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<td>7. Parents’ use of TV as recreational activity (11)</td>
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<td>8. Family co-viewing (11)</td>
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<td>.25***</td>
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<tr>
<td>9. Parents’ restriction of girls’ access to TV (11)</td>
<td>−.19***</td>
<td>−.13*</td>
<td>−.11*</td>
<td>−.11*</td>
<td>−.23**</td>
<td>−.28***</td>
<td>−.13*</td>
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</tr>
</tbody>
</table>

Numbers in parentheses indicate the girls’ age at the time of measurement. Italicized correlations represent stability estimates for each parenting construct.

*P < .05; **P < .01; ***P < .001.
The percentage of parents who watched 1, 1 to 2, and >2 hours of TV per day when the girls were age 9 and 11 is presented in the Figure. Approximately 30% of mothers and fathers reported watching TV for more than 2 hours per day, and this figure increased slightly across time (from 25% to 30% for mothers and from 25% to 34% for fathers).

With respect to the other dimensions of TV-related parenting, less than 1 in 10 parents reported devoting 50% or more of their free time to watching TV, and approximately 25% of parents reported watching TV for more than 2 hours per day, and this figure increased slightly across time (from 25% to 30% for mothers and from 25% to 34% for fathers).

Table I presents associations between parents' TV viewing behaviors and parenting practices across both measurement occasions. Results generally showed that parenting behaviors clustered within families. With the exception of parental restriction, all measures of parenting were significantly correlated within and across measurement occasions. For example, mothers and fathers who watched more TV when their daughters were age 9 relied more heavily on TV as a recreational activity and reported higher levels of family co-viewing when their daughters were age 9 and 11. With respect to parent restriction, mothers (when the girls were 9) who watched more TV were less likely to restrict their daughters’ access to TV when they were age 11.

Table II. Spearman rank correlations assessing cross sectional and longitudinal associations between parents’ TV-viewing behaviors and parenting practices and girls’ TV viewing

<table>
<thead>
<tr>
<th>Correlations with girls’ TV viewing</th>
<th>Mothers’ TV viewing</th>
<th>Fathers’ TV viewing</th>
<th>Parents’ use of TV as recreation</th>
<th>Family co-viewing</th>
<th>Restricting girls’ access to TV</th>
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<tbody>
<tr>
<td>Cross-sectional associations</td>
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<tr>
<td>Parenting and girls’ TV viewing (age 9)</td>
<td>.55***</td>
<td>.37***</td>
<td>.35***</td>
<td>.35***</td>
<td>–</td>
</tr>
<tr>
<td>Parenting and girls’ TV viewing (age 11)</td>
<td>.47***</td>
<td>.39***</td>
<td>.34***</td>
<td>.22**</td>
<td>.18**</td>
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<tr>
<td>Longitudinal associations</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Parenting (age 9) and girls’ TV viewing (age 11)</td>
<td>.23**</td>
<td>.06</td>
<td>.20**</td>
<td>.13</td>
<td>–</td>
</tr>
<tr>
<td>Change in parenting and girls’ TV viewing between age 9 and 11</td>
<td>-.08</td>
<td>.08</td>
<td>.04</td>
<td>.18†</td>
<td>–</td>
</tr>
</tbody>
</table>

All analyses partial out the effects of family income and parent education. Parent restriction was assessed only when the girls were age 11.

*P < .05; **P < .01; ***P < .001.
†Correlations between parenting variables at age 9 and girls’ TV viewing at age 11 controlled for the parent variable at age 11.

Links Between Parents’ And Girls’ Television Viewing Behaviors: A Longitudinal Examination 439
The present study has revealed that parents’ TV-related behaviors and parenting practices are associated with their daughters’ TV viewing at age 9 and 11 years. Approximately 40% of the girls in this sample exceeded the AAP recommendations for TV viewing, mirroring U.S. national rates.1 At age 9 and 11, girls watched significantly more TV when their parents reported higher levels of TV viewing, corroborating findings from previous studies.9-11 Furthermore, girls watched significantly more TV when their parents relied on TV as a recreational activity 2 years earlier, when girls were age 9; these effects were independent of parental risk factors at age 11. Furthermore, total exposure to parental risk factors at age 9 predicted the likelihood of repeatedly exceeding recommendations across age 9 and 11. There was a dose-type pattern such that the girls exposed to 2, 3, or 4 parenting risk factors were approximately 5, 7, and 10 times more likely, respectively, to exceed recommendations at age 9 and 11 than were girls exposed to 1 or fewer parenting risk factors.

Parental behaviors shape family environments that can promote similar behaviors in their children, and these relationships between parent and child behaviors can contribute to familial similarities in risk outcomes. Parents’ eating and activity behaviors are linked with parent-child similarities in adiposity.15,16 Additional studies provide evidence for similarities in parents’ and children’s activity patterns,17 eating behaviors,18 and dietary patterns.19-21 These findings substantiate the crucial role that parents play in shaping their children’s behaviors through modeling and parenting practices.

As with these and other parent-child relationships, similarities in parents’ and children’s behaviors may also be a result of parents responding to children’s behaviors and preferences. In the case of TV viewing, children’s requests to watch TV and their preference for TV over other forms of activity may drive parents’ TV viewing as well. Parents, in wanting to spend time with their children, may choose to participate in activities they know their children enjoy, such as TV viewing. It is also possible that similarities in children’s and parents’ TV viewing could partially reflect a genetic influence on leisure activities.22 Nonetheless, the important

Table III. Results from logistic regression model predicting the likelihood of girls exceeding TV viewing recommendations at age 9 and 11 years based on their exposure to parenting risk factors at age 9

<table>
<thead>
<tr>
<th>Number of parenting risk factors to which the girls were exposed at age 9</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of girls in each category who watched &gt;2 hours of TV per day at age 9 and 11</td>
<td>8</td>
<td>34</td>
<td>42</td>
<td>50</td>
</tr>
<tr>
<td>Likelihood [OR (95% CI)] of girls watching &gt;2 hours of TV per day at age 9 and 11 controlling for covariates</td>
<td>Referent group</td>
<td>5.4 (1.9 to 15.6)</td>
<td>7.3 (2.4 to 21.9)</td>
<td>9.6 (3.1 to 29.8)</td>
</tr>
</tbody>
</table>

Covariates included mothers’ education and family income. An additional analysis was run that included girls’ weight status at age 9 as an additional covariate; see the text for results. A total of 68, 44, 33, and 28 girls were exposed to ≤1, 2, 3, and 4 parenting risk factors, respectively.
role of parents should not be downplayed. It is the parents’ responsibility to create a household environment that facilitates their children’s health and development. The challenge lies in finding ways to encourage parents to turn off the TV and to identify alternatives to TV viewing as a recreational activity for themselves and their children.

Based on findings from this study, advice for parents should include limiting their own TV viewing time and the time they spend watching TV as a family, as well as decreasing their children’s access to TV. At first glance, the recommendation to limit family co-viewing appears to be in conflict with the AAP recommendation that parents watch TV with their children to monitor their viewing content. But the AAP recommendation is focused primarily on young children. In our sample of girls in late childhood, co-viewing was linked with higher levels of TV viewing, suggesting that there can be too much co-viewing and that co-viewing may undermine the AAP recommendation to limit children’s TV viewing to no more than 2 hours per day. In addition, research shows that parents watch TV with their older children because of shared viewing preferences rather than for the purpose of monitoring viewing content.22 Parents can limit their children’s access to TV by removing TVs from children’s bedrooms. In addition to being associated with increased hours of TV viewing,3 the presence of a TV set in a child’s bedroom has been linked to an increased risk of overweight,3 fewer hours of reading,24 and sleep disturbances25 in children. Parents can also limit their children’s TV viewing time by encouraging them to be selective viewers, choosing only a few favorite programs to watch, as suggested by Salon et al.26

Key strengths of this study include its longitudinal design and the broad assessment of TV-related parenting practices. However, findings from this study cannot be generalized beyond white middle-class families. Consequently, our findings are only a first step in understanding the links between parents’ TV-related parenting and children’s TV viewing in American families. Additional research with more diverse samples, and samples including boys, is needed to extend the generalizability of these findings. It is also possible that other factors, including neighborhood safety and access to recreational spaces, are important determinants of children’s TV viewing; however, we did not collect information on these factors. Finally, findings from this study are limited by the use of retrospective parent-reported measures of TV viewing. Future work could build on these findings by using objective measures of TV viewing or repeated measures of TV viewing through the use of time sampling.

Children can learn to choose and prefer activities other than TV viewing, playing video games, and using the computer. The challenge is to provide guidance and support for parents that will promote this objective. To encourage their children to engage in other forms of activity, parents must serve as role models and create environments that allow and encourage their children to engage in alternate activities. The TV Turnoff Network (www.tvturnoff.org) provides guidance for parents in this area. Reducing their children’s TV viewing will require that parents turn off the TV, limit their children’s access to TV in the home, adopt new hobbies that require activity, and find or create outdoor settings that will support their and their children’s engagement in creative and non-TV-related activities.

We express our sincere appreciation to the girls in the study group and their families, who continue to show commitment to the larger longitudinal project. In addition, the services provided by the General Clinical Research Center of the Pennsylvania State University were appreciated. Finally, we thank Simon Marshall for his helpful comments on the statistical analyses.

REFERENCES


50 Years Ago in The Journal of Pediatrics

PEDIATRIC REHABILITATION AND THE PEDIATRICIAN

In 1955, Kanof described his experience of building an interdisciplinary pediatric rehabilitation unit at the Jewish Chronic Disease Hospital in Brooklyn and asked readers to consider the concept of a division of pediatric rehabilitation. In contrast to the adult rehabilitation goal of restoring previously attained skills, pediatric rehabilitation not only strives to restore skills but also assists the child in attaining skills that were never possessed. He noted that rehabilitation medicine had “developed as a team approach, a concept which is the outstanding contribution of this specialty to modern medical practice.”

Today, interdisciplinary teams are central to many care settings, particularly for children with chronic illnesses. The disciplines described in the Kanof article are now essential, valued, and familiar members of the care team. In many departments of pediatrics, rehabilitation is a part of a division of developmental medicine and, regardless of its administrative home, is key to providing comprehensive care. Regional pediatric rehabilitation centers are common and are based on the interdisciplinary care model.

Three other ideas were visions of things to come. Kanof voiced the opinion that the child/patient and the parents were two essential, but separate, members of the team. Child- or family-centered care is now well integrated into pediatric care philosophy. He wrote that the pediatrician has a special role as “surrogate” and “arbiter” for the child, and today we recognize this as the essential role that pediatricians play as child advocates—for the individual patient and in policy settings for child health. Lastly, the importance of clinical research was emphasized with a call for research-quality “controlled observations and clinical experimentation” so that future care would be evidence based.

Issues have changed over 50 years. Today, few children literally grow up in the hospital, although as recently as the 1980s, children with special technology needs for respiratory or nutrition support lived in the hospital for years. With current care and reimbursement realities, the assessment of a child with a complex medical history is likely to be conducted in the outpatient setting and not through admission to the hospital for 2 to 3 weeks’ assessment and observation. As a field, pediatrics embraces the goal of optimal outcomes—both medical and psychosocial—for children with chronic disease, and the ongoing need for interdisciplinary teams and research to guide improvements in care.

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HEALTH-RELATED QUALITY OF LIFE IN OVERWEIGHT AND NONOVERWEIGHT BLACK AND WHITE ADOLESCENTS

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ERICA D. TAYLOR, BS, MARC L. COHEN, MD, DEBORAH YOUNG-HYMAN, PhD, MARGARET KEIL, MS, CRNP,
RONETTE L. KOLOTKIN, PhD, and JACK A. YANOVSKI, MD, PhD

Objectives To assess the impact of obesity on quality of life (QOL) in black and white adolescents.

Study design One hundred ten overweight (body mass index [BMI], 41.7 ± 8.9 kg/m²) and 34 nonoverweight adolescents (BMI, 20.6 ± 2.9 kg/m²) and their parents completed measures of QOL.

Results Overweight was associated with poorer adolescent-reported QOL and parent reports of their children’s QOL. Examining groups by weight status and race, overweight whites reported the greatest impairment on Social/Interpersonal, Self-Esteem, and Physical Appearance QOL (all P < .01), whereas parents of overweight blacks reported the poorest General Health Perceptions scores regarding their children. Interactions between BMI z-score and race were detected for Social/Interpersonal, Self-esteem, Daily Living, Self-Efficacy, Self-regard, and Physical Appearance QOL (all P < .05): Higher BMI in whites was associated with greater impairments in QOL than in blacks. Parents reported similar relations for their children.

Conclusions According to adolescent and parent reports, overweight is associated with poorer QOL in adolescence, regardless of race; however, compared with overweight white adolescents, blacks report less impairment in QOL. Future research is required to determine whether differences in QOL are predictive of treatment success. (J Pediatr 2005;147:443-50)

Pediatric overweight is associated with increased medical morbidity and negative psychosocial functioning. Limited prior research also suggests obesity affects significantly the quality of life (QOL) of adolescents. Six studies have directly examined the relation of childhood obesity and health related QOL (HRQOL), defined as “the physical, psychologic, and social domains of health that are influenced by individual experiences, beliefs, expectations, and perceptions.” One study found that obese youth report lower HRQOL compared with nonoverweight control subjects, with distress at levels similar to children and adolescents with cancer. Several groups have also found parents of overweight children more likely to report poorer child HRQOL than parents of nonoverweight children.

The relation between race and QOL has remained relatively unexplored in overweight children, while yielding mixed findings in the adult literature. Some studies have found obese whites report more impairment on QOL measures than blacks, whereas others have identified no racial differences.

The goal of the current study was to compare the QOL of overweight and nonoverweight black and white adolescents. We hypothesized that both black and white overweight adolescents and their parents would report lower teen HRQOL than their nonoverweight counterparts. Since some data suggested obesity was associated with less psychologic distress in black than white adults, we also hypothesized that overweight black adolescents would report less impairment in quality of life than overweight whites.

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BMI Body mass index
HRQOL Health-related quality of life
QOL Quality of life

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This research was supported by the National Institute of Child Health and Human Development (Z01-HD-00641) and the National Center on Minority Health and Health Disparities, NIH, DHHS.

Submitted for publication Dec 20, 2004; last revision received Apr 27, 2005; accepted May 31, 2005.

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0022-3476/3 - see front matter
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10.1016/j.jpeds.2005.05.039
METHODS

Subjects

Extremely overweight (body mass index [BMI], 41.7 ± 8.9 kg/m²; mean ± SD) adolescents (62 black and 48 white) were assessed before entry into an obesity treatment program. Nonoverweight (BMI, 20.1 ± 2.9 kg/m²) adolescents (n = 34) were assessed before they participated in an exercise physiology study for healthy volunteer adolescents. Subjects were recruited through posted flyers and, in the case of overweight volunteers, newspaper advertisements and letters to local physicians. Inclusion and exclusion criteria are described elsewhere.17 The protocol was approved by the Institutional Review Board of the National Institute of Child Health and Human Development.

Of overweight adolescents screened, 34% (n = 70) did not enter the trial; 23 individuals chose not to participate based on the time commitment required, and 47 teens were ineligible because they lacked evidence of obesity-related comorbid conditions. There were no differences in the race, sex, or age of these groups compared with the studied cohort.

Adolescents completed the following questionnaires.

Impact of Weight on Quality-of-Life, adapted for use with adolescents (IWQOL-A) is a 66-item self-report, condition-specific instrument designed to measure the perceived effect of weight on quality of life. The adolescent version differs from the adult IWQOL in that it generates seven subscales (Health, Social/Interpersonal, Work, Mobility, Self-esteem, Activities of Daily Living, and Comfort with Food) subscales, eliminating the eighth (Sexual Life) subscale. Questions not applicable to adolescents were omitted from the original 74-item questionnaire and terminology was simplified and/or modified for adolescent use. The original IWQOL has demonstrated good construct validity, test-retest reliability, and internal consistency.18,19 Higher scores indicate greater QOL impairment. While a short-form of the IWQOL now exists, it was not available at the time data collection was initiated. To assess the construct validity of the adapted version of the IWQOL-A, we examined correlations of the Self-esteem and Social/Interpersonal subscales to data collected by the Children’s Depression Inventory (CDI).20 The IWQOL-A Self-esteem scale was significantly correlated with the CDI Negative Self-esteem scale (P < .001) and the Social/Interpersonal scale was significantly correlated with the CDI Interpersonal Problems scale (P < .001). Test-retest reliability was assessed over a 6 month interval in a subset of the adolescents by examining the intra-class correlation coefficients in 23 study subjects whose weight remained stable (within ± 0.93 BMI units, equivalent to a 5-lb change in body weight). The IWQOL-A Health (α = 0.66; P < .01), Social/Interpersonal (α = 0.54; P < .05), Work/School (α = 0.56; P = .06), Mobility (α = 0.50; P = .06), and Daily Living (α = 0.56; P < .05) subscales all showed acceptable agreement. We eliminated the Food Comfort scale (α = 0.31, P = .21) from our analyses. The possible subscale score ranges are as follows: Health: 12 to 60, Social/Interpersonal: 11 to 55, Work/School: 6 to 30, Mobility: 10 to 50, Self-esteem: 10 to 50, and Activities of Daily Living: 7 to 35. While no data have been published on the IWQOL adolescent version, of the 6 scales retained for our analyses, 3 scales (Social/Interpersonal, Self-esteem, and Daily Living) maintained all of the individual items and constructs used to generate the same scales for the adult IWQOL. Studies of treatment seeking obese adults have reported the following ranges of mean subscale scores: Social/Interpersonal: 16.3 to 23.2, Self-esteem: 20.0 to 28.2, and Daily Living: 11.9 to 18.3.18,19,21

Health-Related Quality-of-Life (HRQOL)22 is a 55-item HRQOL self-report assessment designed to address the key domains known to be affected by obesity and the subsequent loss of body weight. The questionnaire was developed using scales recommended by quality-of-life researchers, clinicians, obese individuals, and findings in the literature regarding HRQOL in obesity. The HRQOL generates the following subscales: General Health, Comparative Health, Health Efficacy (consisting of three individually scored questions: A, “How much do you believe your weight is harmful to your health?” B, “How much do you think your health will benefit if you achieve or maintain your ideal weight?” C, “How sure are you that you will control your weight in the next year?”), Overweight Distress, Depression, Self-regard, Physical Appearance, Work Productivity/Work Loss, Physical and Social Activities, and Satisfaction with Treatment. A study of normal weight and obese adults demonstrated good internal consistency and test-retest reliability, and adequate construct validity.22 For use with our sample, we adapted the HRQOL by eliminating the age-inappropriate questions that generate the Work Productivity/Work Loss and Physical and Social Activities scales. The Satisfaction with Treatment scale was removed as it was not relevant to our baseline analyses. For the General Health (possible score range: 0 to 100), Comparative Health (possible range: 0 to 100), and Physical Appearance (possible range: 0 to 35) subscales, lower scores indicate greater QOL impairment. For the Depression (possible range: 0 to 60), Health Efficacy (possible range: 1 to 10), Overweight Distress (possible range: 0 to 100), and Self-regard (possible range: 0 to 49) subscales, higher scores are indicative of worse QOL impairment.

In terms of construct validity, the HRQOL Depression scale was significantly correlated with 4 of 5 CDI subscales and with the Total scale (all P ≤ .001). For test-retest reliability assessed over a 6-month interval, 5 of the 7 HRQOL scales showed acceptable agreement: General Health (α = 0.65; P = .01); Health Efficacy B (α = 0.59; P < .05); Depression (α = 0.67; P < .01); Self-Regard (α = 0.60; P < .01); Physical Appearance (α = 0.79; P < .001). The Comparative Health (α = 0.27; P = .24), Health Efficacy A (α = 0.42; P = .12) and C (α = 0.03; P = .53), and Overweight Distress (α = 0.18; P = .33) scales subscales were eliminated because they did not demonstrate adequate reliability.

One parent (almost invariably the mother) completed the Child Health Questionnaire – Parent Report (CHQ-PF50),23 a validated50–item parent-reported measure of...
Physical and psychosocial well-being of children and adolescents that has been shown to be a useful method of assessing children’s health in pediatric and minority populations.²⁵ Scales based on a single-item response were not assessing children’s health in pediatric and minority populations. Among overweight subjects were adolescents with steatosis (black: n = 6, 10%; white: n = 5, 10%). No significant differences were found in the number or type of obesity-related comorbidities for black and white subjects. There were also no significant differences between the mean number of self-reported obesity-related comorbidities for black and white subjects. There were also no significant differences between the mean number of self-reported obesity-related comorbidities for black and white subjects’ mothers (black: 1.1 ± 1.3 vs white: 0.79 ± 1.3, P = .35), fathers (black: 0.85 ± 1.1 vs white: 0.79 ± 0.83, P = .92), or extended families (black: 3.4 ± 1.2 vs white: 3.1 ± 1.4, P = .23).

### RESULTS

#### Subject Characteristics

Overweight subjects were from families with significantly lower Hollingshead socioeconomic class scores (SES) compared with the nonoverweight subjects (Table I). Therefore SES was included as a potential covariate for all analyses.

Among overweight subjects were adolescents with hyperinsulinemia (black: n = 44, 71%; white: n = 37, 77%), hyperlipidemia (black: n = 31, 50%; white: n = 18, 38%), hypertension (black: n = 5, 8.1%; white: n = 7, 15%), type 2 diabetes (black: n = 3, 5%; white: n = 2, 4%), and hepatic steatosis (black: n = 6, 10%; white: n = 5, 10%). No significant differences were found in the number or type of obesity-related comorbidities for black and white subjects. There were also no significant differences between the mean number of self-reported obesity-related comorbidities for black and white subjects. There were also no significant differences between the mean number of self-reported obesity-related comorbidities for black and white subjects’ mothers (black: 1.1 ± 1.3 vs white: 0.79 ± 1.3, P = .35), fathers (black: 0.85 ± 1.1 vs white: 0.79 ± 0.83, P = .92), or extended families (black: 3.4 ± 1.2 vs white: 3.1 ± 1.4, P = .23).

#### Relation Between Adolescent and Parent Reports

Among the many significant relations between adolescent and parent reports, a number of scales sharing constructs demonstrated significant relations. The adolescent–report IWQOL Health scale correlated significantly with the parent report CHQ General Health Perceptions (r = −0.34, P < .05) scale. IWQOL Social scale correlated significantly with the CHQ Role/social Limitations – Physical (r = −0.25, P < .01) and Family Activities (r = −0.17, P < .05) scales. The IWQOL Mobility scale correlated with the CHQ Physical Functioning (r = −0.32, P < .01), Role/social Limitations – Physical (r = −0.18, P < .05), and Bodily Pain (r = −0.30, P < .01) scales. IWQOL

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### Table I. Subjects

<table>
<thead>
<tr>
<th></th>
<th>Overweight</th>
<th>Nonoverweight</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Blacks (n = 62)</td>
<td>Whites (n = 48)</td>
</tr>
<tr>
<td></td>
<td>(19 M/43 F)</td>
<td>(22 M/26 F)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>14.5 ± 1.5</td>
<td>14.3 ± 1.6</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>3.3 ± 1.0a</td>
<td>3.3 ± 1.0b</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>120.3 ± 27.8</td>
<td>105.8 ± 25.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.8 ± 7.7</td>
<td>166.3 ± 8.5</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>44.6 ± 8.6b</td>
<td>38.1 ± 7.9b</td>
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<tr>
<td>BMI-SD</td>
<td>6.1 ± 3.2a</td>
<td>5.0 ± 2.3a</td>
</tr>
<tr>
<td>Fat (%) by ADP</td>
<td>48.3 ± 5.7a</td>
<td>48.3 ± 5.7a</td>
</tr>
</tbody>
</table>

BMI, Body mass index; BMI-Standard Deviation Score (BMI-SD) was calculated from data of Frisancho.³⁶ ADP, air displacement plethysmography. Different subscript letters indicate significant differences between groups. *P < .01; mean ± SD and (range) are given unless otherwise indicated.

†Socioeconomic status calculated according to Hollingshead Index; higher scores represent lower status.
Self-esteem scale correlated with the CHQ Self-esteem scale ($r = 0.25$, $P < .05$). The IWQOL Daily Living scale correlated with the CHQ Physical Functioning ($r = -0.30$, $P < .01$) and Role/social Limitations – Physical ($r = -0.23$, $P < .01$) scales. Of the HRQOL scales, only the General Health subscale appeared to correspond to a CHQ subscale, and was significantly related to the parent-reported General Health Perceptions scale ($r = 0.35$, $P < .01$).

Adolescent Reports

Overweight subjects reported significantly poorer QOL compared with nonoverweight teens on all of the IWQOL and HRQOL subscales, with the exception of the HRQOL Self-regard scale (Means, standard deviations, F-values and significance levels in Table II). Comparing subjects by weight status separated by race (four groups), overweight white teens reported significantly poorer IWQOL Social/Interpersonal and Self-esteem QOL compared with all three groups and overweight blacks reported poorer Self-esteem compared with nonoverweight subjects. Overweight white adolescents endorsed poorer HRQOL Physical Appearance compared with the other three groups, whereas overweight blacks endorsed poorer scores than nonoverweight teens (for all main effects $P < .01$; Table III). No differences were detected on either the IWQOL-A or the HRQOL based upon sex (data not shown).

After controlling for race, only the HRQOL General Health was negatively correlated with age ($r = -0.2$, $P < .05$; older children reported lower QOL), and the HRQOL Depression scale was marginally correlated with SES ($r = 0.2$, $P = .05$; less impairment was associated with lower SES).

Significant interactions between BMI-SD and race were detected on the IWQOL-A Social/Interpersonal, Self-esteem, and Daily Living subscales. For whites, as BMI-SD increased, subjects reported poorer Social/Interpersonal (BMI-SD × race interaction: $F = 7.2$, $P < .01$) and Self-esteem ($F = 5.4$, $P < .05$) QOL. This pattern was not evident for black subjects (Figure, A and B). Although increases in BMI-SD were associated with poorer reports of Daily Living QOL for both races, the increase in scores among whites was significantly more precipitous ($F = 9.8$, $P < .01$; Figure C). Similar

### Table II. Means and standard deviations of quality of life scores based on weight status

<table>
<thead>
<tr>
<th></th>
<th>Overweight (n = 106)</th>
<th>Nonoverweight (n = 34)</th>
<th>F-statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adolescent report</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impact of Weight on Quality-of-Life</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health</td>
<td>23.1 ± 5.6</td>
<td>16.1 ± 3.9</td>
<td>66.4**</td>
</tr>
<tr>
<td>Social/Interpersonal</td>
<td>21.5 ± 9.0</td>
<td>14.7 ± 4.5</td>
<td>29.1**</td>
</tr>
<tr>
<td>Work/school</td>
<td>14.0 ± 5.1</td>
<td>12.1 ± 4.0</td>
<td>4.8*</td>
</tr>
<tr>
<td>Mobility</td>
<td>16.9 ± 6.3</td>
<td>11.2 ± 2.0</td>
<td>67.1**</td>
</tr>
<tr>
<td>Self-esteem</td>
<td>21.93 ± 8.0</td>
<td>14.3 ± 3.8</td>
<td>52.5**</td>
</tr>
<tr>
<td>Daily living</td>
<td>14.1 ± 4.6</td>
<td>7.9 ± 1.4</td>
<td>145.2**</td>
</tr>
<tr>
<td><strong>Health-Related Quality-of-Life</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General health</td>
<td>54.0 ± 25.4</td>
<td>86.7 ± 13.4</td>
<td>90.25**</td>
</tr>
<tr>
<td>Health efficacy B¹</td>
<td>8.2 ± 2.1</td>
<td>6.2 ± 4.1</td>
<td>5.4**</td>
</tr>
<tr>
<td>Depression</td>
<td>12.3 ± 12.4</td>
<td>4.1 ± 3.6</td>
<td>35.6**</td>
</tr>
<tr>
<td>Self-regard</td>
<td>35.8 ± 6.4</td>
<td>36.2 ± 7.7</td>
<td>0.07</td>
</tr>
<tr>
<td>Physical appearance</td>
<td>19.9 ± 7.7</td>
<td>28.3 ± 5.6</td>
<td>44.9**</td>
</tr>
</tbody>
</table>

| **Child Health Questionnaire–Parent Report** |                      |                        |             |
| General health perceptions | 66.6 ± 22.4          | 92.7 ± 9.4             | 43.3**      |
| Physical functioning      | 87.0 ± 21.5          | 99.8 ± 1.0             | 12.0**      |
| Role/social limitations – emotional/behavioral | 91.9 ± 20.2          | 100.0 ± 0.0            | 5.4*        |
| Role/social limitations – physical        | 90.7 ± 25.4          | 99.03 ± 5.7            | 3.4         |
| Bodily pain/discomfort       | 75.5 ± 23.8          | 95.9 ± 9.6             | 23.7**      |
| Behavior                      | 74.6 ± 17.9          | 87.8 ± 11.4            | 16.1**      |
| Mental health                | 75.8 ± 18.1          | 86.3 ± 8.8             | 10.6**      |
| Self-esteem                  | 64.3 ± 19.4          | 107.3 ± 158.7          | 7.4**       |
| Parental impact – emotional   | 62.8 ± 26.8          | 94.2 ± 7.7             | 46.1**      |
| Parental impact – Time        | 88.1 ± 18.8          | 96.7 ± 10.1            | 0.9         |
| Family activities             | 81.1 ± 20.7          | 93.1 ± 10.2            | 10.6**      |

* $P ≤ .05$.
** $P ≤ .01$.

¹How much do you believe your weight is harmful to your health? For the IWQOL-A, higher scores are indicative of greater impairment. For HRQOL, lower scores indicate greater QOL impairment on the General Health and Physical Appearance subscales, whereas higher scores are indicative of worse QOL impairment for Health Efficacy B, Depression, and Self-regard subscales.
interactions were also found between BMI-SD and race on the HRQOL Health Efficacy B scale ($F = 5.8, P < .01$), the Self-Regard ($F = 4.2, P < .05$) and Physical Appearance scales ($F = 8.6, P < .01$): as BMI-SD increased, whites’ reports of impairment increased more precipitously than blacks’ reports.

### Parent Reports

Parents of overweight teens reported significantly poorer CHQ scores compared with parents of nonoverweight subjects on all subscales except the CHQ Role/Social Limitations – Physical and Parental Impact – Time scales (Means, standard deviations, $F$-values and significance levels in Table II). Comparing subjects by weight status separated by race, parents of overweight black teens reported poorer CHQ General Health Perceptions compared with parents of teens in the other three groups; and parents of overweight whites reported poorer scores compared with nonoverweight whites’ parents but not blacks’ parents (main effect, $P < .01$; Table III). No other racial differences between weight subgroups were detected. No differences were detected on the CHQ scale based upon sex or age (data not shown). After controlling for race, only the Role/Social Limitations – Physical scale demonstrated a marginally negative correlation with age ($r = -0.2$, $P = .05$; parents of older teens reported poorer functioning), and the Parental Impact – Time scale was correlated with SES ($r = 0.2$, $P < .05$; less impairment was associated with higher SES).

Significant BMI-SD by race interactions were found for the following CHQ scales: Mental Health, Parental Impact-Emotional, and Family Activities. Although BMI-SD did not appear to be associated with scores on the Mental Health (BMI-SD X race interaction: $F = 4.6, P < .05$),
Parental-Impact Emotional ($F = 7.0, P < .01$), or Family Activities ($F = 5.0, P < .05$) subscales according to black parents, high BMI-SD was associated with poorer QOL in white teens as reported by their parents.

**DISCUSSION**

We found overweight was a potent indicator of poorer weight and health-related QOL for white and black adolescents. Based on their IWQOL responses, overweight teens appeared to struggle with levels of distress regarding Social/Interpersonal, Self-esteem and Daily Living QOL similar to those reported by obese adults seeking weight loss treatment. However, overweight had a greater impact among heavier whites, compared with blacks, with regard to social and psychologic well-being, aspects of daily living, health efficacy, and physical appearance. As white adolescents became heavier, they described greater distress than blacks. These findings are consistent with some but not all adult studies reporting that obese blacks have greater QOL and less impairment than obese whites.

While no prior study has directly compared QOL between overweight black and white adolescents, racial comparisons of body weight and eating concerns have been examined. One study found that compared with black girls of similar body weight, white girls endorsed significantly greater disturbed eating and body weight–related cognitions. Moreover, black college women report less dissatisfaction with weight and less thin body-size ideals. Black girls are also more likely than white girls to misperceive themselves as being thinner than they actually are. Such differences may be a reflection of a greater acceptance of obesity in some subcultures. Other studies have found no differences between black and white adults on measures of body dissatisfaction, with one study suggesting that previous findings of less body concern in black women may be due to other demographic variables. However, the present analyses suggest that the differing impact of overweight on QOL in black teens is not due to such factors.

Black parents of overweight teens reported their children had poorer general health than healthy weight teens or than overweight whites. By contrast, parents of heavier white teens reported poorer QOL psychosocial functioning than normal weight or overweight black adolescents. It is possible that parents of overweight black teens are more generally concerned about their children’s current or future health and susceptibility to illness, while parents of overweight whites focus on the specific psychologic and social impact of obesity. Interestingly, in a weight loss intervention for black adolescent girls, the strongest predictive variable for weight loss was parent report of family satisfaction. This finding was interpreted to mean that the family context has an important influence on the effectiveness of weight loss treatment. Further studies should investigate reports of QOL and parent-reported family satisfaction to determine whether these factors serve as relevant mediators of weight loss for black and white teens. Alternatively, the characteristics of our weight reduction study, requiring an obesity-related medical
complication, may have differentially affected black and white parents. This may lead the parents of black overweight teens to report greater physical problems.

Limitations of this study include the small sample size of nonoverweight subjects. However, the sample size was adequate to find significant differences in QOL between overweight and nonoverweight adolescents. There are also possible sampling biases introduced by the study of overweight adolescents who desired weight loss treatment and healthy weight teens willing to participate in an exercise physiology study. The detected differences between groups may represent the extremes on the spectrum of our measures. However, the comparisons between black and white teens would not be affected by such biases. Because we detected several racial differences that were independent of weight status, it is also possible the measures of QOL we used may in some instances induce differential responses in blacks and whites. In addition, further analysis of the stability of our adapted versions of the QOL measures is required with samples not undergoing treatment, as our pretest–posttest comparison was obtained from a group of adolescents whose weight remained stable after treatment. Validation is also needed to determine whether our overweight subjects reported clinically meaningful levels of distress compared with other samples of overweight teens. Given that the overweight subjects in our sample reported scores similar to overweight adults, the means from the present study may provide potential adolescent cut-off scores on our measure of QOL that are indicative of clinically significant distress. Finally, since our sample only included adolescent subjects, our findings may not generalize to younger overweight children.

Black adults are less successful at weight loss treatment attempts, and the same may be true for black teens. We hypothesize that impairment in QOL may be a motivator for weight loss in adolescents, and that one obstacle to weight loss in black adolescents is their less marked dissatisfaction with weight and QOL. Whether the presence or absence of impairment in QOL is a predictor for the success of obesity treatment in adolescents remains to be determined. Future studies should investigate whether clinicians can more successfully motivate overweight black adolescents by focusing on QOL domains that cause them distress, such as the associated health risks and mobility limitations caused by excess weight.

REFERENCES

A HEAD-TO-HEAD COMPARISON: “CLEAN-VOID” BAG VERSUS CATHETER URINALYSIS IN THE DIAGNOSIS OF URINARY TRACT INFECTION IN YOUNG CHILDREN

DAVID MCGILLIVRAY, MD, FRCP(C), ELISE MOK, MSc, EDWARD MULROONEY, AND MICHAEL S. KRAMER, MD

Objective  To compare the validity of the urinalysis on clean-voided bag versus catheter urine specimens using the catheter culture as the “gold” standard.

Study design  This is a cross-sectional study of 303 nontoilet-trained children under age 3 years at risk for urinary tract infection (UTI) who presented to a children’s hospital emergency department. Paired bag and catheter specimens were obtained from each child and sent for dipstick and microscopic urinalysis. Sensitivity and specificity were compared using McNemar’s $\chi^2$ test for paired specimens and the ordinary $\chi^2$ test for unpaired comparisons.

Results  The bag dipstick was more sensitive than the catheter dipstick for the entire study sample: 0.85 (95% confidence interval [CI] = 0.78 to 0.93) versus 0.71 (95% CI = 0.61 to 0.81), respectively. Both bag and catheter dipstick sensitivities were lower in infants ≤90 days old (0.69 [95% CI = 0.44 to 0.94] and 0.46 [95% CI = 0.19 to 0.73], respectively) than in infants >90 days old (0.88 [95% CI = 0.81 to 0.96] and 0.75 [95% CI = 0.65 to 0.86], respectively). Specificity was consistently lower for the bag specimens than for the catheter specimens: 0.62 (95% CI = 0.56 to 0.69) versus 0.97 (95% CI = 0.95 to 0.99), respectively.

Conclusions  Urine collection methods alter the diagnostic validity of urinalysis. These differences have important implications for the diagnostic and therapeutic management of children with suspected UTI. (J Pediatr 2005;147:451-6)

Urinary tract infection (UTI) occurs in 5% of infants and young children with fever without source.1-4 In selected populations, such as girls with fever >39°C, the prevalence of UTI is as high as 30%.1,6 Early detection can be important in this age group to detect urinary tract anomalies to avoid the risks of urosepsis and possibly prevent the late sequelae of renal scarring, including hypertension and chronic renal failure.3,6-11

The sensitivity and specificity of the dipstick and microscopic urinalysis in detecting UTI in children have been evaluated recently.3,12-24 In these studies, urine collection methods included spontaneously voided (“clean-catch”), catheter, urine bag, diaper urine, and suprapubic aspiration. The success rate of obtaining urine by suprapubic aspiration can be low, whereas bag urine culture specimens are often contaminated.4,5,26 However, urinalysis from a bag specimen is often used to detect UTIs in non–toilet-trained infants through tests used to detect the presence of leukocytes and nitrites. Schroeder et al,27 as part of the PROS (Pediatric Research in the Office Setting) group, recently published an abstract of a study in which they tested the characteristics of urine tests in bag- and catheter-collected urine specimens. The catheter urinalysis was found to be more sensitive and more specific than the bag urinalysis, although urine specimens were not paired and bag and catheter cultures were used as a “gold standard.” The authors called for a paired urine study (ie, both types of specimens from the same child at the same visit) to address this issue. Therefore, in this study, the validity of the bag dipstick and urinalysis was subjected to a head-to-head comparison with urinalyses of catheterized specimens from the same children.

AAP American Academy of Pediatrics  HPF High-power field
CFU Colony-forming unit  UTI Urinary tract infection
CI Confidence interval  WBC White blood cell

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Supported in part by a grant from the Canadian Association of Emergency Physicians.

Submitted for publication Mar 29, 2004; last revision received Apr 8, 2005; accepted May 4, 2005.

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0022-3476/$ - see front matter

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10.1016/j.jpeds.2005.05.007
METHODS

We conducted a prospective cross-sectional study of non–toilet-trained children age <3 years who came to a children’s hospital emergency department between June 15, 2000 and December 31, 2001. The triage nurses judged the potential risk of UTI based on the following criteria: fever without source plus male sex ≤6 months or female sex ≤12 months, uncircumcised boys of any age, past history of UTI or abnormal renal anatomy, and fever >39°C or any fever ≥48 hours in duration. A second set of criteria included a heterogeneous group of infants and children without fever. These children were either ill-appearing without identifiable focus of infection or infants age <3 months, exhibited signs or symptoms of UTI (eg, dysuria, foul-smelling urine, change in urine color), or had unexplained abdominal pain. Children needing urgent medical intervention, such as immediate administration of antibiotics or resuscitation, were excluded from the study, as were those already receiving antibiotics.

After cleaning with liquid soap, a sterile urine collection bag (U-bag; Hollister, Libertyville, Ill) was applied to the child’s perineum. Our previous work demonstrated that changing the bag every 30 minutes does not alter the contamination rates of a bag urine specimen,26 therefore, the urine bag was not changed on a regular basis unless there was evidence of stool contamination or the bag had separated from the skin. We recognize that this information does not apply directly to the sensitivity or specificity of the urinalysis. After collection, the bag urine was transferred to a sterile plastic container and sent to the hospital’s clinical laboratory. The decision to catheterize a child was left to the discretion of the treating physician, because it was impractical and deemed unethical to obtain catheter urine specimens from all children who had a bag urinalysis performed in the absence of specific clinical indications. The physician who ordered the catheter specimens was not blinded to the results of the bag urinalysis. Only those children with bag and catheter urine specimens obtained on the same visit were included. This allowed for comparison of the bag and catheter urinalyses in the same patient, using the results of the catheter culture as the “gold standard.” The automated Clinitek 100/200 (Bayer, Pittsburgh, Pa) analyzer and Multistix 10 SG reagent strip (Bayer, Elkhart, IN) were used for dipstick analysis of leukocyte esterase and nitrites on bag and catheter urine specimens. This method was chosen to standardize the procedure and avoid operator-dependent variability, even if this instrument may not yet be available in all centers. The procedure for microscopic urinalysis included centrifugation of up to 10 mL of urine (if available) at 1800 rpm for 5 minutes, followed by decanting of the supernatant, resuspension of the remaining specimen, and examination under high power (40×) to determine the number of white blood cells (WBCs) per high-power field (HPF). A minimum of 2 mL of urine was required from both the bag and catheter specimens for inclusion in the study. Because of the high risk of contamination in bag urine cultures, no bag urine specimens were sent for culture.26 All catheter specimens were sent for culture.

The bag urine was always the first specimen collected. The perineum was therefore cleaned once before the bag collection and a second time before the catheter collection. To examine the possible effect of the 2 cleanings for catheter urines, we also analyzed the operating characteristics of the catheter urinalysis in a separate group of children in whom a catheter specimen but no bag specimen was obtained.

We defined a positive dipstick test as the presence of greater than trace (Ca 15/mm3) leukocyte esterase or a positive nitrite result. A positive microscopic examination was defined as >5 WBCs/HPF. We defined a combined microscopic and dipstick urinalysis as positive if the dipstick leukocyte esterase was greater than trace or nitrite positive, or if the microscopic analysis revealed >5 WBCs/HPF. The catheter urine culture was considered positive if it yielded >103 colony-forming units (CFU)/mL (or >106 CFU/L) of a single pathogenic organism. These definitions were based on the American Academy of Pediatrics (AAP) practice parameter for the diagnosis, treatment, and evaluation of initial UTI in febrile infants and young children.4

Data were also collected on age, temperature, sex, and circumcision status. Temperature was converted to a “rectal equivalent” by adding 1°C to axillary and 0.5°C to oral temperatures.28 We calculated the sensitivity and specificity and the 95% confidence interval (CI) of the dipstick, microscopic, and combined microscopic and dipstick for the bag and catheter specimens (based on the results of the catheter culture).

The data were stratified by age (age <90 days vs >90 days), sex, and (for boys) circumcision status. To determine whether the relationship of sensitivity and specificity for bag versus catheter specimens was preserved when using different colony counts to define a UTI, we compared the sensitivity and specificity of paired bag and catheter urinalyses using different colony counts for the definition of UTI (>103, >104, and >105 CFU/mL) in children age >90 days. Statistical significance was assessed using McNemar’s χ2 test for paired specimens and the ordinary χ2 test for unpaired comparisons. SPSS version 11.0 (SPSS, Chicago, Ill) was used for all statistical analyses. The Poisson distribution was used to estimate 95% CIs for 0 numerators.29

RESULTS

A total of 303 children were enrolled in the study, including 102 (33.6%) boys and 201 (66.3%) girls. Fifty-four (17.8%) of the children were age <90 days. Overall, 82 (27.1%) of the catheter urine cultures were positive, including 13/54 (24%) of those in children age <90 days and 69/249 (27.7%) of those in children age >90 days. Boys and girls were culture-positive in 28.4% (29/102) and 26.3% (53/201) of the cases, respectively. The 3 most common pathogenic organisms were Escherichia coli (79.2%), Proteus (7.8%), and Klebsiella (2.6%).

The circumcision status was recorded in 69 of 102 boys, of whom 79.7% (55/69) were uncircumcised. Cultures were
positive in 36.4% (20/55) of the uncircumcised boys versus 14.2% (2/14) of circumcised boys ($P < .001$). A rectal equivalent temperature was obtained in 297/303 children, of whom 17.5% (53/297) had a temperature $>39.5^\circ C$. Nitrites were positive in 36% of the children with a positive urine culture. No culture-positive urine specimens were positive for nitrites and negative for leukocyte esterase.

The paired dipstick sensitivities and specificities are given in Table I. In all of the infants and children $<3$ years, the sensitivity of the bag urinalysis was greater than that of the catheter urinalysis: 0.85 (95% CI = 0.78 to 0.93) versus 0.71 (0.61 to 0.81), respectively ($P = .003$). The bag sensitivity was highest in children age $>90$ days. The trend of higher bag versus catheter sensitivity was seen in infants age $<90$ days, children age $>90$ days to 3 years, and in both sexes. The sensitivity of bag and catheter collection methods was low in infants age $<90$ days. Overall, specificity of the bag dipstick for all ages was low (many false-positives) compared with the catheter specimen: 0.62 (95% CI = 0.56 to 0.69) versus 0.97 (95% CI = 0.94 to 0.99), respectively ($P = < .001$).

The results of the combined dipstick and microscopic urinalyses are given in Table II. As expected, the sensitivity of both the bag and catheter specimens increased, whereas specificity decreased, compared with dipstick alone. The relative performance of the bag and catheter urinalyses did not change, however.

The dipstick sensitivity results for paired bag and catheter urinalyses did not differ according to sex, although the larger sample size and higher rate of infection in the girls resulted in lower $P$ values (Table III). In contrast, bag dipstick specificity (Table IV) was much higher in boys than in girls at all ages: 0.86 (95% CI = 0.78 to 0.94) versus 0.51 (95% CI = 0.43 to 0.59), respectively ($P = < .001$; unmatched $\chi^2$ test).

Stratification of data based on circumcision status failed to explain the difference in specificity noted between boys and girls. For the 69 infants and children in whom circumcision status was known, the dipstick specificity of the bag urine was 0.83 (95% CI = 0.62 to 1.00) in circumcised boys and 0.81 (95% CI = 0.68 to 0.93) in uncircumcised boys.

The order of collection of catheter urine specimens did not significantly affect the operating characteristics of the dipstick urinalysis. A group of 218 infants also age $<3$ years who were not part of the 303 children in this study had catheter urine specimens obtained directly and thus had only 1 cleaning before the catheter urinalysis and culture. This

### Table I. Comparison of dipstick sensitivity and specificity in paired bag versus catheter urine specimens, overall and in 2 age groups

<table>
<thead>
<tr>
<th>Bag</th>
<th>Catheter</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall (n = 303)</td>
<td>0.85 (0.78 to 0.93)</td>
<td>0.71 (0.61 to 0.81)</td>
</tr>
<tr>
<td>$\leq 90$ days (n = 54)</td>
<td>0.69 (0.44 to 0.94)</td>
<td>0.46 (0.19 to 0.73)</td>
</tr>
<tr>
<td>$&gt; 90$ days (n = 249)</td>
<td>0.88 (0.81 to 0.96)</td>
<td>0.75 (0.65 to 0.86)</td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall (n = 303)</td>
<td>0.62 (0.56 to 0.69)</td>
<td>0.97 (0.95 to 0.99)</td>
</tr>
<tr>
<td>$\leq 90$ days (n = 54)</td>
<td>0.61 (0.46 to 0.76)</td>
<td>1.00 (0.93 to 1.00)</td>
</tr>
<tr>
<td>$&gt; 90$ days (n = 249)</td>
<td>0.63 (0.56 to 0.70)</td>
<td>0.97 (0.94 to 0.99)</td>
</tr>
</tbody>
</table>

### Table II. Comparison of combined dipstick and microscopic sensitivity and specificity in paired bag versus catheter urine specimens, overall and in 2 age groups

<table>
<thead>
<tr>
<th>Bag</th>
<th>Catheter</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall (n = 287)</td>
<td>0.95 (0.90 to 1.00)</td>
<td>0.83 (0.74 to 0.91)</td>
</tr>
<tr>
<td>$\leq 90$ days (n = 52)</td>
<td>0.77 (0.54 to 1.00)</td>
<td>0.62 (0.35 to 0.88)</td>
</tr>
<tr>
<td>$&gt; 90$ days (n = 235)</td>
<td>0.99 (0.96 to 1.00)</td>
<td>0.87 (0.78 to 0.95)</td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall (n = 287)</td>
<td>0.45 (0.38 to 0.52)</td>
<td>0.95 (0.92 to 0.98)</td>
</tr>
<tr>
<td>$\leq 90$ days (n = 52)</td>
<td>0.54 (0.38 to 0.69)</td>
<td>1.00 (0.92 to 1.00)</td>
</tr>
<tr>
<td>$&gt; 90$ days (n = 235)</td>
<td>0.43 (0.35 to 0.50)</td>
<td>0.94 (0.90 to 0.98)</td>
</tr>
</tbody>
</table>
group was compared with our study group, all of whom had a catheter urine specimen obtained after bag collection (the 2-cleaning group).

Overall sensitivity was 0.63 (95% CI = 0.56 to 0.69) for the 1-cleaning group versus 0.71 (95% CI = 0.66 to 0.76) for the 2-cleaning group. Specificity for the 2 groups was 0.96 (95% CI = 0.93 to 0.99) and 0.97 (95% CI = 0.94 to 0.98), respectively.

**DISCUSSION**

Urine cultures by catheterization or suprapubic aspiration are necessary to detect UTI in infants. The question has arisen as to whether a “catheterize all” strategy or a “selective catheterization” strategy should be used in the search for a UTI in non–toilet-trained infants and children. The AAP practice parameter for the diagnosis, treatment, and evaluation of the initial UTI in febrile infants and children suggests 2 options for managing the infant or young child (age 2 months to 2 years) with unexplained fever who is assessed as not being so ill as to require immediate antimicrobial therapy. One option is the “catheterize all” approach; the other is to “obtain a urine specimen by the most convenient means and perform a urinalysis. If the urinalysis suggests a UTI, obtain and culture a urine specimen, collected by SPA or transurethral catheterization.”

If the urinalysis is negative, then the AAP suggests that it is “reasonable to follow the clinical course without initiating antimicrobial therapy, recognizing that a negative urinalysis does not rule out a UTI.”

Recent research has focused on the utility of rapid diagnostic tests, such as dipstick and microscopic urinalysis, for diagnosing UTI and making early patient management decisions pending culture. We have compared the performance of diagnostic tests for UTI using bag versus catheter urinalyses to determine their ability to detect a UTI in the non–toilet-trained infant and young child. The unique aspect of our study is that we documented the sensitivity of bag urinalyses compared with catheter urinalyses in the same children based on the catheter culture as the gold standard. Other unique aspects of our study are that it stratifies the results according to 2 different age groups and according to different levels of colony count definitions for a positive urine culture in the 3- to 36-month age category.

Children in this study had a high prevalence of UTI (26%). The main reason for this high prevalence is that treating physicians were aware of the urinalysis results of the bag specimens when deciding whether to order a catheter specimen for urinalysis and culture. Other factors that may account for the high prevalence include the fact that the nurses and physicians have an awareness of which infants and children are at risk for UTI, the low circumcision rate in our study sample (20%), and the large number of referred children. As shown in Table V, a low threshold for the definition of UTI (>10^3 CFU/mL, or >10^6 CFU/L) also increases the

| Table III. Comparison of dipstick sensitivity in paired bag versus catheter urine specimens, overall and in 2 age groups by sex |
|-----------------|-----------------|-----------------|
| Bag | Catheter | P value |
| Boys | | |
| Overall (n = 102) | 0.86 (0.74 to 0.99) | 0.69 (0.52 to 0.86) | .131 |
| ≤90 days (n = 25) | 0.73 (0.46 to 0.99) | 0.45 (0.16 to 0.75) | .248 |
| >90 days (n = 77) | 0.94 (0.84 to 1.00) | 0.83 (0.66 to 1.00) | .617 |
| Girls | | |
| Overall (n = 201) | 0.85 (0.75 to 0.95) | 0.72 (0.60 to 0.84) | .023 |
| ≤90 days (n = 29) | 0.50 (0.00 to 1.00) | 0.50 (0.00 to 1.00) | 1.00 |
| >90 days (n = 172) | 0.86 (0.77 to 0.96) | 0.73 (0.60 to 0.85) | .023 |

| Table IV. Comparison of dipstick specificity in paired bag versus catheter urine specimens, overall and in 2 age groups by sex |
|-----------------|-----------------|-----------------|
| Bag | Catheter | P value |
| Boys | | |
| Overall (n = 102) | 0.86 (0.78 to 0.94) | 0.99 (0.96 to 1.00) | .027 |
| ≤90 days (n = 25) | 1.00 (0.79 to 1.00) | 1.00 (0.79 to 1.00) | 1.00 |
| >90 days (n = 77) | 0.83 (0.73 to 0.93) | 0.98 (0.95 to 1.00) | .027 |
| Girls | | |
| Overall (n = 201) | 0.51 (0.43 to 0.59) | 0.97 (0.94 to 1.00) | <.001 |
| ≤90 days (n = 29) | 0.41 (0.22 to 0.59) | 1.00 (0.89 to 1.00) | <.001 |
| >90 days (n = 172) | 0.53 (0.44 to 0.62) | 0.96 (0.92 to 0.99) | <.001 |
Specificity for the definition of a UTI

In The Diagnosis Of Urinary Tract Infection In Young Children

A Head-To-Head Comparison: “Clean-Void” Bag Versus Catheter Urinalysis

The work by Hellerstein suggests that a catheter urine culture with a colony count of 10³ or 10⁴ CFU/mL is suspicious for a UTI and should be repeated.³⁰

All cultures in this study were obtained by catheterization. In general, lower colony counts are accepted for the diagnosis of UTI compared with colony counts on midstream urine specimens. This figure is also supported by the AAP practice parameter on UTIs.⁴ Moreover, the results in this study demonstrate that even when a higher colony count is chosen as the threshold for defining a UTI, the relationship between bag and catheter urinaries (higher sensitivity and lower specificity in bag urinalysis vs lower sensitivity and higher specificity from catheter urinalysis) does not change.

Sensitivity and specificity of the bag urine specimens were chosen as the main outcome measures, because the high prevalence of UTIs in our study sample would affect the positive and negative predictive values. Sensitivity and specificity were felt to illustrate the performance characteristics of the test itself, independently of the specific population or setting. The most important test characteristic for urinalysis is sensitivity, because it ensures that children with a negative result are at very low risk of a UTI and can thus be spared the discomfort of a catheterization.

The higher bag versus catheter dipstick sensitivity may result from the presence of WBCs or bacteria in the urethra or introitus in girls and under the foreskin in boys. The same presence of WBCs in the perineal skin, vaginal introitus, or urethra would likely explain the lower specificity of the bag urine in females. However, the higher specificity of the bag urine from boys despite circumcision status suggests that the presence of periurethral WBCs or bacteria is less common in males. The lower sensitivity of the bag urine in infants age <90 days could be caused by either a decreased inflammatory response or the frequent voiding and low “dwell time” in neonates and other young infants versus older infants. But the lack of a significant difference in sensitivity among infants receiving immediate catheterization versus catheterization postbag (presumably with a lower “dwell time”) argues against the “dwell time” theory. Although our conclusions are limited by the small sample size of infants age <90 days, the low sensitivity of the dipstick urinalysis in both bag and catheter specimens reinforces the recommendation that a catheter sample for culture is needed in this age group.⁴

Every non–toilet-trained child truly at high risk for UTI should be catheterized to obtain both a urinalysis and culture. In children with fever without source and at low risk for UTI (no previous history of UTI, no anatomic abnormalities, not immunosuppressed, no urinary symptoms), a “selective catheterization” strategy as outlined in the AAP practice parameter appears reasonable, based on our findings.

In our study, a “selective catheterization” strategy would have led to catheterization of 138/255 (54%) in the 3- to 36-month age group, compared with 100% with a “catheterize all” strategy (a reduction of 46%). In a population of children with a lower prevalence of UTI, the proportion of children not requiring catheterization would be even higher. The bag dipstick and microscopic urinalysis are thus a useful screen for UTI in non–toilet-trained infants age >90 days because of its practicality, the difficulty of obtaining catheterized specimens in nonpediatric centers and office settings, and the high sensitivity compared with catheter specimens.

However, using a dipstick screening strategy for UTI in infants age >90 days will miss 4% to 12% of UTIs, in the subsequent catheterized specimen, depending on the threshold chosen for colony count. This is true both for high-risk and low-risk children. For children at high risk (eg, recurrent UTI, reflux, impaired renal function), the adverse consequences of such a false-negative rate may well be unacceptable; in such children, a catheter specimen should be sent for culture. Based on our previous studies, bag urine specimens should never be sent for culture.²⁶

In low-risk children, serious consequences of infection are far less likely, and the risks of missing a UTI are likely to be outweighed by the risks of catheterization, including pain, false-positive result, trauma, or even introduction of infection. The physician thus may choose to use a bag urine screening strategy to reduce the number of unnecessary catheterizations. The consequences of insisting on a “catheterize all” strategy

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### Table V. Comparison of dipstick sensitivity and specificity in paired bag versus catheter urine specimens in 249 non–toilet-trained infants age >90 days, using different colony counts (from a catheterized specimen) for the definition of a UTI

<table>
<thead>
<tr>
<th>Percent positive</th>
<th>Bag</th>
<th>Catheter</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;10³ CFU/mL (n = 69)</td>
<td>27.7% (69/249)</td>
<td>0.88 (0.81 to 0.96)</td>
<td>0.75 (0.65 to 0.86)</td>
</tr>
<tr>
<td>&gt;10⁴ CFU/mL (n = 58)</td>
<td>23.3% (58/249)</td>
<td>0.93 (0.87 to 1.00)</td>
<td>0.81 (0.73 to 0.90)</td>
</tr>
<tr>
<td>&gt;10⁵ CFU/mL (n = 46)</td>
<td>18.5% (46/249)</td>
<td>0.96 (0.90 to 1.00)</td>
<td>0.83 (0.74 to 0.91)</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;10³ CFU/mL (n = 180)</td>
<td>72.3% (180/249)</td>
<td>0.63 (0.56 to 0.70)</td>
<td>0.97 (0.94 to 0.99)</td>
</tr>
<tr>
<td>&gt;10⁴ CFU/mL (n = 191)</td>
<td>76.7% (191/249)</td>
<td>0.61 (0.54 to 0.68)</td>
<td>0.94 (0.91 to 0.97)</td>
</tr>
<tr>
<td>&gt;10⁵ CFU/mL (n = 203)</td>
<td>81.5% (203/249)</td>
<td>0.59 (0.52 to 0.65)</td>
<td>0.90 (0.86 to 0.94)</td>
</tr>
</tbody>
</table>
for all nontoxic-appearing children with fever without source may have the potential to lead to test resistance by nurses, physicians, and parents who are concerned about a painful procedure and the unknown risk of introducing infection.

Formal decision and cost-benefit analyses of this versus other strategies might be helpful to compare alternative approaches. Moreover, further studies with larger numbers are needed to confirm our findings obtained for children age < 90 days.

REFERENCES

AMINO ACID ADMINISTRATION TO PREMATURE INFANTS
DIRECTLY AFTER BIRTH

FRANS W. J. TE BRAAKE, M SC, CHRIS H. P. VAN DEN AKKER, M SC, DARCOs J. L. WATTIMENA, JAN G. M. HUIJMANS, PHD, AND JOHANNES B. VAN GOUDOEVER, MD, PHD

Objectives To test the hypothesis that the administration of 2.4 g amino acids (AA)/(kg d) to very low birth weight infants is safe and results in a positive nitrogen balance.

Study design We conducted a randomized, clinical trial. Preterm infants with birth weights <1500 g received either glucose and 2.4 g AA/(kg d) from birth onward (n = 66) or solely glucose during the first day with a stepwise increase in AA intake to 2.4 g AA/(kg d) on day 3 (n = 69). Blood gas analysis was performed daily during the first 6 postnatal days; blood urea nitrogen levels were determined on days 2, 4, and 6; AA plasma concentrations and nitrogen balances were determined on days 2 and 4. Student t tests, Mann-Whitney tests, and χ² tests were performed to compare groups.

Results Infants supplemented with AA had no major adverse side effects. Their blood urea nitrogen levels were higher, nitrogen balance turned positive upon AA administration, and more AA concentrations were within reference ranges.

Conclusions High-dose AA administration to very low birth weight infants can be introduced safely from birth onward and results in an anabolic state. (J Pediatr 2005;147:457-61)

A fter birth, very low birth weight (VLBW) infants are dependent on externally administered nutrients, as hardly any stored energy is at their disposal. Both fat tissue and glycogen levels are limited, especially in small-for-gestational age (SGA) VLBW infants. Consequently, without adequate exogenous nutrient supply, protein breakdown will increase in these infants, resulting in a catabolic state.

Despite a growing body of literature regarding the safety and efficacy of early amino acid (AA) administration, there is still wide variability in practice. Often, carbohydrates are still the only exogenous nutrients administered in the immediate postnatal period. In the past, AA were often withheld since formerly used AA mixtures were found to result in metabolic acidosis and hyperammonemia. In utero, fetuses are supplied with large amounts of AA, which not only are used for protein synthesis but also serve as an important fuel source. It seems logical, therefore, to supply newborn infants with adequate amounts of both energy and growth substrates to meet energy requirements and to promote protein accretion for ongoing growth. Indeed, several studies indicate that the currently used crystalline solutions seem well suited for the preterm infant, who may benefit from the anabolic effects. However, in most of these studies, either low amounts of AA were administered, administration started only after the first day of life, infants with higher birth weights were studied, or the number of infants studied was small.

Hypothesizing that premature infants may benefit from the anabolic effects of AA without metabolic derangement, we investigated the safety and efficacy of relatively large amounts of AA supplied postnatally to a large group of VLBW infants.

METHODS

A randomized, blinded trial was performed in the neonatal intensive care unit (NICU) of the Erasmus MC-Sophia Children’s Hospital, Rotterdam, the Netherlands.
For logistic reasons, it was not possible to perform the study using a double-blinded design. The trial was investigator-initiated, with no funding from the pharmaceutical industry. The study protocol was approved by the Erasmus MC Medical Ethical Committee, and parental consent was obtained before random assignment and subsequent enrollment in the study.

### Study Design

Prematurely born infants with birth weights equal or less than 1500 g born between March 2003 and September 2004 in the hospital and admitted to the NICU were randomly assigned to receive one of two parenteral nutritional schemes, as indicated in Table I. The amount of 2.4 g AA/(kg d) was chosen because that was the amount that resulted in a positive nitrogen balance in an earlier study.1

After the third day of life, all nutrient intakes, including enteral feedings, were the decision of the attending neonatologist. Minimal enteral nutrition (6 to 12 feedings of 1.0 mL) was whenever possible started on postnatal day 2 to day 3 and advanced to full enteral nutrition in the subsequent days if tolerated. We recorded birth weight, gestational age, percentage of SGA infants (< 2 SD), sex ratio, number of prenatal corticosteroid doses (0, 1, or 2), and severity of illness at entry to the study with Apgar and CRIB scores.10 Exclusion criteria were known congenital abnormalities, chromosome defects, metabolic diseases, and endocrine, renal, or hepatic disorders.

### Analysis

#### SAFETY

We analyzed blood gas and glucose concentrations (mmol/L) 12 hours after delivery, followed by daily measurements at 8 AM until day 6. Blood urea nitrogen (BUN) concentrations (mmol/L) were monitored on days 2, 4, and 6 (Roche Hitachi 912, Roche Diagnostics, Basel, Switzerland). On days 2 and 4, we determined plasma AA concentrations (mmol/L) (Biochrom 20, Biochrom Ltd, Cambridge, England) in a subset of patients (intervention group n = 17, control group n = 14) to identify possible hyperaminoacidemia (ie, above reference ranges, as defined in Reference 21). We also recorded fluid intakes and medications.

#### EFFICACY

Efficacy of early AA administration was studied by quantifying the nitrogen balance in both groups on postnatal days 2 and 4. Because most nitrogen leaves the body in urine, we collected urine during a 12-hour period on both study days. Urinary nitrogen content was measured with a CHN elemental analyzer (ANA 1500; Carlo Erba Strumentazione, Milan, Italy). By subtracting the calculated nitrogen excretion rates from the precisely recorded nutritional intakes, nitrogen balances could be defined under the assumption that 1 g of nutritional AA equals 160 mg of nitrogen. Although 24-hour collections of urine are preferable, 12-hour or even 6-hour collections can be used to establish reasonable estimates of nitrogen excretion.10 Many investigators used 12-hour urine collections accordingly.10,13,14,18 Finally, to express efficacy in terms of a measurable clinical variable, we recorded on which postnatal day infants regained their birth weight.

#### STATISTICS

Differences between groups were tested by Student t tests, Mann-Whitney tests, and \( \chi^2 \) tests, using SPSS version 11.0 (SPSS Inc, Chicago, IL). Depending on

### Table I. Targeted intravenous macronutrient intake in mg/(kg-min) (glucose) or g/(kg d) (AA and lipids)

<table>
<thead>
<tr>
<th>Day</th>
<th>Glucose</th>
<th>AA*</th>
<th>Lipids†</th>
<th>Glucose</th>
<th>AA*</th>
<th>Lipids†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.5</td>
<td>2.4</td>
<td>0</td>
<td>5.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5.6</td>
<td>2.4</td>
<td>1.4</td>
<td>5.6</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>3</td>
<td>5.7</td>
<td>2.4</td>
<td>2.8</td>
<td>5.7</td>
<td>2.4</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>7.1</td>
<td>2.4</td>
<td>2.4</td>
<td>7.1</td>
<td>2.4</td>
<td>2.4</td>
</tr>
</tbody>
</table>

If enteral feedings were tolerated, parenteral glucose intake was decreased.

*Primene 10%, Baxter, Clintec Benelux NV, Brussels, Belgium.
†Intralipid 20%, Fresenius Kabi BV, ’s-Hertogenbosch, the Netherlands.

### Table II. Clinical characteristics of the infants in the intervention and control group

<table>
<thead>
<tr>
<th></th>
<th>Intervention</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (male/female)</td>
<td>66 (34/32)</td>
<td>69 (31/38)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1039 ± 235</td>
<td>989 ± 252</td>
</tr>
<tr>
<td>Gestational age</td>
<td>28.4 ± 2.0</td>
<td>28.4 ± 1.9</td>
</tr>
<tr>
<td>SGA infants (&lt;−2 SD)</td>
<td>20%</td>
<td>29%</td>
</tr>
<tr>
<td>CRIB score</td>
<td>3 (0–13)</td>
<td>4 (0–14)</td>
</tr>
<tr>
<td>Apgar (5 #) score</td>
<td>9 (1–10)</td>
<td>8 (2–10)</td>
</tr>
<tr>
<td>Prenatal corticosteroids</td>
<td>18/18/64</td>
<td>39/19/42</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± SD.
†Values are expressed as median (min-max).

For logistic reasons, it was not possible to perform the study using a double-blinded design. The trial was investigator-initiated, with no funding from the pharmaceutical industry. The study protocol was approved by the Erasmus MC Medical Ethical Committee, and parental consent was obtained before random assignment and subsequent enrollment in the study.
Table III. Blood gas analysis and whole blood glucose concentrations in the intervention and control groups 12 hours postnatally and on postnatal day 2

<table>
<thead>
<tr>
<th></th>
<th>12 h</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intervention</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.33 ± 0.08</td>
<td>7.34 ± 0.08</td>
</tr>
<tr>
<td>BE (mmol/L)</td>
<td>-4.8 ± 3.1</td>
<td>-3.7 ± 3.3</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L)</td>
<td>20.5 ± 2.6</td>
<td>21.5 ± 2.6</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.7 ± 3.2</td>
<td>6.1 ± 2.4</td>
</tr>
</tbody>
</table>

|                      | Intervention| Control     |
|                      |             |             |
|                      |             |             |

Values represented as mean ± SD and tested with Student t test.
*Statistically significant; P < .05.

Distribution and type of test, values are expressed as mean ± SD, as median (min-max), or as percentage, respectively. Significance level was set at P < .05. However, because of multiple variables assessed on single samples, differences in AA concentrations were considered to be statistically significant at P < .01. From previous findings, we calculated that with a power of 0.80, group size needed to be at least 26 to detect a difference in the nitrogen balance of 150 mg N/(kg/d), with a standard deviation of 120 mg N/(kg/d). However, as we intended to study safety aspects as well, we continued to include patients for the full 18 months.

RESULTS

We included 66 infants in the intervention group and 69 in the control group; all infants were included on the basis of intention to treat (Table II). Despite random assignment, infants in the intervention group were more frequently exposed to prenatal corticosteroids (P = .017). According to study design, the infants in the intervention group received AA within 2 hours after birth (median, 1 hour, 33 minutes). Nonprotein energy intakes did not differ between groups, except on day 5 (68 ± 14 [intervention] vs 63 ± 14 [control] kcal/[kg-d]; P = .033) (Figure 1).

Safety

Results of blood gas analysis and whole blood glucose levels 12 hours after birth and on the second day are shown in Table III. Between postnatal days 3 and 6, there were no differences. BUN levels are shown in Table IV.

Table V shows individual plasma AA concentrations on the second day of life. No statistical differences between the two groups were found on the fourth postnatal day.

Medications, including sodium bicarbonate for metabolic acidosis, were not different between groups.

Efficacy

As follows from study design, nitrogen intake on the second day was higher in the intervention group (Figure 2). On the fourth day, intakes were similar between groups. Nitrogen excretion rates in the intervention group exceeded excretion rates in the control group on both day 2 and day 4. Furthermore, within the intervention as well as within the control group, rates of excretion did not change between days 2 and 4. Consequently, nitrogen balance was higher in the intervention group on day 2 as compared with the control group, which had a negative nitrogen balance. On the fourth day, nitrogen balances in both groups were positive. However, in the control group, the balance was more positive than in the intervention group. There was no correlation between antenatal steroid administration and nitrogen excretion or balance.

Fluid intakes were higher in the intervention group on both postnatal day 1 and day 2 due to the administration of AA. On all other days, fluid intakes were similar. Fluid balances, determined on postnatal days 2 and 4, did not differ between groups. Age to regain birth weight was not statistically different; newborn infants in the intervention group regained their birth weight at day 8 (2-25) (median and [min-max]), those in the control group at day 10 (2-26) (P = .286).

DISCUSSION

The currently available AA solutions are safe and can be administered to premature infants during the first few days of life.8-14 We performed the largest study to date confirming the safety and anabolic effects of early AA administration beginning within 2 hours after birth. Unlike most other reports, we did find modestly altered blood gas values and increased BUN levels with early AA administration. This could be due to the inclusion of fewer infants in other studies, with subsequently the possibility of reduced statistical power. Another explanation could be the early start of AA.
administration in our study, which was within 2 hours instead of 24 hours after birth or even later. In addition, others used a smaller amount of AA (1.5 g/[kg/d]) or included infants with higher birth weights.

We found that early AA administration normalized the plasma concentrations of most AA and that nitrogen balance was positive on day 2 of life, despite a relatively low energy intake (<40 kcal/[kg/d]). BUN levels were higher in the intervention group, which theoretically could have increased urine production but in fact did not (data not shown). Besides, fluid balance is usually tightly controlled in NICUs. To our knowledge, no other potential side effects of increased BUN levels have been reported. The higher BUN levels are a reflection of a higher AA oxidation rate. This resembles the intrauterine situation in which AA seem to be a key nutrient for energy generation and where BUN reference values for human umbilical cord blood are 7.5 to 14.3 mmol/L (21.0 to 40.1 mg/dL).

In conjunction with the higher BUN levels, the higher amounts of excreted nitrogen in the intervention group also indicate a higher oxidation rate. Higher BUN levels should, therefore, not be interpreted as a sign of AA intolerance but rather as a reflection of AA oxidation, just like in utero, where the AA are partly oxidized and partly used for protein synthesis.

Many of the infants in the intervention group had on average less hyperglycemia than did the control group, which might be explained by higher insulin concentrations triggered by relatively higher plasma arginine and leucine concentrations. In addition to these two AA, all essential AA levels, except for threonine and most of the nonessential AA concentrations, were higher and were within the reference range in the intervention group on the second day of life. Although the plasma concentrations of valine, lysine, and asparagine exceeded the reference values measured postnatally in term breast-fed infants, the former two AA concentrations fit within intrauterine reference ranges.

The nitrogen balance was calculated by subtracting nitrogen excretion from nitrogen intake. However, nitrogen excretion is often modestly underestimated, because of incomplete urine collections and stool, breath, and skin losses, which are not accounted for. Furthermore, although nitrogen balance measurements demonstrate net loss or accretion of protein, they do not reveal the mechanisms underlying these conditions. Previously performed studies using stable isotope techniques showed that premature infants supplemented with AA have an improved balance, which is due to increased protein synthesis, while proteolysis is not suppressed.

Inasmuch as premature infants cannot survive without growth, we conclude that the administration of AA soon

### Table V. AA profile in the intervention and control groups on postnatal day 2

<table>
<thead>
<tr>
<th>AA</th>
<th>Intervention mean ± SD in μmol/L</th>
<th>Control mean ± SD in μmol/L</th>
<th>Reference range in μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine**</td>
<td>148 ± 43</td>
<td>47 ± 13</td>
<td>86 – 171</td>
</tr>
<tr>
<td>Isoleucine**</td>
<td>88 ± 33</td>
<td>18 ± 8</td>
<td>31 – 124</td>
</tr>
<tr>
<td>Valine**</td>
<td>281 ± 90</td>
<td>88 ± 23</td>
<td>56 – 154</td>
</tr>
<tr>
<td>Threonine</td>
<td>125 ± 48</td>
<td>123 ± 63</td>
<td>67 – 143</td>
</tr>
<tr>
<td>Lysine**</td>
<td>345 ± 144</td>
<td>98 ± 34</td>
<td>65 – 282</td>
</tr>
<tr>
<td>Histidine**</td>
<td>103 ± 53</td>
<td>52 ± 19</td>
<td>25 – 126</td>
</tr>
<tr>
<td>Methionine*</td>
<td>42 ± 22</td>
<td>22 ± 9</td>
<td>21 – 55</td>
</tr>
<tr>
<td>Phenylalanine**</td>
<td>92 ± 31</td>
<td>58 ± 10</td>
<td>35 – 112</td>
</tr>
<tr>
<td>Cystine</td>
<td>31 ± 79</td>
<td>22 ± 12</td>
<td>33 – 55</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>83 ± 43</td>
<td>122 ± 57</td>
<td>48 – 122</td>
</tr>
<tr>
<td>Alanine**</td>
<td>265 ± 139</td>
<td>124 ± 67</td>
<td>137 – 362</td>
</tr>
<tr>
<td>Proliné*</td>
<td>175 ± 89</td>
<td>102 ± 56</td>
<td>79 – 227</td>
</tr>
<tr>
<td>Serine*</td>
<td>186 ± 89</td>
<td>116 ± 49</td>
<td>79 – 227</td>
</tr>
<tr>
<td>Glycine</td>
<td>282 ± 161</td>
<td>205 ± 70</td>
<td>66 – 432</td>
</tr>
<tr>
<td>Arginine**</td>
<td>70 ± 19</td>
<td>29 ± 12</td>
<td>11 – 88</td>
</tr>
<tr>
<td>Glutamine</td>
<td>507 ± 296</td>
<td>313 ± 153</td>
<td>147 – 623</td>
</tr>
<tr>
<td>Glutamate**</td>
<td>64 ± 34</td>
<td>22 ± 9</td>
<td>76 – 551</td>
</tr>
<tr>
<td>Asparagine</td>
<td>39 ± 23</td>
<td>49 ± 24</td>
<td>16 – 21</td>
</tr>
<tr>
<td>Aspartate</td>
<td>35 ± 16</td>
<td>18 ± 14</td>
<td>5 – 46</td>
</tr>
<tr>
<td>Taurine</td>
<td>150 ± 87</td>
<td>106 ± 112</td>
<td>20 – 84</td>
</tr>
<tr>
<td>Citrulline</td>
<td>54 ± 67</td>
<td>31 ± 44</td>
<td>39 – 386</td>
</tr>
<tr>
<td>Ornithine**</td>
<td>180 ± 87</td>
<td>40 ± 13</td>
<td></td>
</tr>
<tr>
<td>OH-Proline</td>
<td>47 ± 26</td>
<td></td>
<td>46 ± 28</td>
</tr>
</tbody>
</table>

Reference values from healthy term breast-fed infants on postnatal day 1.
*Statistically significant; P < .01.
**Statistically significant; P < .001.
after birth with the aim of promoting anabolism is safe and effective.

REFERENCES

Objective To test the hypothesis that cytokines might distinguish critically ill infants with bacterial sepsis or necrotizing enterocolitis (NEC) from those with sepsis syndrome and that these elevations would be correlated with clinical variables of inflammation and mortality.

Study design We measured plasma and tracheal aspirate (TA) levels of interleukin-8 (IL-8), epithelial neutrophil activating peptide (ENA-78), IL-10, and IL-18 in 84 neonates with suspected sepsis or NEC. Thirty-one infants had bacterial sepsis, 19 had NEC, and 34 infants with negative results on cultures had sepsis syndrome.

Results Plasma IL-8 and IL-10 levels were significantly increased in infants with bacterial sepsis compared with those in infants with sepsis syndrome. Plasma IL-8, ENA-78, and IL-10 levels were elevated in infants with NEC compared with those in infants with sepsis syndrome. TA IL-8 and IL-10 levels were also increased in infants with bacterial sepsis; TA ENA-78, and IL-18 were not elevated in infants with sepsis or NEC when compared with infants with sepsis syndrome. Plasma and TA cytokine levels correlated with hematologic parameters. Plasma cytokine levels were higher in infants who did not survive than in infants who did survive.

Conclusions Plasma and TA cytokine levels are elevated in critically ill infants with bacterial sepsis or NEC compared with those in infants with sepsis syndrome. Our results suggest distinct patterns of cytokine elaboration in different disease states.

Bacterial sepsis is an important determinant of morbidity and mortality in the newborn intensive care unit. This most likely results from physiologic deficiencies in the immune system that diminish the newborn inflammatory response. Cytokines are important endogenous proteins that mediate the cardiorespiratory abnormalities and end-organ dysfunction associated with sepsis and shock. Recent studies suggest that interactions between pro- and anti-inflammatory cytokines regulate the inflammatory response.

Studies in adults have found markedly elevated blood levels of the pro-inflammatory cytokines tumor necrosis factor (TNF), interleukin-1 (IL-1), and IL-6 in sepsis and shock. Elevations of anti-inflammatory mediators, in particular IL-10, correlate with both the severity of the inflammatory insult and the plasma concentration of pro-inflammatory cytokines. The chemokines IL-8 and epithelial neutrophil activating peptide (ENA-78) have an important regulatory role in neutrophil influx in the lung during inflammation. These mediators are important in plasma during sepsis and in tracheal lavage fluid of adults with acute respiratory distress syndrome (ARDS). A recently described member of the IL-1 family, the pro-inflammatory cytokine IL-18 induces
chemokine and cytokine production with neutrophils and promotes neutrophil accumulation. Elevated plasma IL-18 levels were found in adults with sepsis. Thus, both pro- and anti-inflammatory cytokines are augmented in severe sepsis.

In earlier studies, we noted increased plasma levels of TNF and IL-6 in critically ill infants and children with bacterial sepsis and NEC compared with infants with sepsis syndrome; elevations of IL-6 correlated with mortality. Additional studies have demonstrated elevations of both IL-8 and IL-10 in infected neonates. However, the role of TNF and IL-6 in critically ill infants and children with bacterial sepsis and NEC compared with infants with sepsis syndrome; elevations of IL-6 correlated with mortality. Additional studies have demonstrated elevations of both IL-8 and IL-10 in infected neonates. However, the role of TNF and IL-6 in critically ill infants and children with bacterial sepsis and NEC compared with infants with sepsis syndrome; elevations of IL-6 correlated with mortality. Additional studies have demonstrated elevations of both IL-8 and IL-10 in infected neonates. However, the role of TNF and IL-6 in critically ill infants and children with bacterial sepsis and NEC compared with infants with sepsis syndrome; elevations of IL-6 correlated with mortality.

We hypothesized that differential patterns of cytokine expression in blood and TA fluid would distinguish critically ill infants with sepsis or NEC from infants with sepsis syndrome. Furthermore, we suspected that these elevations would be associated with clinical parameters of inflammation and mortality. The purpose of this study was to: 1) determine plasma and TA cytokine levels in infants <6 months of age with bacterial sepsis or NEC and compare them with values in a population of critically ill infants with sepsis syndrome; 2) correlate cytokine levels with clinical parameters of inflammation and mortality; and 3) examine differences in cytokine levels in relation to outcome.

**METHODS**

**Study Population**

Infants with suspected sepsis were recruited from the newborn intensive care units at the Children’s Hospital of Philadelphia and the University of Virginia during the 4-year period from September 1998 to September 2002. Inclusion criteria included the following: 1) a risk factor for infection; 2) either respiratory or circulatory dysfunction demonstrated by means of a physical examination; and 3) physical signs of infection. Exclusion criteria included: 1) maternal or infant administration of antibiotics within 48 hours of the sepsis evaluation; 2) multiple congenital anomalies; 3) administration of any corticosteroid medication to the infant within the previous 48 hours; and 4) confirmed viral or fungal sepsis.

**Risk factors for infection included at least 1 of the following: prematurity, rupture of membranes >24 hours, maternal fever >38°C, chorioamnionitis, maternal colonization with group B streptococcus, or the presence of a central venous line or a foreign body such as an endotracheal tube, thoracostomy tube, or ventriculoperitoneal shunt. Respiratory dysfunction was evidenced by the presence of grunting, flaring, retractions, tachypnea, or apnea. Circulatory dysfunction was evidenced by the presence of tachycardia, bradycardia, oliguria, poor perfusion, or hypotension. Physical signs of infection included at least 1 of the following: respiratory distress, feeding intolerance, abdominal distension, lethargy, irritability, or temperature instability.

Although infants were selected for possible inclusion into the study at the time of the evaluation for sepsis, the study protocol required that samples be obtained within 12 hours of the sepsis work-up. For these reasons, not all eligible infants were enrolled. Infants with bacterial sepsis were subsequently identified on the basis of positive peripheral blood culture results. Infants with NEC were those with negative blood culture results with bloody stools and the pathognomonic radiographic features of pneumatosis intestinalis, portal venous air, or free intraperitoneal air. Infants who met the inclusion criteria, whose blood culture results were negative for bacteria, and who did not have NEC were defined as sepsis syndrome subjects. Infants were classified as non-survivors when they died within 7 days of enrollment into the study.

Data recorded included birth weight, gestational age, age at the time of study, principal diagnoses, and outcome. Laboratory data included culture results, complete blood count, differential, and platelet count, fraction of inspired oxygen (FiO₂) and mean airway pressure (MAP). An oxygen index (OI) was calculated for all ventilated infants in which OI = FiO₂ × MAP/PaO₂ × 100. A Score for Acute Neonatal Physiology-Perinatal Extension-II (SNAPPE-II) score was calculated for each infant as a measure of severity of illness at presentation. The study was approved by the Committees for the Protection of Human Subjects at The Children’s Hospital of Philadelphia and the University of Virginia. Informed written consent was obtained from parents of all study subjects.

**Cytokine Determinations**

Blood and TA samples for cytokine determinations were collected from infants at the time of onset of suspected sepsis. In infants who were not receiving mechanical ventilation, a TA specimen was not obtained (n = 8). Plasma samples were collected as described previously. TA samples were collected into Leukens traps by instilling 1 mL of sterile saline in 2 aliquots, followed by suctioning through the endotracheal tube. TA samples were centrifuged at 1000 g for 10 minutes to remove cellular debris, stored in small aliquots at −70°C, and thawed once at the time of analysis. Cytokine concentrations were determined by using enzyme linked immunosorbent assay (R & D Systems). The limits of detection were: 10 to 2000 pg/mL of IL-8, 15 to 2000 pg/mL of ENA-78, 4 to 500 pg/mL of IL-10, and 12.5 to 1000 pg/mL of IL-18. Because the volume recovered in TA samples was not uniform, cytokine values were normalized for epithelial lining fluid volume by using the urea method.

**Statistical Analysis**

The study was designed to detect a 2-fold change in IL-8 levels with 80% power and a type 1 error of 0.05. The effect size was estimated to be 0.6, and the sample size was 90 subjects with a 1:2 ratio of infected patients to control patients. The final data indicate that the variance was smaller than...
expected, so the number of subjects required was <90. Histograms of continuous outcome variables were examined for normality of distribution, and appropriate transformations (eg, logarithmic) were made. For normally distributed variables (raw or log transformed), group means were compared with analysis of variance, and pairwise comparisons were made with t tests. Plasma IL-18 was skewed and resistant to normalization by using logarithmic transformation, and it correlated with gestational and postnatal age. Therefore, we examined analysis of covariance models, using the square root transformation for IL-18 and including gestational and postnatal age as covariates. Pearson and Spearman correlation coefficients were used to correlate cytokine levels with clinical parameters of inflammation. Box plots were used to provide graphic representation of cytokine levels, including the median, quartiles, and outliers. Mann-Whitney tests and t tests were used to examine differences between groups. The statistical significance level was set at 0.05. The P values have not been adjusted for multiple comparisons.

RESULTS

Study Population

Eighty-four of the 134 infants with suspected sepsis enrolled into the study met the inclusion criteria. Neonates were excluded when they had received antibiotics (n = 15) or corticosteroids (n = 13), had viral or fungal sepsis (n = 14), or had a genetic syndrome (n = 1). Six parents declined study participation and, in 1 infant who died, consent was not requested.

The study population was divided in 3 groups (Table). Among the 31 infants with bacterial sepsis, 13 had early-onset sepsis, 10 had nosocomial or late-onset sepsis, 6 had infection associated with the presence of a foreign body, and 2 had positive blood culture results and NEC. Fifteen infants had Gram-negative infections, 14 had Gram-positive infections, and 2 had polymicrobial sepsis. Organisms isolated included group B streptococci (n = 9), Escherichia coli (n = 8), coagulase-negative staphylococci (n = 5), Klebsiella pneumoniae (n = 5), citrobacter species (n = 2), enterobacter species (n = 2), Staphylococcus aureus (n = 1), Serratia marcescens (n = 1), and Hemophilus influenzae (n = 1). Two infants had polymicrobial sepsis, 1 with E coli and group B streptococci, and the other with multiple Gram-negative organisms. The sepsis syndrome group included 34 infants with these diagnoses: asphyxia with pulmonary hypertension (n = 8), respiratory distress syndrome (n = 7), aspiration pneumonia (n = 5), cardiac failure (n = 3), seizures (n = 2), apnea (n = 2), chronic lung disease (n = 2), dehydration (n = 2), pulmonary hemorrhage (n = 1), cerebral thrombosis (n = 1), and omphalocoele (n = 1).

The infants with bacterial sepsis and the infants with NEC were significantly less mature but older than patients with sepsis syndrome (Table). There were 4 deaths from Gram-positive organisms and 3 from Gram-negative organisms. The time of death (±SD) from sepsis or its complications for the 7 infants who didn’t survive was 1.4 ± 1.3 days after presentation (range, 0.5-4 days). One infant in the sepsis syndrome group with a diagnosis of birth asphyxia expired at 5 days when support was withdrawn.

Plasma and TA Cytokine Levels

Plasma IL-8 levels were significantly elevated in infants with bacterial sepsis and NEC in comparison with levels in infants with sepsis syndrome (Figure 1). In contrast, plasma ENA-78 levels were significantly elevated only in the infants with NEC compared with infants with bacterial sepsis and sepsis syndrome. Plasma IL-10 levels were also elevated in infants with bacterial sepsis and NEC in comparison with infants with sepsis syndrome. Elaboration of plasma IL-8, ENA-78, and IL-10 was independent of birth weight, gestational age, and postnatal age. However, plasma IL-18 levels were directly correlated with postnatal age (r = .42, P < .0005), and inversely correlated with birth weight (r = -.28, P < .025). When adjusted for age by means of analysis of covariance, plasma IL-18 levels were significantly higher in infants with bacterial sepsis (672 ± 148 pg/mL, n = 27) than in infants with NEC (324 ± 66 pg/mL, n = 19, P < .05), but not compared with infants with sepsis syndrome (229 ± 53 pg/mL, n = 30). Plasma IL-18 levels were not significantly different in infants with sepsis syndrome than in infants with NEC. Moreover, elevations of plasma cytokine levels were independent of the Gram stain characteristics of the infecting organisms.

TA IL-8 levels were significantly elevated in infants with bacterial sepsis compared with infants with sepsis syndrome and infants with NEC (Figure 2). TA IL-8 levels were not significantly different in infants with NEC compared with infants with sepsis syndrome. TA ENA-78 levels were significantly higher in the infants with bacterial sepsis when compared with infants with NEC, but not compared with infants with sepsis syndrome. TA IL-10 levels were significantly increased in infants with bacterial sepsis compared with infants with sepsis syndrome, but not compared with infants with NEC. In contrast, TA IL-18 levels were not significantly different in the 3 patient groups.

There was an association of plasma and TA cytokines that was independent of the diagnostic group. Plasma IL-8 levels were directly correlated with plasma ENA-78 levels (r = .51, P < .0005), and IL-10 levels (r = .71, P < .0005). Plasma ENA-78 and IL-10 levels were also positively correlated (r = .40, P = .001), suggesting coordinated up-regulation of these cytokines. In contrast, there was no correlation of plasma IL-18 levels with the other cytokines. Similar to the findings in plasma, TA IL-8 levels were directly correlated with TA ENA-78 levels (r = .52, P < .001). However, in contrast to plasma IL-18, TA IL-18 was correlated with TA IL-10 (r = .37, P < .01).

Correlation with Clinical Parameters of Inflammation and Mortality

There was a significant inverse relationship between white blood cell (WBC) counts and plasma IL-8 levels

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(r = −.58, P < .005), ENA-78 levels (r = −.41, P = .001), and IL-10 levels (r = −.42, P = .001), and a positive correlation to immature/total neutrophil (I/T) ratios for plasma IL-8 levels (r = .47, P < .0005), ENA-78 levels (r = .40, P = .001) and IL-10 levels (r = .49, P < .0005). Plasma IL-8 levels were also inversely related to platelet counts (r = −.37, P < .005). There was no significant association of plasma IL-18 levels with WBC or I/T ratios; however, plasma IL-18 levels and platelet counts were inversely correlated (r = −.25, P < .05). There was also a significant inverse association between WBC counts and TA IL-10 levels (r = −.27, P < .005) and between platelet counts and TA ENA-78 levels (r = −.39, P < .025). Furthermore, there was no correlation among OI, SNAPPE-II scores, and plasma or TA cytokine levels.

We noted an association of cytokine elaboration and outcome that was independent of the diagnostic group. Plasma IL-8, IL-10, and IL-18 levels were significantly higher in infants who died than in infants who lived (IL-8: 1269 ± 239 pg/mL, n = 7, versus 523 ± 95 pg/mL, n = 61, P < .0005; IL-10: 435 ± 136 pg/mL, n = 7, versus 131 ± 61 pg/mL, n = 56, P < .0005; IL-18: 691 ± 213 pg/mL, n = 6, versus 416 ± 64 pg/mL, n = 70, P < .05). However, plasma ENA-78 and TA cytokine levels were unrelated to outcome (data not shown).

**DISCUSSION**

Our results demonstrate a markedly different pattern of both pro- and anti-inflammatory cytokine elaboration in infants with bacterial sepsis compared with infants with either sepsis syndrome or NEC. These findings are consistent with an earlier study, in which we found differential expression of plasma IL-6, but not TNF, in infants with bacterial sepsis compared with infants with sepsis syndrome. A similar increase in circulating IL-8 levels has been reported in infants with bacterial sepsis compared with infants with sepsis syndrome. IL-10 levels are elevated in neonatal sepsis, but differences in bacterial sepsis compared with sepsis syndrome have not been previously explored. Similar to our findings, plasma IL-10 levels in adults were useful in distinguishing septic shock in bacterial disease from circulatory shock of non-septic origin. To the best of our knowledge, this is the first study to investigate IL-18 and ENA-78 levels in infected neonates.

Elevations of both pro- and anti-inflammatory cytokines have been reported in infants with NEC, reflecting a response to circulating bacteria and their byproducts and local mediator release associated with tissue inflammation. Increased IL-10 levels in pre-term infants with NEC have been reported, and similar to our findings, these levels were lower than in infants with bacterial sepsis. In our study, levels of ENA-78 were significantly elevated only in infants with NEC, whereas levels of IL-8 were significantly elevated in both infants with NEC and infants with bacterial sepsis, suggesting differential regulation of these 2 chemokines. ENA-78 has not been previously examined in infants with NEC-78 and TA cytokine levels were unrelated to outcome (data not shown).
NEC; however, its role in inflammatory bowel disease has been demonstrated in adults. In contrast, plasma IL-18 levels were not significantly different in infants with NEC than in infants with sepsis syndrome.

Most infants enrolled in this study required mechanical ventilation. Thus, we wanted to determine whether the different disease entities had different patterns of cytokine elaboration in the pulmonary compartment. The chemokines IL-8 and ENA-78 and the pro-inflammatory cytokine IL-18 have an important role in the development of lung injury in inflammatory diseases. Consistent with previous studies, IL-8 and ENA-78 concentrations in TA were significantly higher than plasma levels, suggesting compartmentalization of cytokine responses. The differences between the patterns of cytokine elaboration in plasma and in tracheal fluids is even more striking in infants with NEC. Both IL-8 and ENA-78 were significantly decreased in the TA fluids of infants with NEC as compared with those in infants with bacterial sepsis. This is in sharp contrast to plasma levels of IL-8 and ENA-78, in which infants with NEC had the highest concentrations of these chemokines. These results suggest that plasma levels may not adequately represent the local production of pro- and anti-inflammatory cytokines in selected compartments.

Both plasma and TA IL-8, ENA-78, and IL-10 levels were directly correlated with one another, suggesting coordinated up-regulation of these cytokines. Correlations of plasma levels of both the pro-inflammatory (TNF, IL-6, IL-8) and anti-inflammatory (IL-10) cytokines have been reported previously in both newborn infants and adults with sepsis. In adults, an association between the chemokines IL-8 and ENA-78 in tracheal aspirates has been reported during the evolution of pulmonary inflammation in ARDS. In contrast, but similar to our findings, plasma levels of IL-18 did not correlate with other inflammatory mediators in adults with sepsis. These findings suggest selective, independent regulation of this pro-inflammatory cytokine, although differences in timing of production and other cytokine effects cannot be excluded.

In this study, plasma and TA cytokine levels correlated with hematologic parameters including WBC and platelet counts, and neutrophil I/T ratios. In adults, peak IL-8 concentrations correlated with leukopenia in sepsis. Although the usefulness of the complete blood cell count in the diagnosis of neonatal sepsis is controversial, abnormalities of WBC, platelet counts, and I/T ratios were predictive of bacterial sepsis or NEC. Thus, the correlation of selective cytokines with clinical parameters of inflammation and the association of bacterial sepsis or NEC with hematologic abnormalities suggest the interdependent nature of the acute phase response.

In our study, plasma IL-8, IL-18, and IL-10 levels were significantly related to outcome. Correlations between IL-6, IL-8, and IL-18 levels and mortality in sepsis have been reported previously. In contrast, mortality in sepsis has been correlated to either depressed or enhanced IL-10 levels. In this study, plasma IL-10 levels were significantly elevated in infants who died, which suggests that the adverse outcome in these infants was not the result of failure to mount an anti-inflammatory response. However, the small number of infants who died in our study limits the predictive value of cytokine concentrations.

In our patient population, there were no differences in severity of illness across the groups and no correlation of OI or
SNAPPE-II scores with cytokine levels. Thus the observed differences in cytokine levels are likely related to the pathophysiology of sepsis or NEC rather than disease severity. In adults, plasma IL-8 levels correlated with the complications of septic shock, including lactic acidosis and disseminated intravascular coagulation. Furthermore, a negative correlation between alveolar lavage IL-8 levels and PaO2/FiO2 ratios has been reported in adults with ARDS.

Our data have important strengths and limitations. In contrast to previous studies, we measured patterns of cytokine elaboration, rather than single cytokines, and compared values to critically ill infants with sepsis syndrome. However, infants with sepsis syndrome were more mature but younger at the onset of illness than the infants with sepsis or NEC. Because all infants were enrolled in the study at the time of evaluation for suspected sepsis, these group differences became apparent only after blood culture results and diagnoses were established. In addition, although our method of determining TA cytokines may not provide a truly quantitative measurement of epithelial lining fluid, results were similar to uncorrected values and to values reported in the literature (data not shown). Limitations in sample volumes also prevented the analysis of all cytokines in all infants. Finally, we studied only infants in the neonatal intensive care unit, so the results are biased toward infants with respiratory or cardiovascular abnormalities. Although this may limit the generalization of our findings to all infants, we do not believe that it diminishes their importance.

In conclusion, both pro- and anti-inflammatory cytokines are augmented in plasma in critically ill infants with bacterial sepsis or NEC. Furthermore, TA cytokines are accentuated in the presence of bacterial infection. Plasma and TA IL-8, ENA-78, and IL-10 levels were highly correlated with each other and with clinical indices, suggesting the interdependent nature of cytokine expression during inflammation. This association likely reflects the magnitude of the underlying inflammatory response. IL-18 regulation, in contrast, appears independent of the other cytokines measured in this study. Our study also suggests distinct patterns of cytokine elaboration in response to different disease states.

REFERENCES


Objective To evaluate prenatal treatment with hydroxycobalamin (OH-Cbl) in a pregnancy at risk for a severe form of the cobalamin C defect and postnatal treatment of the affected child.

Study design Observational study with non-randomized intervention.

Results In contrast to reported pregnancies with affected fetuses in which maternal methylmalonic aciduria was found in the last trimester of pregnancy, there was no maternal methylmalonic aciduria in our case, given prenatal treatment with intramuscular OH-Cbl. We did not find that the concentration of odd long-chain fatty acids in cord blood erythrocytes reflects fetal methylmalonic academia. After birth, the infant was treated with intramuscular OH-Cbl and oral carnitine. Oral folate and betaine were added as adjunct therapy to decrease plasma total homocysteine. Because of inadequate metabolic control, a diet reduced in natural protein was introduced. The child had normal developmental milestones but had nystagmus, hyperpigmented retinopathy, and discrete truncal muscular hypotonia.

Conclusions Despite prenatal and postnatal treatment, adequate metabolic control, absence of metabolic crises, and normal developmental milestones, this patient with the cobalamin C defect had characteristic symptoms of the disease. (J Pediatr 2005;147:469-72)

The cobalamin C (cblC) defect is an inborn error of intracellular cobalamin (Cbl) metabolism presenting as combined methylmalonic aciduria (MMA-uria) and hyperhomocysteinemia. The defect is located within the cytosol and has been suggested to involve the hydroxylation of Cbl (III) to hydroxycobalamin (OH-Cbl) (III). OH–Cbl (III) is transformed to Cbl (II) and consecutively to methylcobalamin, which serves as cofactor for the cytosolic enzyme methionine synthase (E.C. 2.1.1.13). Methionine synthase is a key enzyme in the remethylation of homocysteine (Hcy) to methionine. Therefore, hyperhomocysteinemia is a hallmark of the cblC defect, and methionine concentrations are usually low. Cbl is transported into the mitochondria and is metabolized stepwise to adenosylcobalamin, the essential cofactor for the enzyme methylmalonyl CoA mutase. Adenosylcobalamin deficiency causes a functional enzyme deficiency leading to MMA-uria.3 The gene locus for the cblC defect has not yet been identified. The mode of inheritance is considered to be autosomal recessive.2 Disease manifestations are highly variable and range from death within the first weeks of life to late-onset forms with first symptoms after the second decade.3 Affected newborn infants may present with metabolic acidosis, neurologic deterioration, lethergy, hypotonia, bone marrow suppression, and renal and hepatic failure. Congenital malformations such as microcephaly, heart disease, and dysmorphic features have been described.4,6 Late-onset forms may present with mental retardation, epilepsy, hypotonia, unsteady gait, impaired speech, pigmentary retinopathy, megaloblastic anemia, impaired renal and liver function, or psychiatric symptoms.2,5 Prenatal treatment with cyano-Cbl or OH-Cbl has been tried in only a few cases, with Cbl-responsive, isolated MMA-uria caused by defective adenosylcobalamin synthesis (eg, cblA or cblB defect) and in a single case with combined MMA-uria and hyperhomocysteinemia (ie, in the cblC or cblD defect). We present our experience with prenatal and postnatal treatment for the cblC defect.

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Submitted for publication Dec 7, 2004; revision received Apr 4, 2005; accepted Apr 15, 2005.

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0022-3476/$ - see front matter
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10.1016/j.jpeds.2005.04.040
CASE 1

The second son of healthy, consanguineous (first cousins) Turkish parents with a healthy 7-year-old son was admitted at the age of 2 weeks because of recurrent vomiting. Birth weight was 2900 g (<3rd percentile). Newborn screening had been performed on day of life 3 during the implementation phase of tandem mass spectrometry into the Austrian Newborn Screening Program. In MMA-uria, elevated propionylcarnitine (C3) concentrations and C3/acylcarnitine (C2) ratio are characteristic. The screening results were as follows: C3 = 3.9 μmol/L (upper cutoff, 7); C2 = 8.8 μmol/L (lower cutoff, 8); and C3/C2, 0.4 μmol/L (upper cutoff, 0.3). These results were considered normal by the screening center. Weight on admission was 2840 g (<3rd percentile); head circumference was 34 cm (10th percentile). On admission, repeated blood gas analyses revealed normal acid base status. Neurologic impairment of the patient was not obvious, and the patient had no dysmorphic stigmata. Abdominal ultrasound showed evidence for pyloric hypertrophy resulting in surgery at 30 days of age. After surgery, the child was admitted to the pediatric intensive care unit with respiratory insufficiency and the general impression of an abnormally prolonged recovery with vomiting, refusal of feedings, and drowsiness. Laboratory investigations for sepsis revealed neutropenia and mild acidosis. Subsequent metabolic investigations on day 35 of life revealed MMA-uria (1675 mmol/mol creatinine; reference, 0.1 to 10), homocystinuria (227.6 mmol homocystine/mol creatinine), and increased plasma total homocysteine (tHcy) (287 μmol/L; reference, <15). Plasma methionine concentration was low, with 3.4 μmol/L (reference, 10 to 60), whereas the C3 concentration in dried blood spots was 4.9 μmol/L (reference, <4). Guanidinoacetate in urine was normal (61 mmol/mol creatinine; reference, 32 to 367 mmol/mol creatinine). Protein restriction and treatment with OH-Cbl (1 mg/d IM), carnitine (200 mg/kg per day IV), folate (5 mg/d PO), and betaine (100 mg/kg per day PO) was initiated and resulted in an immediate metabolic response. MMA-uria decreased to 64 mmol/mol creatinine and plasma tHcy to 106 μmol/L. Nevertheless, renal function continuously deteriorated, and, despite peritoneal dialysis, the child died of multiorgan failure. The cblC/D defect was identified in skin fibroblasts, based on deficient methionine and serine synthesis and low propionate incorporation, both of which responded to supplementation of culture medium with OH-Cbl as well as low total Cbl uptake and deficient synthesis of methyl- and adenosyl-Cbl.7,8 The cblC/D defect was established by somatic complementation analysis in fibroblasts by measuring the incorporation of propionate in cells fused with known cblC and cblD cell lines using polyethylene glycol.9

CASE 2

In the third pregnancy, prenatal diagnosis was undertaken at the 16th week of gestation. In amniotic fluid, tHcy (23.2 μmol/L; control subjects, 3.1 and 1.0 μmol/L), MMA (17.8 μmol/L; control subjects, 0.36, 0.49, and 0.53 μmol/L, respectively), and propionylcarnitine (2.76 μmol/L; control subjects, 0.19, 0.24, and 0.39 μmol/L, respectively) were all significantly elevated. The cblC defect was confirmed in cultured amniotic fluid cells by demonstration of reduced synthesis of methionine in cells grown in unsupplemented medium which increased in cells grown in OH-Cbl supplemented medium. At 24 weeks of gestational age, before treatment, maternal levels of plasma tHcy (5.2 μmol/L) and methionine (23.7 μmol/L) were normal and MMA-uria was within the normal range. OH-Cbl, 1 mg IM, was administered twice weekly to the mother from the 24th gestational week until delivery, and both plasma tHcy concentrations and MMA in urine remained normal. A boy without dysmorphic features was born at term (birth weight, 3270 g; length, 49 cm; both 50th percentile; head circumference, 34.8 cm; 25th percentile; APGAR, 9/10/10) after an uneventful delivery. MMA (393 mmol/mol creatinine) and propionyl carnitine (2.0 mmol/mol creatinine) in the first urine (6 hours after delivery) and plasma tHcy (160.9 μmol/L) were significantly elevated, whereas methionine was slightly below normal (9.1 μmol/L). The level of odd long-chain fatty acids (OLCFAs) was elevated to 4.6% of total in umbilical cord blood (reference mean ± SD, 1.43% ± 0.42%). The newborn screening results on the third day of life were as follows: C3 = 3.5 μmol/L (upper cutoff, 3.5); C2 = 16.1 μmol/L (lower cutoff, 8); and C3/C2 = 0.2 μmol/L (upper cutoff, 0.3). Treatment with carnitine (100 mg/kg per day) was started on the first day of life, and 1 mg OH-Cbl IM was given daily for 3 days followed by 3 injections per week. MMA excretion decreased to 82.7 mmol/mol creatinine and tHcy in plasma decreased to 79.7 μmol/L. Folate (1.25 mg/d) and betaine (100 mg/kg per day) were added to the treatment, and the tHcy concentration further decreased to 49.2 μmol/L. During the following 4 weeks, tHcy concentrations fluctuated between 50 and 90 μmol/L. The frequency of OH-Cbl administration was increased to 1 mg 4 times and then 5 times per week, but tHcy concentrations remained unchanged. At 4 months of age, the child had gastroenteritis, was hospitalized, and received glucose infusions to avoid catabolism. He had a rapid clinical recovery, but urinary MMA excretion and plasma tHcy increased to 180 mmol/mol creatinine and 170 μmol/L, respectively, despite daily OH-Cbl during the period of hospitalization. Methionine concentrations remained normal. After this episode, the child was started on a diet restricted in natural protein (1.5 g/kg per day) and supplemented with a methionine-free, threonine-free, valine-free, isoleucine-low infant formula (daily protein intake derived from amino acids, 1 g/kg per day). Plasma tHcy concentrations decreased to 40 to 50 μmol/L and MMA excretion remained stable below 30 mmol/mol creatinine (Figure). The biochemical profile remained stable during severe infections such as rotavirus enteritis and bronchitis at 10 months of age. At 12 months of age, plasma creatine (21.4 μmol/L) (reference, 58.96 ± 22.30) was slightly below normal. The creatine/creatinine ratio (0.7; reference, ≤2,1)
and urine guanidinoacetate (152 mmol/mol creatinine) were normal, indicating no obvious secondary impairment of creatine metabolism. Hematologic abnormalities were never observed. Ophthalmologic examination at week 6 of life showed no evidence of retinopathy. Repeated electroencephalography and ultrasound of the abdomen and heart were normal. At 5 months, horizontal nystagmus was observed and ophthalmologic examination now revealed hyperpigmentation of the retina in the foveal region, as is often seen in patients with the cblC defect. Discrete truncal muscular hypotonia became obvious at the age of 6 months. The boy was able to sit without support and had a pincer grasp at 10 months. At age of 15 months, the boy was sociable and expressed single words. He walked and drank from a cup without support. Weight, length, and head circumference (10.800 g; 80 cm; 46.5 cm,) were normal for age (all 50th percentile).

**DISCUSSION**

We found a benefit of prenatal treatment in a fetus affected with the cblC defect. In Cbl-responsive MMA-uria without hyperhomocysteinemia caused by defective adenosylcobalamin synthesis (cblA or cbl B defect), different regimens of prenatal treatment have been reported. Three fetuses were treated from weeks of pregnancy 21,10 27,11 or 3112 until delivery at term with OH-Cbl or cyanocobalamin given orally or by intramuscular injection. Cbl was elevated in umbilical cord blood, indicating adequate transport into the fetus. In a fetus with combined MMA-uria and hyperhomocysteinemia caused by the cblC or cblD defect, prenatal treatment with OH-Cbl (5 mg IM twice per week and 1 mg PO the other days) between week 17 and delivery of prenatal and postnatal treatment with OH-Cbl. The child was born symptom-free and without impairment of intrauterine growth. After delivery, OH-Cbl treatment is essential. Depending on the severity of the disease, carnitine, betaine, folate, and protein restriction may be added.
The analysis of odd long chain fatty acids in cord blood was performed by Prof U. Wendel, Department of Pediatrics, Heinrich Heine University, Düsseldorf, Germany.

REFERENCES
EFFECTS OF PRENATAL ALCOHOL EXPOSURE ON INFANT VISUAL ACUITY

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Objective  To examine the effects of prenatal alcohol exposure ascertained prospectively on infant visual acuity across a range of exposures and factors that mediate or moderate these effects.

Study design  Infant visual acuity was examined in 131 Cape Coloured (mixed ancestry) maternal-infant pairs in Cape Town, South Africa. Drinking patterns were documented by maternal reporting during pregnancy. Grating acuity was assessed with Teller Acuity Cards (TAC) at 6.5 months after term. Data were analyzed by correlation, multiple regression, and analysis of variance.

Results  Greater average daily prenatal alcohol exposure was related to poorer acuity, as indicated by lower TAC scores. The effect of alcohol on acuity was significant primarily for infants born to mothers ≥30 years of age at delivery, in comparison to infants born to younger mothers. This effect was not mediated by gestational age or birth size or attributable to alcohol-related neurocognitive deficits.

Conclusions  This study linked prenatal alcohol exposure ascertained prospectively to poorer visual acuity in infancy. The results are consistent with clinical and animal evidence of alcohol-related disruption of the visual system. (J Pediatr 2005;147:473-9)

In the early 1970s, Jones and Smith described a syndrome of prenatal growth deficiency, developmental delay, and specific craniofacial dysmorphology, which they termed fetal alcohol syndrome (FAS). Disorders of the eye were noted in the earliest reports of FAS, and case studies have documented numerous ophthalmologic abnormalities. In a case study of 30 children with FAS referred for ophthalmologic evaluation, 90% had some ophthalmologic abnormality, and more than half of those affected had impaired visual acuity. Another study found poorer acuity in 9 of 10 children with FAS.

Previous studies on prenatal alcohol exposure reported poorer visual acuity but were limited to children diagnosed with FAS, only included small numbers of infants, and lacked quantitative documentation of maternal drinking levels. We examined the effects of heavy prenatal alcohol exposure in a prospective, longitudinal study in which maternal alcohol use was ascertained during pregnancy. Because prenatal alcohol exposure is associated with reductions in birth size and length of gestation, which can be related to developmental disabilities, we examined prenatal and postnatal growth and gestational age as potential mediators of observed effects of prenatal alcohol on visual acuity. Performance on the Fagan Test of Infant Intelligence (FTII), a measure of visual recognition memory and information processing speed predictive of later intellectual development, was included in the analyses to test the hypothesis that poorer attention or developmental delay may mediate the effect of prenatal alcohol exposure on acuity. Case studies of white and Native American women found that each successive child born to a heavy-drinking mother was more severely impaired than the previous one, a pattern caused by maternal aging rather than parity in controlled animal experiments. Because maternal age was identified as an important moderator of fetal alcohol effects on a broad range of deficits in...
infancy and school-aged children in our Detroit and Cape Town cohorts, we examined whether maternal age moderated the relation between alcohol exposure and visual acuity.

Recent studies have documented a very high prevalence of FAS in the Cape Coloured (mixed ancestry) population in the Western Cape Province of South Africa. The Cape Coloured, mainly descendents of white European, Malaysian, Khoi (Hottentot), and black African ancestors, have historically comprised the large majority of workers in the wine-producing and fruit-growing region of the Western Cape. The high prevalence of FAS is a consequence of the very heavy maternal drinking during pregnancy commonly found in this community.

METHODS

Sample

The sample consisted of 131 infants (73 males, 58 females) born to women from the Cape Coloured community in Cape Town, South Africa, who are participating in a prospective study on the effects of heavy prenatal alcohol exposure on neurobehavioral development. The mothers were recruited between July 1999 and January 2002 at the antenatal clinic of a midwife obstetric unit that serves an economically disadvantaged, predominantly Cape Coloured population. This clinic was selected for its high prevalence of heavy alcohol use on the basis of data collected from 6 midwife obstetric units in the Peninsula Maternity and Neonatal Service, which is associated with the University of Cape Town and serves 59.3% of the population.

Each woman was interviewed regarding alcohol consumption both at the time of recruitment and at conception, using a timeline follow-back interview approach. Any woman averaging at least 1.0 oz absolute alcohol per day (AA/day), the equivalent of 2 standard drinks or about 30 mL AA/day, during the first trimester of pregnancy or reporting a history of at least 2 incidents of binge drinking per month was invited to participate in the study. Binge drinking was defined as consumption of at least 5 standard drinks on 1 occasion. The next woman initiating antenatal care at this clinic who drank <0.5 oz AA/day, did not binge drink during the first trimester, and whose gestational week of pregnancy was within 2 weeks of that of the previously recruited heavy-drinking participant was also invited to participate in the study. Women <18 years of age and those with diabetes, epilepsy, or cardiac problems requiring treatment were not invited to participate. Religiously observant Muslim women were also excluded because their religious practices prohibit alcohol consumption. Infants exclusionary criteria were major chromosomal anomalies, neural tube defects, multiple births, and seizures. Among the 131 infants for whom Teller Acuity Card (TAC) data were collected at 6.5 months after term, 61 (46.6%) were born to heavy-drinking women and 70 (53.4%) to abstainers and low level drinkers. Although heavy-drinking women were overrepresented in this sampling design, prenatal alcohol exposure was treated as a continuous variable in all of the data analyses. Informed consent was obtained from each mother, and approval for human research was obtained from both the Wayne State University and the University of Cape Town human investigation committees.

Procedure

Infants were evaluated for visual acuity at 6.5 months, corrected for gestational age in cases of preterm birth. At the end of each visit, the mother received a small monetary compensation, a gift for her infant, and a photograph of herself with her infant.

Visual Acuity

Binocular visual acuity was assessed at 6.5 months on a resolution acuity test, the TAC, which uses preferential looking to determine whether an infant can resolve individual lines in successively finer vertical gratings. Resolution acuity develops quickly during the first 6 months of life and then more slowly until adult acuity is reached at age 3 to 5 years. Trained testers were masked with respect to the infants’ prenatal alcohol exposure. The TAC consists of a series of 16 rectangular cards, 28 cm by 60 cm in size, each of which contains a uniform gray background on which is imposed a 12.5-cm by 12.5-cm patch of black-and-white square-wave grating (black and white vertical stripes) that is located to the left or right of a central 4-mm peep hole. The spatial frequency of the grating increases (ie, stripe width decreases) on successive cards from 0.23 to 38.0 cycles/cm in 0.5-octave steps. (An octave is a halving or doubling of stripe width).

During testing, the infant sat on the mother’s lap and was shown the cards in order from wider stripes (0.64 cycles/cm) to finer and finer stripes at a distance of 55 cm. The examiner, who did not know which side the grating is on, looked through the peep hole and judged which side of the card the infant preferentially fixated. The examiner then rotated the card 180 degrees and checked to see whether the infant looked to the opposite side of the card. The examiner could rotate the card by 180 degrees several times until a consistent preference was or was not exhibited. If a preference was exhibited, the examiner then verified whether the grating was on the side where the infant looked. When the infant failed to differentiate the grating from the gray background, the examiner retested the infant by going back to the previous card to confirm that the infant could make that discrimination. The examiner then returned to the card on which the infant failed to confirm that failure. The spatial frequency (in cycles/degree) of finest grating that the examiner judged that the infant could differentiate from the gray background was recorded as the infant’s visual acuity score.

Visual acuity scores were converted to octaves by means of logarithmic (base2) values. Binocular acuity norms derived by Salomão and Ventura from infants with no significant ocular disease were used to identify infants in our cohort with acuity scores below the fifth percentile at 6.5 months (ie, <3.38
cycles/degree). Correlational and regression analyses were performed on the octave scores; means are reported as cycles/degree and standard deviations in octaves.

Alcohol and Drug Use Data

Each mother was interviewed regarding her pregnancy alcohol and drug use at recruitment, at a follow-up antenatal visit, and when the infant was 1 month old. Almost all of the interviews were conducted in Afrikaans. During recruitment, the mother was asked about her drinking on a day-by-day basis during a typical 2-week period around the time of conception, with recall linked to specific times of day and activities. If her drinking had changed since conception, she was also asked about her drinking during the past 2 weeks and when her drinking had changed. At the follow-up antenatal visit, the mother was again asked about her drinking during the previous 2 weeks. If there were any weeks since the recruitment visit when she drank greater quantities, she was asked to report her drinking for those weeks as well. At the 1-month post-term visit, the mother was asked about her drinking during a typical 2-week period during the latter part of pregnancy, as well as her drinking during any weeks during that period when she drank greater quantities. Volume was recorded for each type of alcohol beverage consumed each day and converted to oz of absolute alcohol (AA) using multipliers proposed by Bowman et al. Six summary measures were constructed—average AA/day at conception, AA/day averaged during pregnancy, AA per drinking day (quantity per occasion) at conception and during pregnancy, and proportion drinking days (frequency) at conception and during pregnancy.

In addition to the quantitative alcohol interview, the alcohol module of the Diagnostic Interview Schedule was administered to each mother at the antenatal interview to determine whether she met Diagnostic and Statistical Manual of Mental Disorders–Fourth Edition (DSM-IV) criteria for alcohol abuse or dependence. Each mother was also asked at both the antenatal and postnatal interviews how many cigarettes she smoked per day and how often she used marijuana, inhalants (eg, glue or solvents), heroin, cocaine, mandrax, sedatives, or other drugs during pregnancy.

FAS Diagnosis

The infants were examined at 1, 12, or 13 months for presence of alcohol-related dysmorphic features by 2 dysmorphologists trained by Kenneth L. Jones, MD, and his colleagues to assess FAS in a previous South African study. Frontal and side view facial photographs taken at 12 months post-term of each of the infants were also reviewed by Dr. Jones and Nathaniel Khaole, MD, for alcohol-related craniofacial dysmorphology. Children with the facial features characteristic of FAS (small palpebral fissures, flat midface, and smooth philtrum), significant growth retardation (<10th percentile for height and weight or <3rd percentile for head circumference), and evidence of poor central nervous system function were diagnosed as having FAS.

Control Variables

Data were obtained on a broad range of control variables, including maternal age, years of education, marital status, parity, maternal depression, and infant sex. Prenatal control variables also included maternal smoking and illicit drug use during pregnancy. Birth weight and head circumference were obtained from hospital medical records. Gestational age at birth was calculated from early pregnancy ultrasound examination or expected date of confinement when ultrasound data were not available. Weight, length, head circumference, and the FTII were measured at 6.5 months. The FTII consists of 10 problems, in which the infant is shown 2 identical target photographs for a fixed period of time and is then shown the familiar target paired with a novel one. Novelty preference (the proportion of looking time devoted to the novel stimulus) provides an index of visual recognition memory. The mean duration of the infant’s visual fixations to the stimuli provides a measure of information processing speed.

Data Analysis

Before analysis, all variables were checked for normality of distribution. Average alcohol use per day at conception and during pregnancy were positively skewed (skew >3.0) and were normalized by means of log (X + 1) transformation. Pearson correlation analysis was used to determine which control variables should be included in multivariate analyses to control for potential confounding. Because a control variable cannot be the true cause of an observed deficit unless it is related both to exposure and outcome, association with either exposure or outcome can be used as the criterion for inclusion in a multivariate analysis to control for confounding. In this study, control variables were selected in relation to outcome, which has the additional advantage of increasing precision by also including covariates unrelated to exposure. We planned to assess the effect of each alcohol measure on acuity in a multiple regression analysis, adjusting for the effects of the potential confounders, as necessary. To assess mediating effects of gestational age and prenatal and postnatal growth, each regression analysis was rerun, adding each of the hypothesized mediators (gestational age at birth, birth weight and head circumference, and weight, length, and head circumference at 6.5 months) in separate analyses. In these analyses, the potential confounding variables and drinking during pregnancy were entered at the first step, the potential mediator at the second step. To determine whether maternal age moderated the effect of alcohol exposure on visual acuity, Pearson correlation analysis was used to determine the relation of alcohol exposure to visual acuity in infants whose mothers were either < or ≥ 30 years of age at delivery.

RESULTS

Sample

The mothers in the cohort were very poorly educated; only 24 (18.3%) had completed high school (Table I). Almost
a third (30.5%) were 30 years of age or older at delivery, 47 (35.9%) were primiparous, and the majority (93.0%) breastfed their infants. To date, among the Cape Coloured (35.9%) were primiparous, and the majority (93.0%) breastfed their infants. To date, among the Cape Coloured population, there is a much lower incidence of HIV than in black African mothers and, to the best of our knowledge, none of the infants in the sample was HIV positive.

Twenty-four infants (18.3%) were born preterm (gestational age <37 weeks). Twenty-two infants (16.8%) met criteria for a diagnosis of FAS. Six of these infants (27.3%) had TAC scores below the fifth percentile, compared with only 10 of the 107 (9.3%) infants without FAS. The children in the bottom 5th percentile all had acuity scores of 3.2 cycles/degree or lower.

### Alcohol and Drug Use During Pregnancy

Half of the women (51.9%) reported drinking at conception, with slightly more (56.5%) drinking during pregnancy (Table II). As in other studies of pregnant women, these mothers reported reducing their alcohol consumption during pregnancy. However, although they reduced the number of days they drank by about a third, the women continued to drink at risk levels of on average 6.4 drinks per occasion during pregnancy. Eleven percent met DSM-IV criteria for alcohol abuse and an additional 22.9% for alcohol dependence. More than two thirds of the women (70.2%) reported smoking cigarettes, with one fifth smoking an average of 10 or more cigarettes per day. Marijuana and mandrax use was rare, and no women reported using inhalants (eg, glue or solvents), heroin, cocaine, sedatives, or other drugs during pregnancy.

### Alcohol Effects on Visual Acuity

Alcohol exposure during pregnancy was significantly related to reductions in birth weight and 6.5-month weight and length but not to gestational age at birth (Table III). Twenty-two infants (16.8%) met criteria for a diagnosis of FAS. Six of these infants (27.3%) had TAC scores below the fifth percentile, compared with only 10 of the 107 (9.3%) infants without FAS, $\chi^2 (1) = 7.80$, $P < .005$. The children in the bottom 5th percentile all had acuity scores of 3.2 cycles/degree or lower.

When visual acuity was examined as a continuous measure, none of the control variables were related to it at $P < .10$, and therefore none were controlled statistically in the data analyses. Poorer acuity at 6.5 months was associated with higher average daily alcohol consumption at conception and

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**Table I. Sample characteristics (n = 131)**

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>Mean or %</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
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<td>Age at delivery</td>
<td>27.3</td>
<td>6.4</td>
<td>18.4-43.8</td>
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<tr>
<td>Years of school completed</td>
<td>8.7</td>
<td>2.5</td>
<td>0.0-12.0</td>
</tr>
<tr>
<td>Married (%)</td>
<td>32.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Parity</td>
<td>2.2</td>
<td>1.3</td>
<td>1.0-8.0</td>
</tr>
</tbody>
</table>

**Infant characteristics**

<table>
<thead>
<tr>
<th>Birth</th>
<th>Mean or %</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% female)</td>
<td>44.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>38.6</td>
<td>2.3</td>
<td>29.1-43.0</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>2879.1</td>
<td>587.0</td>
<td>1130-4240</td>
</tr>
<tr>
<td>Weight percentile</td>
<td>32.8</td>
<td>29.5</td>
<td>0.5-90.3</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>32.8</td>
<td>2.0</td>
<td>23.0-36.0</td>
</tr>
<tr>
<td>Head circumference percentile</td>
<td>20.0</td>
<td>20.9</td>
<td>0.0-77.6</td>
</tr>
</tbody>
</table>

**6.5-month visit**

| Postnatal age at visit (mo) | 7.1 | 0.6 | 5.6-9.4 |
| Corrected age at visit (mo) | 6.8 | 0.5 | 5.2-7.9 |
| Weight (kg) | 7.8 | 1.0 | 5.1-10.0 |
| Weight percentile | 42.7 | 32.0 | 0.0-98.3 |
| Length (cm) | 67.4 | 2.6 | 60.0-73.0 |
| Length percentile | 44.6 | 30.8 | 0.0-97.9 |
| Length for weight percentile | 50.5 | 31.8 | 0.0-99.2 |
| Head circumference (cm) | 43.5 | 1.2 | 39.8-46.2 |
| Head circumference percentile | 48.4 | 31.7 | 0.0-99.4 |
| Number of weeks breast-fed | 24.3 | 11.4 | 0.0-37.3 |
| Visual acuity: cycles/degree | 6.4 | 0.6 | 2.4-13.0 |

---

**Table II. Maternal alcohol, drug use, and smoking**

<table>
<thead>
<tr>
<th>Daily average (oz AA)*</th>
<th>N</th>
<th>Mean</th>
<th>%</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>At conception</td>
<td>68</td>
<td>1.5</td>
<td>1.7</td>
<td>0.02-11.6</td>
<td></td>
</tr>
<tr>
<td>During pregnancy</td>
<td>74</td>
<td>0.9</td>
<td>1.0</td>
<td>0.01-10.3</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Average per drinking day (oz AA)*</th>
<th>N</th>
<th>Mean</th>
<th>%</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>At conception</td>
<td>68</td>
<td>4.3</td>
<td>2.7</td>
<td>0.3-15.4</td>
<td></td>
</tr>
<tr>
<td>During pregnancy</td>
<td>74</td>
<td>3.2</td>
<td>2.0</td>
<td>0.2-10.3</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of drinking days/week*</th>
<th>N</th>
<th>Mean</th>
<th>%</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>At conception</td>
<td>68</td>
<td>2.1</td>
<td>1.4</td>
<td>0.02-7.0</td>
<td></td>
</tr>
<tr>
<td>During pregnancy</td>
<td>74</td>
<td>1.4</td>
<td>1.4</td>
<td>0.004-7.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alcohol abuse (%)† ‡</th>
<th>N</th>
<th>Mean</th>
<th>%</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol dependent (%)† ‡</td>
<td>109</td>
<td>11.0</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Cigarettes smoked per day*</td>
<td>92</td>
<td>7.5</td>
<td>6.0</td>
<td>0.4-40.0</td>
<td></td>
</tr>
<tr>
<td>Marijuana use (days/week)</td>
<td>14</td>
<td>2.4</td>
<td>2.0</td>
<td>0.03-7.0</td>
<td></td>
</tr>
</tbody>
</table>

*Consumers only.
† Based on DSM-IV criteria.
‡ Missing for 22 women.

No sex-related differences were found for acuity, $t (131) = -0.492$, $P > .20$. The children in the bottom 5th percentile all had acuity scores of 3.2 cycles/degree or lower.

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*Based on percentiles from the Centers for Disease Control and Prevention.
† Not reported for 24 infants who were premature (gestational age at birth <37 weeks).
‡ Not assessed for 1 infant.
§ Based on TAC test. The values for mean and range have been back transformed to cycles/degree; SD in octaves.

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during pregnancy (Table III). Although the amount of alcohol consumed on a given occasion was not related to acuity, infants born to mothers who binge drank were more likely to have acuity values below the 5th percentile than infants born to mothers who did not binge drink, $\chi^2(1) = 7.80, P < .005$.

Among the 10 infants with low TAC scores who did not meet the diagnostic criteria for FAS, 4 were born to mothers who reported binge drinking during pregnancy and 1 to a mother who drank an average of 1.9 oz AA/day (3.8 standard drinks) during pregnancy. The mother of 1 infant, who was diagnosed with FAS and whose TAC score was 2.4 cycles/degree, reported consuming 5.1 oz AA (10.2 standard drinks) during pregnancy. The mother of 1 infant, with a TAC score of 2.4 denied drinking during pregnancy, had DSM-IV criteria for alcohol abuse and reported very heavy drinking (5.8 oz AA/occasion) during pregnancy. Octave, which was computed by performing a logarithmic (base2) transformation on cycles/degree, has been back transformed to the original cycles/degree units on the y-axis. Group Ns are shown in parentheses.

![Figure](image.png)

**Figure.** Relation of binocular visual acuity to four levels of drinking during pregnancy. Octave, which was computed by performing a logarithmic (base2) transformation on cycles/degree, has been back transformed to the original cycles/degree units on the y-axis. Group Ns are shown in parentheses.

Given that prenatal alcohol exposure is associated with reduced fetal and postnatal growth, the hypothesis that the effect of this exposure on acuity might be mediated by impaired somatic growth was tested by multiple regression analysis. The relation of pregnancy drinking to acuity was virtually unchanged when gestational age and birth size were included in the analysis but was reduced slightly by the inclusion of 6.5-month size, indicating that the poorer acuity seen in relation to prenatal alcohol exposure might relate, in part, to delayed postnatal development associated with slow somatic growth (Table IV). By contrast, the inclusion of measures of cognitive performance on the FTII did not mediate the effect of prenatal alcohol exposure on acuity, suggesting that the effect on acuity was not due to developmental delay, poorer attention, or ability to respond appropriately to the visual assessment procedure.

Table V examines maternal age as a potential moderator of the effect of prenatal alcohol on acuity. Although infants born to older mothers comprised only about one third of the cohort, the relation of daily alcohol exposure to visual acuity was seen primarily in the infants born to mothers 30 years of age or older. As expected, maternal age was highly associated with years of drinking in this cohort, $r = .59, P < .0001$. However, there were no significant differences in average daily alcohol intake between the 2 groups at conception, t (129) = −0.54, or during pregnancy, t (129) = −0.24, or in
consumption per occasion at conception, \( t (129) = 0.75, \) or during pregnancy, \( t (129) = 0.10, \) indicating that the greater vulnerability of the infants born to older mothers was not attributable to heavier drinking in older mothers.

**DISCUSSION**

This study is the first to prospectively document the impact of very heavy alcohol use during pregnancy by segments of the Cape Coloured population on infant development. The large number of infants with FAS born to the heavy drinking women in this cohort is consistent with the very high incidence reported in a retrospective study of Cape Coloured children entering elementary school, which was conducted in a more rural, grape-growing region of South Africa, \(^2\) and extends those findings to show that heavy alcohol use and alcoholism during pregnancy persists among some subgroups of this population who have moved to urban areas.

We found that greater average daily prenatal alcohol exposure is related to poorer acuity, as indicated by lower TAC scores. Because a complete eye examination was not conducted on these infants, it is not possible to know the source of the alcohol-related lower acuity scores, such as refractive error, retinal changes, or central nervous system abnormalities. Our results are consistent with clinical case reports of poor visual acuity in children with FAS.\(^3\)\(^4\) We examined whether lower TAC scores among alcohol-exposed infants might be due to a developmental delay that affects the infant’s ability to attend appropriately to the visual acuity cards. But the effect of prenatal alcohol exposure on acuity was independent of the infant’s performance on the FTII, a valid measure of infant visual information processing that also depends on the infant’s ability to attend to visual stimuli. The failure of gestational age and birth size to mediate the effect of prenatal alcohol on acuity suggests that the acuity deficit was not attributable to poorer prenatal somatic growth. On the other hand, the acuity deficit seen at 6.5 months might be due to a maturational deficit or delay in the visual system and might therefore not be permanent. Predictive validity of the TAC for later acuity has been demonstrated by Mash and Dobson.\(^3\)\(^3\) However, the utility of the 6-month TAC as a screening instrument for alcohol-related ocular deficits depends on the degree to which TAC performance is related to ocular status at 6 months and predictive of later visual system anomalies, which needs to be assessed by ophthalmologic examination during childhood.

The effect of alcohol exposure on acuity was seen primarily in infants whose mothers were 30 years or older at delivery, even though the older mothers did not drink larger quantities of alcohol. This finding is consistent with previous research implicating maternal age as an important moderator of alcohol effects in animal studies,\(^1\) case reports of both white\(^1\)\(^2\) and Native American\(^1\)\(^3\) children with FAS, and our previous research conducted on a moderate-to-heavily exposed, longitudinal Detroit cohort indicating increased vulnerability on numerous endpoints in children born to mothers 30 years of age or older.\(^1\)\(^4\)\(^5\) This increased vulnerability may be attributable to physiological changes in the mother relating to the aging process or to consequences of chronic drinking over a more prolonged period. Age-related increases in the ratio of maternal body fat to water lead to higher peak blood alcohol concentrations per unit dose of ethanol consumed, exposing the fetus of the older mother to higher doses.\(^3\)\(^4\) A recent laboratory study found that exposure of female mice to daily heavy doses of alcohol before conception resulted in lower offspring body weights, ovarian anomalies, and fewer follicles that reached maturity, even when the animals were not exposed to alcohol during gestation,\(^3\)\(^5\) suggesting that a longer history of alcohol abuse may reduce embryonic and fetal viability.

These findings suggest that the TAC is a useful tool for screening for adverse effects of prenatal alcohol exposure on acuity. Particular attention needs to be given to screening infants born to older mothers, who are at higher risk of damage to the ocular system from their mothers’ alcohol use during pregnancy. An in-depth ophthalmologic evaluation of the

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### Table IV. Effect of alcohol (AA/day during pregnancy) on acuity controlling for hypothesized mediating variables (n = 131)

<table>
<thead>
<tr>
<th>Controlling for</th>
<th>R</th>
<th>β*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, birth weight, and head circumference</td>
<td>−.23†</td>
<td>−.25‡</td>
</tr>
<tr>
<td>6.5-month weight, length, and head circumference §</td>
<td>−.25†</td>
<td>−.21†</td>
</tr>
<tr>
<td>FTII—novelty preference and duration visual fixation</td>
<td>−.23†</td>
<td>−.22†</td>
</tr>
</tbody>
</table>

*Effect of alcohol on acuity, controlling for potential mediators.
†P < .05.
‡P < .01.
§Data missing for 1 infant.

### Table V. Maternal age ≥ 30 years as a moderator of the effect of prenatal alcohol exposure on infant visual acuity

<table>
<thead>
<tr>
<th></th>
<th>Age &lt; 30 (N = 91)</th>
<th>Age ≥ 30 (N = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute alcohol/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At conception</td>
<td>−.14</td>
<td>−.33†</td>
</tr>
<tr>
<td>During pregnancy</td>
<td>−.09</td>
<td>−.40‡</td>
</tr>
<tr>
<td>Drinks/occasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At conception</td>
<td>−.05</td>
<td>−.25*</td>
</tr>
<tr>
<td>During pregnancy</td>
<td>−.03</td>
<td>−.30*</td>
</tr>
<tr>
<td>Number of drinking days/week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At conception</td>
<td>−.13</td>
<td>−.35†</td>
</tr>
<tr>
<td>During pregnancy</td>
<td>−.09</td>
<td>−.43†</td>
</tr>
</tbody>
</table>

Values are Pearson r's.
*P < .10.
†P < .05.
‡P < .01.
infants in this study during childhood is necessary to determine the nature of the visual abnormalities associated with fetal alcohol exposure and the degree to which the screening information from the TAC is indicative of these deficits.

We wish to thank Velma Dobson, who consulted on the administration of the TAC procedure; Kenneth Lyons Jones, Nathaniel Khaleo, and Sally Zief for their diagnostic evaluations of the infants and Andrea Hay, Anna–Susan Marais, Deborah Price, Magdalene September, Julie Croxford, Raucha Corobana, Neil Dodge, and Dickie Naude for their contributions to this research. Portions of this research were presented at the 2001 and 2002 meetings of the Research Society on Alcoholism.

REFERENCES

SUBSTRATE UTILIZATION AND KINETICS OF SURFACTANT METABOLISM IN EVOLVING BRONCHOPULMONARY DYSPLASIA

KIMBERLY L. SPENCE, MD, JAMES C. ZOZOBRADO, MD, BRUCE W. PATTERSON, PHD, AND AARON HAMVAS, MD

Objectives To use stable isotopically labeled precursors of pulmonary surfactant phospholipids to measure precursor utilization and surfactant turnover in premature infants who required mechanical ventilation at birth, 2 weeks, and >4 weeks of age.

Study design Infants of ≥28 weeks’ gestation received simultaneous 24-hour intravenous infusions of \([1,2,3,4-{^13}C_4]\) palmitate and \([1-{^13}C_1]\) acetate at birth, 2 weeks, and ≥4 weeks of life. Disaturated phospholipids were extracted from sequential tracheal aspirate samples obtained over a period of 2 weeks. Fractional catabolic rate (a measure of total turnover) and the fractional synthetic rates from plasma palmitate and de novo synthesis (acetate) were measured.

Results The fractional catabolic rate increased from 25.3% ± 7.0% per day at birth to 53.8% ± 14.4% per day at 4 weeks (\(P = .001\)). The combined contribution from plasma palmitate and de novo synthesis to total synthesis increased from 44.2% ± 19.8% at birth to 85.2% ± 32.8% at 4 weeks (\(P = .03\)).

Conclusions Total surfactant turnover increased in premature infants with evolving bronchopulmonary dysplasia. The increasing contributions from acetate and plasma palmitate suggest a decrease in surfactant phospholipid recycling. (J Pediatr 2005;147:480-5)

Pulmonary surfactant is a phospholipid-protein complex synthesized by alveolar type 2 cells. Surfactant is composed of approximately 80% phospholipid, with palmitate comprising 60% to 80% of the fatty acid composition. The palmitate in pulmonary surfactant phospholipid may originate from different sources, including plasma, surfactant recycling, or de novo synthesis from acetate in type II cells. Animal studies have yielded conflicting results regarding the relative contributions of each of these sources to surfactant palmitate.1-4 Although the importance of these relative contributions to disease is unknown, pigs with palmitate-deficient diets had decreased pulmonary compliance suggestive of an alteration in surfactant function.4

The critical role for surfactant in lung function is highlighted in preterm infants in whom surfactant deficiency results in hyaline membrane disease. The role of pulmonary surfactant in the development of bronchopulmonary dysplasia (BPD) is unknown. Studies of surfactant composition in infants with BPD have demonstrated decreased ratios of surfactant protein A to saturated phosphatidylcholine, which suggests altered surfactant metabolism.5 In fact, Cogo et al6 found increased disaturated phosphatidylcholine (DSPC) catabolism in infants who had bronchopulmonary dysplasia. On the other hand, Janssen et al7 studied evolving BPD in a premature baboon model with stable and radioactive isotopes of dipalmitoyl phosphatidylcholine and direct pool size measurements. They determined that surfactant turnover did not change during the first 8 days in the development of BPD, and pool size was constant after surfactant therapy. Studies using both intravenously and intratracheally administered stable isotopes have described surfactant metabolism in several populations of infants, including those with evolving BPD, and have reached slightly different conclusions about the associated alterations in surfactant metabolism.
metabolism.6,8-14 However, these studies did not investigate changes in precursor utilization longitudinally. This study uses simultaneous infusions of stable isotopically labeled acetate and palmitate to interrogate the differential contribution of these substrates to surfactant synthesis as hyaline membrane disease evolves into BPD.

METHODS

Study Design
Infants admitted to the Neonatal Intensive Care Unit at St Louis Children’s Hospital were eligible if they were ≤28 weeks’ estimated gestational age (EGA) and required mechanical ventilation. Infants were studied at <4 days of life (n = 15, newborn group), at 12 to 15 days of life (n = 7, intermediate group), and at >28 days of life (n = 8, BPD group). Six infants received more than one infusion. Infants who were expected to be extubated in less than 3 days or who had documented infection, pulmonary hemorrhage, chromosomal anomalies, or imminent death were excluded.

After informed consent from parents, all infants received simultaneous 24-hour infusions of 2.9 μmol/kg of [1-13C1] acetate (Cambridge Isotope Laboratories, Inc, Andover, MA) and 58 μmol/kg of [1,2,3,4-13C4] palmitate (Isotec, Miamisburg, OH), prepared in 5% dextrose, heated to 60°C, and then filtered for sterility into 25% albumin. Protein, fat, and glucose administration were held constant during the infusion. Tracheal aspirates were collected in a standardized fashion before the tracer infusion and every 6 to 12 hours for 2 weeks or until extubation. Blood samples were obtained approximately every 6 hours during the infusion. Tracheal aspirates and plasma were stored at −70°C. The Washington University Human Studies Committee approved the study.

Analytical Procedures
Disaturated phospholipids (DSPL) were extracted from tracheal aspirates by means of chloroform-methanol osmium tetroxide oxidation.8,15,16 The isotopic enrichments of methyl palmitate from surfactant and plasma were measured by gas chromatography and mass spectrometry (GC/MS).15 Enrichment is expressed as tracer to tracee ratio (TTR), representing the molar ratio of labeled to unlabeled palmitate in the sample. Indexes of surfactant metabolism were determined from the (m+1) and (m+4) isotopic enrichment curves representing the enrichment of palmitate that has incorporated one 13C-acetate unit and palmitate that was incorporated directly from plasma, respectively.3,4,10-15,17 Palmitate was extracted from plasma by solid-phase chromatography and enrichment determined by GC/MS.18

Measurements
The fractional synthetic rate (FSR) is the fraction of the surfactant phospholipid pool that is formed from a particular precursor (%/day).8,12,15,17 FSRacetate was calculated from the slope of (m+1) palmitate and the enrichment of acetyl CoA and de novo synthesized palmitate as determined by mass-isotopomer distribution analysis.15,17,19,20 FSRpalmitate was calculated from the downslope of the (m+1) palmitate time-enrichment curve after logarithmic transformation (Figure 1, A). The fractional catabolic rate (FCR) was calculated from the downslope of the (m+1) palmitate time-enrichment curve (Figure 1, B). The FCR represents the total fractional turnover rate from all production pathways as tracer-labeled surfactant is replaced by unlabeled surfactant. The ratio FSR/FCR for a particular precursor reflects the proportion that precursor contributes to the total production of surfactant.

Severity of lung disease was determined from the average daily FiO₂ × mean airway pressure over time, divided...
by number of days studied. Based on the standard deviations of previous studies in this population, sample sizes were calculated to be approximately 5 infants per group to detect a 2 SD difference in the fractional catabolic rate, with 80% power and an α value of 0.05. However, as data from the individual groups were analyzed, it became obvious that larger sample sizes would be needed because the variance in FCR in the study groups was larger than our previous studies suggested. The data were analyzed as a cross-sectional study. Two-tailed Student t tests, paired t tests, analysis of variance (ANOVA), and regression and correlation analyses were performed with SPSS (version 11.5, SPSS Inc, Chicago, IL). Results shown are mean ± SD.

RESULTS

The clinical characteristics of each group are displayed in Table I. Two patients from the BPD group died 2 and 12 weeks after study completion. All patients in the newborn group received exogenous surfactant from 20 to 90 hours before the start of the isotope infusion; 11 infants received two doses and 4 received a single dose. As anticipated, FCRacetate was not significantly different from FCRpalmitate at each time point (paired t test, \(P = .14\)). FCR increased with age from 25.3% ± 7.0% to 43.6% ± 14.0% to 53.8% ± 14.4% in the newborn, intermediate, and BPD groups, respectively (ANOVA, \(P = .001\)). Six infants who underwent at least two sequential studies demonstrated this increase in FCR (Figure 2). In each group, FSRpalmitate was significantly greater than FSRacetate (Table II), suggesting that DSPL palmitate was derived from plasma to a greater extent than from de novo synthesis. FSR from both acetate and palmitate increased over time and correlated with one another (Pearson coefficient = 0.823, \(P = .007\)). To determine if the surfactant metabolic indexes in the immediate newborn group would differentiate those infants who no longer required mechanical ventilation from those who would still need ventilation at 28 days, we performed an independent-samples t test. FSRpalmitate approached statistical significance in differentiating the infants in the newborn group who would have BPD [6.1% ± 3.5% (BPD) versus 11.0% ± 5.8% (no BPD), \(P = .07\)].

The increase in FSR from each substrate over time contributed to a significantly increasing proportion of new surfactant synthesis being accounted for by our tracers (Figure 3). For example, at birth [(FSRacetate + FSRpalmitate)/FCR] accounted for 44.2% ± 19.8% of new surfactant synthesis, whereas at 4 weeks, it increased to 85.2% ± 32.8% (ANOVA, \(P = .03\)).

Because many factors may influence changes in surfactant metabolism over time, we used linear regression models to identify covariates that could have contributed to the differences in FCR and the FSR/FCR ratio. We did not detect any racial differences in these indexes in any of our previous studies. Because surfactant administration might lead to an unstable surfactant pool size, kinetic parameters and substrate

---

Table I. Study population demographics

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Newborn (n = 15)</th>
<th>Intermediate (n = 7)</th>
<th>BPD (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight at study start</strong> (grams)</td>
<td>829 ± 170</td>
<td>874 ± 208</td>
<td>1201 ± 481</td>
</tr>
<tr>
<td><strong>Gestational age</strong> (wks)*</td>
<td>26 ± 1</td>
<td>25 ± 1</td>
<td>26 ± 3</td>
</tr>
<tr>
<td><strong>Age at study start</strong></td>
<td>55 ± 20 hours</td>
<td>15 ± 3 days</td>
<td>40 ± 15 days</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>7 males</td>
<td>3 males</td>
<td>3 males</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td>8:5:2</td>
<td>2:3:2</td>
<td>3:4:0</td>
</tr>
<tr>
<td><strong>Disease severity score</strong></td>
<td>4.0 ± 2.2</td>
<td>4.5 ± 2.0</td>
<td>5.7 ± 3.0</td>
</tr>
<tr>
<td>(average MAP* FiO_{2} during study period)</td>
<td>Antenatal- 13/15</td>
<td>2/7</td>
<td>4/8</td>
</tr>
<tr>
<td><strong>Steroids Exposure (%)</strong></td>
<td>9/15 (60%)</td>
<td>7/7 (100%)</td>
<td>8/8 (100%)</td>
</tr>
<tr>
<td><strong>Ventilated at 28 days</strong></td>
<td>12/15 (80%)</td>
<td>6/7 (71%)</td>
<td>7/8 (88%)</td>
</tr>
<tr>
<td><strong>Oxygen at 36 weeks</strong></td>
<td>100</td>
<td>100</td>
<td>75</td>
</tr>
</tbody>
</table>

*at birth.

**prior to or during study.
utilization were evaluated with respect to dosing and time from surfactant administration until study time. There were no correlations between dosing and time from surfactant administration with fractional synthetic or catabolic rates of DSPL.

Given the small sample size, results were not stratified for race, as it would compromise the statistical power of this study. Disease severity was not significantly different across groups. However, weight, caloric intake, and age were tightly linked covariates that limited the ability of linear regression to correlate any single variable with the surfactant metabolic indexes.

In these studies, we presume that the \( (m+1) \) palmitate detected in surfactant DSPL is being derived directly from acetate incorporation into palmitate in the type II cell. However, some \( (m+1) \) palmitate may be synthesized in other tissues such as the liver and may be incorporated from the same plasma pool labeled by our tissues such as the liver and may be incorporated from the same plasma pool labeled by our tissues labeled by our (m+4) palmitate. Mass-isotopomer distribution analysis would not distinguish between the two sources of \( (m+1) \) palmitate and would lead to an overestimation of strictly alveolar FSRacetate. Knowing FSRpalmitate and the plasma \( (m+1) \) palmitate enrichment, we determined that on average, 13% of the \( (m+1) \) upslope was derived from plasma \( (m+1) \) palmitate in 5 infants. This resulted in a negligible influence on the FSR measurement: Instead of 4.0% per day, the average FSRacetate for the infants studied at birth would be corrected to 3.5% per day. [The \( (m+4) \) upslope and FCR measurements are not influenced by this phenomenon.] All calculations that utilize FSRacetate are based on actual measurements and not corrected because plasma \( (m+1) \) palmitate enrichment was not measured in all infants.

**DISCUSSION**

We used stable isotopically labeled acetate and palmitate to determine their contributions to endogenous surfactant synthesis in premature infants with evolving BPD. In contrast to previous studies in premature infants that measured surfactant metabolism at only one time point, this is the first study in which surfactant kinetics were followed as independent cross-sectional studies (and 6 infants longitudinally) over the course of a month.\(^8\) Surfactant turnover, as measured by FCR, was slow at birth, with only one quarter of the pool turning over per day, but it doubled by 1 month of age.

Interestingly, the FCR of 54% per day for the 1-month-old infants in the present study is similar to that of term infants with normal lungs at our institution reported by Bohlin et al\(^8\) (half-life was 28 hours, which translates to an FCR of 59% per day). The question then arises as to whether surfactant synthesis was “normal” in the BPD group. The results we are reporting are the “fractional” measurements, or the relative contributions of each substrate and the fraction of the pool turnover over per day. Understanding whether the absolute synthetic or turnover rates were changing over time requires measurement of the surfactant pool size, something that can only be estimated with the use of an airway administered tracer, as Cogo et al\(^{13}\) and Torresin et al\(^{14}\) have previously done. Drawing on those studies, we can attempt to estimate absolute surfactant synthesis in our patients. Torresin et al\(^{14}\) estimated that the DSPC pool size in infants approximately 30 weeks’ gestation was 17 ± 14 mg/kg at the time of the second dose of surfactant, similar to when our newborn group was studied. Cogo et al\(^{13}\) estimated that the DSPC pool size was 136 ± 21 mg/kg in infants of 26 weeks’ gestation with evolving BPD studied at 26 days of age, a population similar to our BPD group. Using these estimates of pool size yields an absolute total surfactant synthetic rate (FCR × pool size) for the newborn group of 4 mg/kg per day, which increased to 73 mg/kg per day in the BPD group. In comparison, Cogo et al\(^{13}\) found a DSPC pool size of 58 ± 9 mg/kg in term infants

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**Table II. Cross-sectional analysis of study group kinetic parameters**

<table>
<thead>
<tr>
<th>Population</th>
<th>FSRacetate %/day</th>
<th>FSRpalmitate %/day</th>
<th>P-value*</th>
<th>FSRacetate /FCR, %</th>
<th>FSRpalmitate /FCR, %</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEWBORN</td>
<td>4.05 ± 2.1</td>
<td>7.6 ± 4.6</td>
<td>0.006</td>
<td>15.5 ± 7.8</td>
<td>28.7 ± 16.3</td>
<td>0.006</td>
</tr>
<tr>
<td>INTERMEDIATE</td>
<td>8.0 ± 5.3</td>
<td>21.3 ± 15.7</td>
<td>0.02</td>
<td>18.0 ± 11.5</td>
<td>47.8 ± 34.3</td>
<td>0.02</td>
</tr>
<tr>
<td>BPD</td>
<td>14.9 ± 6.8</td>
<td>30.5 ± 16.3</td>
<td>0.01</td>
<td>27.5 ± 9.6</td>
<td>61.3 ± 34.9</td>
<td>0.02</td>
</tr>
<tr>
<td>P-values (ANOVA between groups)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>N/A</td>
<td>0.03</td>
<td>0.03</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*paired t-test, FSRacetate vs. FSRpalmitate;
**paired t-test, FSRacetate vs. FSRpalmitate/FCR.

**Figure 3.** Percent contribution from plasma palmitate and de novo synthesis (acetate) to DSPL production. Contribution from unlabeled sources (recycling) decreases over time.
without lung disease, which we can extrapolate into a total synthetic rate of 34 mg/kg per day in our previously published normal infants. Although there are no direct comparisons of half-life or FCR measurements using simultaneous endogenous and exogenous tracers, our findings are consistent with Cogo et al that FCR is higher in infants older than 3 weeks with evolving BPD than in the newborn period, but they contrast with the Janssen et al findings in the baboon model during the early development of BPD (8 days). Cogo et al conclude that “BPD leads to increased DSPC catabolism,” although we would contend that it is actually surfactant turnover (both synthesis and catabolism) that is increased.

The increase in surfactant fractional turnover rate over time (newborn infants = “intermediate” BPD) may be due to increased surfactant production, or it may reflect a depression of FCR in the newborn group caused by the administration of exogenous surfactant and increased surfactant pool size. This is consistent with the findings of Kramer et al, who found that the biological half-life of exogenous radiolabeled surfactant in mice was increased (hence, the FCR was decreased) after exogenous surfactant administration.

Associated with this increase in total surfactant synthesis, we also found a greater contribution of FSRacetate/FCR and FSRpalmitate/FCR to total surfactant DSPL production, along with a significant decrease in the contribution from additional unlabeled sources. The sources of unlabeled palmitate may include plasma triglycerides, cellular lipids, or recycled surfactant phospholipids. We cannot determine from these studies, though, how much recycling actually contributes to the unlabeled pool. Studies in preterm, term, and adult rabbits and/or sheep have suggested developmental changes in the proportion of recycling: from 90% in term newborn rabbits to 50% in adult rabbits. Our data suggest that approximately 50% of surfactant production in the newborn group was derived from sources that were not labeled by plasma palmitate and acetate tracers. If the major contribution of unlabeled pool came from recycling, this proportion would be similar to those derived from animal studies. However, in contrast to studies in animals with normal lungs that suggested increased recycling at term, we found that recycling actually decreased (to approximately 10%) as these premature newborn infants aged. Whether this decrease in recycling is unique to preterm infants whose lung disease is more severe and evolving into BPD is unknown and difficult to study because of the lack of an appropriate control group. Furthermore, it is difficult to determine if this decreased recycling is a primary or secondary factor in these infants’ continuing need for mechanical ventilation resulting from alveolar type 2 cell dysfunction.

Alternatively, it may not be a matter of decreased surfactant recycling but rather improved substrate availability and utilization as well as maturity of type 2 cells that promote new surfactant synthesis with less reliance on recycled phospholipids. Fat and total caloric intake increased with age in our study population. Regardless of the quantity or composition of the fat and energy supply, incorporation of plasma palmitate into surfactant DSPL was approximately twice that from de novo synthesis at all time points. Our findings are similar to those in adult pigs in which the flux of plasma palmitate into lung surfactant phosphatidylcholine was greater than that from de novo synthesis. Furthermore, the availability of palmitate in the diet of the study pigs influenced surfactant composition and function. As mentioned before, study age, weight, and nutritional intake in our study were tightly linked covariates, making it difficult to determine the role that dietary substrates play in surfactant metabolism in these infants with evolving lung disease.

A detailed discussion of assumptions and limitations underlying stable isotope methodology and its role in the study of surfactant metabolism is described in detail elsewhere. A major assumption underlying these stable isotope studies is that the surfactant pool size remains constant during the course of the study, an assumption that is difficult to validate. Animal studies indicate that surfactant pool size increases rapidly within the first few days of life. However, the log linear nature of the decrease from peak TTR (Figure 1, B) is consistent with a constant pool size over time. Furthermore, the mass of DSPL in tracheal aspirates, as determined by quantitative gas chromatography, did not increase during the turnover studies (data not shown). The relative contributions of acetate, palmitate, and unlabeled sources (ie, recycling) to total surfactant synthesis are independent of pool size and are unaffected.

Even though these groups of infants were similar in their clinical characteristics, there may be a variety of mechanisms that resulted in preterm birth or their continuing need for mechanical ventilation, possibly explaining the variances in the indexes of surfactant synthesis. Despite these large variances, the 6 infants who had sequential measurements all had similar changes in their indexes of synthesis over time (Figure 2).

In conclusion, we have demonstrated that surfactant turnover increased in a group of premature infants who required mechanical ventilation for the first month of life. This increased turnover was primarily due to new surfactant synthesis with very little contribution from surfactant recycling. Adding an exogenous tracer that can be used simultaneously with the endogenous labels will permit a complete interrogation of surfactant metabolism in vivo, which will in turn lead to better understanding of the role of surfactant metabolism in the development of bronchopulmonary dysplasia in premature newborn infants.

The authors thank Junyoung Kwon for GC/MS analysis, Frieda Custodio for sample preparation, the pharmacy staff, especially Kristina Bryowsky, PharmD, and Rachel Weerasooriya, PharmD, for tracer preparation, the Neonatal Intensive Care Unit staff at St Louis Children’s Hospital for support and sample collection, and Sarah Boslaugh for statistical assistance.

REFERENCES

Substrate Utilization And Kinetics Of Surfactant Metabolism
In Evolving Bronchopulmonary Dysplasia

Objective To determine the impact of respiratory distress syndrome (RDS) on wheezing illnesses and re-hospitalizations in children as old as 2 years of age.

Study design We observed 2 geographically defined cohorts of children with RDS born after 26 weeks of gestation during 1990 to 1995 and 1996 to 1999 and gestationally paired control subjects. Recurrent wheezing illness and the re-hospitalizations caused by a respiratory condition were recorded.

Results In the first year of life, 47 of 224 infants with RDS and 18 of 224 control subjects born in 1990 to 1995 had recurrent wheezing illness ($P<.005$) compared with 21 of 109 infants with RDS and 14 of 109 control subjects in the latter cohort ($P=.27$). A higher number of infants with RDS were readmitted to the hospital (25% versus 13%, $P=.002$) in the former period, and they spent more days in hospital during both periods. The frequencies of wheezing remained constant in the second year of life, but hospital admissions decreased. Siblings at home, male sex, and bronchopulmonary dysplasia were additional risk factors of wheezing illnesses.

Conclusion RDS increases the incidence of wheezing illnesses during the first 2 years of life. Changes in the management of RDS during the 1990s was associated with a decreased incidence of subsequent RDS-associated respiratory morbidity. 

Although respiratory distress syndrome (RDS) remains a major problem among pre-term infants, improved treatment practices have decreased concomitant neonatal mortality and pulmonary and cerebral complications. However, chronic pulmonary and neurodevelopmental sequelae have remained considerable. Pre-term infants who survive with bronchopulmonary dysplasia (BPD) have symptoms and signs of obstructive lung disease and lung function abnormalities even in adulthood. Likewise, very low birth weight infants have recurrent wheezing illnesses more often than full-term infants, and they also have more re-hospitalizations for respiratory causes during early childhood. However, infants with RDS in the neonatal period have not been studied as a group, to evaluate their long-term outcome. Recent studies have focused on particular subsets of very low birth weight or very pre-term infants. The control groups have varied according to birth weight and gestational age, or the studies were carried out before the introduction of surfactant therapy. Novel treatments such as the use of surfactants and especially natural surfactant, antenatal steroids, gentler ventilation strategies, and inhaled nitric oxide have changed the consequences of RDS, possibly resulting in changes in persistent or recurrent respiratory symptoms after the neonatal period.

The aim of this investigation was to evaluate the impact of RDS on the occurrence of wheezing illnesses and re-hospitalizations for respiratory causes in infants as old as 2 years of age during 2 consecutive periods. We hypothesized that the progress in the management of RDS in the 10-year period from 1990 to 1999 may have resulted in improvement in subsequent lung morbidity.

METHODS

The study population was derived from a regional cohort of 488 infants with RDS born between 1990 and 1999 and treated at Oulu University Hospital. Of these, 142

| BPD | Bronchopulmonary dysplasia | RDS | Respiratory distress syndrome |
| NEC | Necrotizing enterocolitis   | RSV | Respiratory syncytial virus   |
| PPROM | Pre-term premature rupture of membranes |
infants were prospectively excluded on the basis of severe primary diseases or extreme abnormalities that hampered the selection of appropriate control subjects: malformations (n = 17), early-onset sepsis (n = 9), feto-fetal transfusion syndrome (n = 4), severe birth asphyxia (n = 5), very early rupture of membranes (n = 2), birth weight <500 g (n = 3), and gestation ≤26 weeks (n = 86). Sixteen very pre-term infants could not be matched. The final study group consisted of 346 infants with RDS (236 in years 1990-1995 and 110 in years 1996-1999) and 346 gestationally paired control subjects. All the infants were from a homogenous population of Caucasian origin. The total numbers of newborns were 36,595 and 22,395 during the periods 1990 to 1995 and 1996 to 1999, respectively. Both the number of deliveries and the incidence of RDS declined in the latter period compared with the former period. Only minimal changes in gestational age or birth weight were seen in the 2 time periods, however (Table 1).

The study was conducted in accordance with the Declaration of Helsinki.

The infants with RDS were matched 1-to-1 to infants without RDS on the basis of gestational age within 7 days of the nearest born infant. Otherwise, the same exclusion criteria that applied to the infants with RDS were used. The records of these mothers and infants were reviewed in the same manner as those of the infants with RDS.

The definition of gestational age, maternal diseases, and the diagnostic criteria for RDS, patent ductus arteriosus, infections, necrotizing enterocolitis (NEC), central nervous system abnormalities, and retinopathy of prematurity were as described elsewhere.19 BPD was defined as supplemental O2 requirement at the post-conceptional age of 36.0 weeks in infants born <32 weeks or more.20 At the beginning of the 1990s, antenatal steroids were administered to mothers from 26 to 28 until 32 weeks of gestation in threatened pre-term birth (excluding diabetes mellitus, severe pre-eclampsia, pre-term premature rupture of membranes [PPROM], and chorioamnionitis), whereas in the latter period, steroids were given between 24 and 34 weeks (excluding patients with clinical chorioamnionitis). Prophylactic antibiotics were administered to mothers in cases of PPROM in the latter period, whereas in the former study period antibiotics were administered only when signs of infection appeared.

Surfactant therapy was given only in a rescue mode, but the timing tended to become earlier toward the end of the decade. During the latter half of the 1990s, natural surfactant became preferred, whereas before that, mainly synthetic surfactant was used. Mechanical ventilation was provided with a pressure-limited ventilator. During the latter period, ventilatory management using lower pressures, higher frequencies, synchronous intermittent mandatory or patient-triggered ventilation, and nasal continuous positive pressure ventilation became more common. Inhaled nitric oxide and high-frequency ventilation were used in some severe cases during the latter period of study. Postnatal steroids were often indicated when the weaning from mechanical ventilation was unsuccessful during the first 10 days of life and no contraindications were evident. All infants with symptoms were screened for infection at admission, and laboratory screening was done daily in the acute phase and 1 to 2 times a week later or when suspected clinical signs of infection appeared.

Follow-up

All infants born before 33 gestational weeks and other infants at high risk were prospectively observed in the outpatient clinic of the hospital at 1- to 4-month intervals for as long as 1 year, and all infants with symptoms and at high risk were observed until 2 years of corrected age, when the final evaluation was made. Altogether 237 infants with RDS and 221 control subjects were regularly observed. Thirty-six additional mature infants with RDS who were at low risk and 52 control subjects were recalled. The follow-up data of 53 infants with RDS and 53 control subjects were collected from other hospitals or family health clinics or by presenting questionnaires to the parents. Thus, 13 pairs (children with RDS or control subjects) could not be traced at the age of 2.

We used recurrent wheezing as a primary outcome measure. Wheezing was defined as an infant having 2 or more wheezing episodes associated with or without lower respiratory tract infections in the first year of life. Wheezing illness was considered to have been present when wheezing continued or appeared during the second year. Events were confirmed for the infants seen in the outpatient clinic of the hospital, and the hospital records were checked for infants who were seen regularly during follow-up. Acute laryngitis causing inspiratory wheezing was not considered to be wheezing in the current setting. The number of infants with continuous inhaled steroid treatment was recorded, as was the number of infants who were re-hospitalized and the duration of re-hospitalizations caused by any non-surgical respiratory causes. Criteria for prescribing continuous inhaled steroids were ≥3 wheezing episodes within 6 months or prolonged symptoms or signs. The dosage and the method of administration were adjusted individually. Antibody prophylaxis against respiratory syncytial virus (RSV) infection for pre-term infants born at or before 32 weeks of gestation was started October 1999 in our hospital. Thus, only 10 infants with RDS and 8 control subjects received RSV prophylaxis.

Statistics

Statistical analyses were performed with SPSS for Windows version 11.5 (SPSS, Chigaco, Ill). Continuous variables were tested with the Student t test for paired samples, and categorical data were analyzed by the χ²-test to find out the differences in outcome between the groups. Logistic regression was used to investigate further whether RDS explained the risk of recurrent wheezing regardless of confounding factors such as maternal asthma, chorioamnionitis, antenatal steroids, mode of delivery, sex, and siblings at home. Adjusted odds ratios were calculated for each variable.
RESULTS

Most pregnancy complications were equally frequent among the infants with RDS and the control subjects. However, premature rupture of membranes had occurred less frequently in the group of infants with RDS than in the control group (Table I). The antenatal steroid therapy was not significantly different in the groups, although its use increased during the latter study period ($P < .001$). The infants with RDS had been delivered significantly more often via Caesarean section than the control subjects (Table I). The neonatal characteristics were similar in the 2 groups in both periods. However, Apgar scores at 5 minutes were significantly lower for the infants with RDS than for the reference group in both periods. The male predominance of RDS cases did not reach statistical significance (Table I).

Neonatal Treatment and Associated Morbidity

Four of the 236 infants with RDS died during the neonatal period in the years 1990 to 1995, but none of the 110 infants born in the years 1996 to 1999 died. All the infants without RDS survived. The underlying cause of death was verified to be RDS in 3 cases and NEC in 1 case.

The treatment requirements and neonatal morbidity in the groups with and without RDS are summarized in Table II. Oxygen supplementation and ventilation support lasted longer in the group with RDS than in the group without RDS in both periods, although the duration of supplemental oxygen decreased in the latter period ($P = .03$). About every third infant without RDS required respiratory support for a short period for different causes, such as transient tachypnea, apnea, or birth asphyxia. Infants with RDS had BPD 8 times more often than infants in the control group, although their proportion remained similar in both periods. The rate of surfactant treatment increased from 56% to 80% of infants with RDS. PDA and pneumothorax occurred 5 to 6 times more often in infants with RDS than in infants without RDS. Interstitial emphysema was not seen in any infant without RDS. The incidence of proven infection did not differ significantly in the groups during the former period (15% versus 9%; $P = .09$), but the rate of infection declined in the control group, probably because of prophylactic antibiotic

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<table>
<thead>
<tr>
<th>Table I. Current pregnancy and perinatal characteristics of infants with and without respiratory distress syndrome in the years 1990 to 1995 and 1996 to 1999</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Years 1990-1995</strong></td>
</tr>
<tr>
<td><strong>Infants</strong></td>
</tr>
<tr>
<td><strong>with RDS (n = 236)</strong></td>
</tr>
<tr>
<td><strong>Infants</strong></td>
</tr>
<tr>
<td><strong>with RDS (n = 110)</strong></td>
</tr>
<tr>
<td><strong>Mother</strong></td>
</tr>
<tr>
<td>Maternal age (years)</td>
</tr>
<tr>
<td>29.5 ± 6.1</td>
</tr>
<tr>
<td>Current asthma</td>
</tr>
<tr>
<td>9 (3.8)</td>
</tr>
<tr>
<td>Hypertensive disorders</td>
</tr>
<tr>
<td>59 (25.0)</td>
</tr>
<tr>
<td>PROM &gt;24 hours before delivery</td>
</tr>
<tr>
<td>29 (12.3)</td>
</tr>
<tr>
<td>Clinical chorioamnionitis</td>
</tr>
<tr>
<td>12 (5.1)</td>
</tr>
<tr>
<td>Antenatal steroids</td>
</tr>
<tr>
<td>33 (14.0)</td>
</tr>
<tr>
<td>Caesarean delivery</td>
</tr>
<tr>
<td>141 (79.7)</td>
</tr>
<tr>
<td>Infant</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
</tr>
<tr>
<td>32.7 ± 2.7</td>
</tr>
<tr>
<td>Birth weight (g)</td>
</tr>
<tr>
<td>1962 ± 688</td>
</tr>
<tr>
<td>Small for gestational age (&lt;2 SD)</td>
</tr>
<tr>
<td>49 (20.8)</td>
</tr>
<tr>
<td>Male/female</td>
</tr>
<tr>
<td>143/93</td>
</tr>
<tr>
<td>Singleton</td>
</tr>
<tr>
<td>175 (74.2)</td>
</tr>
<tr>
<td>Apgar score 1 minute</td>
</tr>
<tr>
<td>6.6 ± 2.1</td>
</tr>
<tr>
<td>Apgar score 5 minutes</td>
</tr>
<tr>
<td>7.3 ± 2.0</td>
</tr>
<tr>
<td>Siblings at home</td>
</tr>
<tr>
<td>143 (60.2)</td>
</tr>
</tbody>
</table>

Data are shown as mean (SD) or number of cases (%).
treatment after PPROM in the latter period (9% versus 2%; \( P = .03 \)). The frequencies of intraventricular hemorrhage, periventricular leukomalasia, retinopathy of prematurity, and NEC were low in the infants with and without RDS, and there were no detectable differences in the groups.

### Outcome Up To Two Years of Age

Three infants with RDS in the earlier period died because of BPD at 3, 5, and 9 months of age, whereas all the others survived. Follow-up data were available for 333 pairs of 339 surviving infants (98.2%) at 1 year and for 326 pairs (96.2%) at 2 years of corrected age.

The outcomes and treatments of children with and without RDS as old as 2 years of corrected age are presented in Table III. Altogether, 21% of the infants with RDS and 8% of the control subjects had recurrent wheezing illnesses during the early period (\( P < .005 \)). During the latter period, the incidence of wheezing illnesses decreased slightly from 21% to 19% in infants with RDS, whereas a small increase was evident in the control groups (from 8% to 13%). The difference between the groups was no longer significant (\( P = .27 \)). No detectable decrease was observed in the rates of wheezing during the second year of life.

### Risk Factors

Logistic regression analysis showed that siblings at home, male sex, RDS, and prolonged need for supplemental oxygen in the neonatal period were independently associated with an increased risk of wheezing illnesses as long as 2 years of age. Maternal history of asthma, clinical chorioamnionitis, or other factors analyzed had no influence on the occurrence of wheezing in this series of children (Table IV).

### DISCUSSION

This study showed that RDS was associated with recurrent wheezing illness and hospitalizations caused by respiratory problems during early infancy, and that these problems tended to decrease during the 10-year study period. Only infants born after 26 completed gestational weeks were included in this survey, because almost all infants younger than that age suffer from RDS, and so very few gestational

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**Table II. Morbidity and treatment requirements in infants with and without respiratory distress syndrome during initial hospitalization in the years 1990 to 1995 and 1996 to 1999**

<table>
<thead>
<tr>
<th></th>
<th>Years 1990-1995</th>
<th>OR or mean difference (95% CI)</th>
<th>Years 1996-1999</th>
<th>OR or mean difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants with RDS (n = 236)</td>
<td>Infants without RDS</td>
<td></td>
<td>Infants with RDS (n = 110)</td>
<td>Infants without RDS</td>
</tr>
<tr>
<td>Duration of FiO2 &gt;21%, days</td>
<td>7 (1-438)</td>
<td>0 (0-56)</td>
<td>-1.2 (-1.5--1.0)</td>
<td>5 (0-180)</td>
</tr>
<tr>
<td>Ventilatory support</td>
<td>231 (97.9)</td>
<td>82 (34.7)</td>
<td>86.8 (34.4-218.9)</td>
<td>108 (98.2)</td>
</tr>
<tr>
<td>Duration of ventilatory support</td>
<td>4.5 (0-87)</td>
<td>0 (0-25)</td>
<td>-1.8 (-1.9--1.6)</td>
<td>4 (0-46)</td>
</tr>
<tr>
<td>Systemic steroids</td>
<td>31 (13.1)</td>
<td>7 (3.0)</td>
<td>4.9 (2.1-11.5)</td>
<td>18 (16.4)</td>
</tr>
<tr>
<td>Patent ductus arteriosus</td>
<td>43 (18.2)</td>
<td>9 (3.8)</td>
<td>5.6 (2.7-11.8)</td>
<td>17 (15.5)</td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>20 (8.5)</td>
<td>4 (1.7)</td>
<td>5.4 (1.8-16.0)</td>
<td>6 (5.5)</td>
</tr>
<tr>
<td>Interstitial emphysema</td>
<td>18 (7.6)</td>
<td>0</td>
<td>19.3 (2.6-146.0)</td>
<td>4 (3.6)</td>
</tr>
<tr>
<td>BPD</td>
<td>21 (8.9)</td>
<td>3 (1.3)</td>
<td>7.7 (2.3-26.3)</td>
<td>8 (7.2)</td>
</tr>
<tr>
<td>Infection</td>
<td>35 (14.8)</td>
<td>22 (9.3)</td>
<td>1.7 (1.0-3.0)</td>
<td>10 (9.1)</td>
</tr>
<tr>
<td>Proven sepsis</td>
<td>8 (3.4)</td>
<td>2 (0.9)</td>
<td>4.1 (0.9-19.5)</td>
<td>4 (3.6)</td>
</tr>
<tr>
<td>Primary hospital stay, days</td>
<td>31.5 (1-266)</td>
<td>30.0 (0-110)</td>
<td>-0.1 (-0.3-0)</td>
<td>37.0 (7-112)</td>
</tr>
</tbody>
</table>

Median and range or number of cases (%).
Recurrent wheezing illness

<table>
<thead>
<tr>
<th></th>
<th>Years 1990-1995</th>
<th></th>
<th>Years 1996-1999</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infants with RDS</td>
<td>Infants without RDS</td>
<td>OR or mean difference (95% CI)</td>
<td>Infants with RDS</td>
</tr>
<tr>
<td>&lt;1 year, n (%)</td>
<td>47 (21.0)</td>
<td>18 (8.0)</td>
<td>3.0 (1.7-5.4)</td>
<td>21 (19.3)</td>
</tr>
<tr>
<td>From 1 to 2 year, n (%)</td>
<td>44 (20.0)</td>
<td>19 (8.6)</td>
<td>2.6 (1.5-4.7)</td>
<td>19 (17.9)</td>
</tr>
</tbody>
</table>

Continuous inhaled steroids

<table>
<thead>
<tr>
<th></th>
<th>Years 1990-1995</th>
<th></th>
<th>Years 1996-1999</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 year, n (%)</td>
<td>15 (6.7)</td>
<td>5 (2.2)</td>
<td>3.1 (1.1-8.8)</td>
<td>16 (14.6)</td>
</tr>
<tr>
<td>From 1 to 2 year, n (%)</td>
<td>18 (8.2)</td>
<td>10 (4.5)</td>
<td>1.9 (0.8-4.1)</td>
<td>16 (15.1)</td>
</tr>
</tbody>
</table>

Re-hospitalized

<table>
<thead>
<tr>
<th></th>
<th>Years 1990-1995</th>
<th></th>
<th>Years 1996-1999</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 year, at least once n (%)</td>
<td>57 (25.4)</td>
<td>30 (13.4)</td>
<td>2.2 (1.4-3.6)</td>
<td>31 (28.4)</td>
</tr>
<tr>
<td>Twice or more often</td>
<td>24 (10.7)</td>
<td>9 (4.0)</td>
<td>3.0 (1.4-6.7)</td>
<td>14 (12.8)</td>
</tr>
<tr>
<td>From 1 to 2 years, at least once, n (%)</td>
<td>23 (10.5)</td>
<td>17 (7.7)</td>
<td>1.4 (0.7-2.7)</td>
<td>15 (14.2)</td>
</tr>
<tr>
<td>Twice or more often</td>
<td>7 (3.2)</td>
<td>3 (1.4)</td>
<td>2.5 (0.6-9.8)</td>
<td>2 (1.9)</td>
</tr>
</tbody>
</table>

Duration of re-hospitalization

<table>
<thead>
<tr>
<th></th>
<th>Years 1990-1995</th>
<th></th>
<th>Years 1996-1999</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 year/patient, days</td>
<td>2.8</td>
<td>1.1</td>
<td>2.6 (2.1-3.4)</td>
<td>1.8</td>
</tr>
<tr>
<td>From 1 to 2 years/patient, days</td>
<td>0.6</td>
<td>0.2</td>
<td>2.7 (1.9-4.0)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table IV. Adjusted odds ratios and 95% CI of significant risk factors for recurrent wheezing in the first 2 years of life

<table>
<thead>
<tr>
<th>Variables</th>
<th>All infants</th>
<th>Infants with BPD excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Siblings at home</td>
<td>2.5 (1.5-4.3)</td>
<td>.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>2.5 (1.5-4.3)</td>
<td>.001</td>
</tr>
<tr>
<td>RDS</td>
<td>1.8 (1.1-3.0)</td>
<td>.02</td>
</tr>
<tr>
<td>BPD</td>
<td>6.0 (2.6-13.6)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Other variables included in the model: maternal asthma, chorioamnionitis, antenatal steroid (full 2 doses/no), Caesarean versus vaginal delivery, gestational age (<32 weeks versus ≥32 weeks).

control subjects would have been available. The reference group made it possible to look for complications associated with RDS other than those expected on the basis of prematurity per se. To eliminate confounding variables further, we excluded infants with blood culture-positive infections within the first week after birth, those with malformations, and some other minor groups (see Methods section). In addition to infection, another issue concerns early treatment with exogenous surfactant. During this survey, surfactant therapy was used only with established RDS in our unit; that is, surfactant was given only to oxygen-treated infants with typical changes on chest radiography. Synthetic surfactant became available in the early 1990s, and natural surfactant became the treatment of choice during the second half of the decade, and timing tended to become earlier. We have not explored the exact timing, however. Synchronized mechanical ventilation with lower pressures and higher frequencies and high-frequency ventilation, nasal continuous positive pressure ventilation, and inhaled nitric oxide became more common toward the late 1990s. Presumably by decreasing neonatal lung injury, these new treatment modalities lower subsequent respiratory morbidity.6,17 Although the population characteristics remained stable during the study period, the factors causing improvement in respiratory outcome cannot be identified with certainty. The use of antenatal steroid therapy increased in the late 1990s, but it had no effect on wheezing illness according to logistic regression analysis.

Most pulmonary follow-up studies have concentrated on infants with BPD. Some of the variation in the incidence of BPD may be caused by the differences in population and diagnostic criteria.20-22 When considering lung damage in infants in whom BPD develops compared with that in infants who do not, one should keep in mind that BPD is a continuum rather than a dichotomous disease. Infants in whom severe BPD develops represent the worst end of the spectrum. Histological examination of infants who died of BPD has revealed a change in the pattern of lung damage in the surfactant era. Less striking inflammation and fibrosis in the Airways and the airway sacculles with poorly developed alveoli are currently seen, compared with the pre-surfactant era. This may be because of lower oxygen requirements and gentler ventilation strategies.23,24 The common concept is that...
inflammation interferes with lung development, resulting in an arrest of acinar development. Chorioamnionitis did not associate with wheezing illness in this study, however. There is evidence that RDS even at an early stage is associated with significant generation of oxygen radicals and an inflammatory response of the airways and lung parenchyma. Animal studies have shown that even a few deep breaths after birth cause lung injury in a surfactant-deficient lung. Narrow and dysplastic airways may be more easily obstructed when infected, and this may be a major cause of wheezing illness. Thus, it was an expected finding that the incidence of wheezing illnesses and the rate of hospitalization was more common among the infants with RDS as a group compared with the control subjects.

In a study of infants born at <33 gestational weeks, the rate of wheezing illness (defined as ≥2 episodes of wheezing) was 24% during the first year of life, whereas in another study among very low birth weight infants, it was 36% to 40% (defined as ≥1 or more episodes of wheezing) during the first 2 years of life. In a recent study among infants born at <33 gestational weeks, the overall re-hospitalization rate was 49% for the infants with BPD and 23% for the rest of the cohort during the first year of life, and the most common causes for re-hospitalization were respiratory illnesses. The availability of a temporally defined control group made it possible for us to control for possible seasonal variations or respiratory syncytial virus epidemics, which could increase respiratory morbidity, or possible changes in the prevention and management of wheezing illness during the years. The antibody prophylaxis against RSV infection in small pre-term infants started first in October 1999. Therefore, only a few infants in this study received RSV prophylaxis. These results showed that the differences in pulmonary morbidity between the infants with and without RDS became smaller toward the end of the study, suggesting that the changes in treatment practices led to a decrease of RDS-associated obstructive lung disease.

The prevalence of wheezing lower respiratory tract illnesses and the high rates of hospitalization reportedly decrease after the first few years of life, whereas subnormal lung function and increased airway reactivity may persist until adolescence. In this series, the prevalence of wheezing illnesses remained similar in the first and second years, but hospital admissions decreased during the second year.

In addition to RDS, the only risk factors for wheezing illnesses found in this series were siblings at home, male sex, and BPD. The increased risk of male infants is consistent with the increased risk of RDS and the possible delay in the differentiation of the pulmonary defense systems in male compared with female infants. Neonatal RDS-associated complications, such as patent ductus arteriosus or pneumothorax, had no detectable influence per se on the risk for later wheezing. When infants with BPD were excluded, RDS remained a significant risk factor for wheezing. Maternal asthma did not associate with an increased risk for wheezing. The risk factors for wheezing illnesses in this series differed somewhat from those identified in older children and in the general population, but are in line with most studies involving similar populations of infants born pre-term. We propose that adverse neonatal events leading to airway damage are prominent in this early age group. Later in life, atopy and some exogenous factors become more predominant risk factors for wheezing illnesses. We propose that lung damage in RDS predisposes infants to obstructive airway disease in early childhood. Further prevention of lung immaturity and development of less-invasive treatment strategies for RDS remain the challenges for the future.

REFERENCES


NEWBORN SCREENING FOR CONGENITAL ADRENAL HYPERPLASIA
HAS REDUCED SENSITIVITY IN GIRLS
TODD S. VARNES, MD, MPH, DAVID B. ALLEN, MD, AND GARY L. HOFFMAN, BS

Objectives To characterize Wisconsin-born infants with 21-hydroxylase deficiency-congenital adrenal hyperplasia (21-OH-D-CAH) who were not identified by the newborn screening for 21-OH-D-CAH, and to examine male and female screening 17-hydroxyprogesterone (17-OHP) levels.

Study design Information on infants with false-negative results was gathered. Results of the Wisconsin newborn screening for 21-OH-D-CAH from January 1, 2000, to June 30, 2003, were analyzed to detect possible differences between male (n = 119,842) and female (n = 114,951) infants.

Results Six of 7 female infants with false-negative results had genital masculinization, and 4 of 8 infants with false-negative results had laboratory evidence of salt-wasting. None died, had a salt-wasting crisis, or was assigned the wrong sex. A significant difference in the mean 17-OHP levels between male (17.5 ng/mL) and female (15.4 ng/mL) infants (P < 0.0001) was detected. The sensitivity of newborn screening for female infants was 60%, compared with 80% for male infants.

Conclusions Male and female infants have significantly different mean 17-OHP levels on newborn screening, and female infants comprise most of the infants with false-negative results. Although health professionals should not assume that newborn screening for 21-OH-D-CAH is a means of identifying all affected infants, the primary goals of newborn screening for CAH (prevention of salt-wasting crises and sex misassignment) are fulfilled. (J Pediatr 2005;147:493-8)

Congenital adrenal hyperplasia (CAH) is a family of autosomal recessive disorders characterized by a deficiency in 1 of the enzymes necessary for the synthesis of cortisol. The most prevalent form of the disorder is 21-hydroxylase deficiency-congenital adrenal hyperplasia (21-OH-D-CAH), which accounts for >90% of the cases1. Since the advent of a reliable laboratory assays for 21-OH-D-CAH,2 routine newborn screening has been widely implemented to facilitate early detection and treatment of 21-OH-D-CAH and prevention of life-threatening salt-wasting crises.3-12 There has also been a decrease in the number of virilized female infants who were initially identified as males.3-12 Various strategies have been devised to decrease the economic and psychological impact of false-positive test results.13-18 In particular, adjustment of 17-OHP cutoff values on the basis of gestational age or birthweight significantly decreases the rates of false-positive and false-negative results of the newborn screening.

In March 1993, the Wisconsin newborn screening program added screening for 21-OH-D-CAH. In October 1993, the program began to use weight-adjusted criteria for 17-OHP levels in screening for 21-OH-D-CAH to decrease false-positive results in low birth weight infants. Since that time, 21-OH-D-CAH has been diagnosed in 8 infants (all full term, 7 female) born in Wisconsin, despite their having 17-OHP results below the threshold on the newborn screening for 21-OH-D-CAH (“false negatives”). This sex discrepancy in false-negative test results raised the question of whether sex differences existed in newborn 17-OHP levels and, if so, whether sex-adjusted thresholds would further optimize the efficiency of screening for CAH.

METHODS

In Wisconsin, newborn screening for CAH is mandated. Whole blood samples are collected on Schleicher and Schuell 903 filter paper, dried, and assayed for 17-OHP with a

| CAH | Congenital adrenal hyperplasia |
| PPV | Positive predictive value |
| 21-OH-D-CAH | 21-hydroxylase deficiency-congenital adrenal hyperplasia |
| 17-OHP | 17-hydroxyprogesterone |
time-resolved fluoroimmunoassay (DELFIA; PerkinElmer Life and Analytical Sciences). The results of the CAH screening are interpreted according to the birthweight of the infant (Table I). A pediatric endocrinologist then provides confirmatory examination and testing of all at-risk infants to provide the final diagnosis of CAH.

Eight infants not identified with the newborn screening subsequently received a diagnosis of 21-OH-D-CAH. Medical records of these patients were reviewed to obtain the following information: 17-OHP newborn screening level (ng/mL), 17-OHP level at diagnosis (ng/mL), age at diagnosis, birthweight, sex, genital abnormality, sex misassignment (yes/no), sodium level (mmol/L), potassium level (mmol/L), and renin activity (ng/mL/hour). Evidence of salt-wasting was determined by means of an examination of the sodium, potassium, and renin activity. This information was recorded in a non-identifiable manner to protect the confidentiality of the patients and their families.

Additionally, results of the Wisconsin newborn screening for 21-OH-D-CAH were obtained for 239,879 infants consecutively born in Wisconsin from January 1, 2000, through June 30, 2003. These data included the frequency of true-positive, false-positive, true-negative, and false-negative test results by using 17-OHP for each sex.

The mean, SD, and median 17-OHP concentration were calculated (with statistical software Stata, version 6.0, Stata Corp, College Station, Texas) for each sex, and the means were compared with a 2-sample t test with unequal variance. The sensitivity, specificity, positive-predictive value (PPV), and negative-predictive value of the screening were calculated for each sex, as was the prevalence and natural frequency of CAH for each sex. The definition of positive screening test results can be found in Table I. This project was reviewed and approved by the University of Wisconsin Health Sciences Institutional Review Board.

RESULTS

Review of False-Negative Results

All 8 infants were full term and weighed >2200 grams (Table II). The newborn screening 17-OHP levels of the infants ranged from 8 to 55 ng/mL, and only 3 infants had a

<table>
<thead>
<tr>
<th>Birth weight</th>
<th>17-OHP result (ng/mL)</th>
<th>Action</th>
</tr>
</thead>
</table>
| ≤1299g       | ≥190                  | 1. Reexamine genitalia. Verify sex if ambiguous.  
2. Repeat 17-OHP test at 1 month of age.  
3. If repeat test ≥190, obtain urgent endocrine consult.  
4. If repeat test <190, follow-up PRN. |
| 1300-1699g   | 115-134               | 1. Reexamine genitalia. Verify sex if ambiguous.  
2. Repeat 17-OHP test at 1 month or at discharge.  
3. If repeat test ≥previous result, obtain urgent endocrine consult.  
4. If repeat test <previous result, follow-up PRN. |
| 1300-1699g   | ≥135                  | 1. Repeat immediately. Overnight delivery to WSLH.  
2. If repeat test ≥135, obtain urgent endocrine consult.  
3. If repeat test <135, follow-up PRN. |
| 1700-2199g   | 80-99                 | 1. Reexamine genitalia. Verify sex if ambiguous.  
2. Repeat 17-OHP test at 1 month or at discharge.  
3. If repeat test ≥previous result, obtain urgent endocrine consult.  
4. If repeat test <previous result, follow up PRN. |
| 1700-2199g   | ≥100                  | 1. Repeat immediately. Overnight delivery to WSLH.  
2. If repeat test ≥previous result, obtain urgent endocrine consult.  
3. If repeat test <previous result, follow-up PRN. |
| ≥2200g       | 55-89                 | 1. Reexamine genitalia. Verify sex if ambiguous.  
2. Repeat 17-OHP test in approximately 2 weeks.  
3. If repeat test ≥previous result, obtain urgent endocrine consult.  
4. If repeat test <previous result, repeat biweekly until <55 ng/mL. |
| ≥2200g       | ≥90                   | 1. Repeat immediately. Overnight delivery to WSLH.  
2. If repeat test ≥previous result, obtain urgent endocrine consult.  
3. If repeat test <previous result, repeat biweekly until <55 ng/mL. |

WSLH = Wisconsin State Laboratory of Hygiene.
17-OHP level >45 ng/mL (approximately 3 SD from patient mean). Full CAH laboratory panels were obtained to confirm the diagnosis of CAH for each patient, and this panel included a 17-OHP level with a ng/dL assay. For the sake of comparison, Table II includes these diagnostic laboratory results as ng/mL.

Seven of the 8 infants with false-negative results were female. Of the 7 female infants with false-negative results, 6 had an identifiable genital abnormality as determined by a pediatric endocrinologist. Four of the 8 infants had laboratory evidence of salt-wasting. None of the infants died, none was treated for a salt-wasting crisis, and none of the infants was assigned the wrong sex. The age at diagnosis ranged from birth to 54 months, with 5 infants identified by 3 months of age, and the other 3 identified after 12 months.

Individual Clinical Presentations

Five of the 7 female infants (#1–#5) were referred to an endocrinology clinic for evaluation of a genital abnormality. The 1 male infant (#6) was evaluated at the age of 54 months for precocious adenarche. Two of the infants were evaluated quite serendipitously. One infant (#7) had a repeat newborn screen at 1 week of life for evaluation of jaundice that revealed an elevated 17-OHP level, whereas the other infant (#8) had a repeat newborn screen for an abnormal immunoreactive trypsinogen level that revealed an elevated 17-OHP level.

Sex Differences in 17-OHP Levels

The mean 17-OHP concentration for male infants is 17.5 ng/mL, whereas the mean concentration for female infants is 15.4 ng/mL (P <.0001; Table III). The mean 17-OHP level for the infants (n = 5086, 2.1%) with unrecorded sex did not differ from the mean for all the infants combined. Furthermore, when the 17-OHP results for infants with unrecorded sex were included in the mean for either male or female infants, there was no statistical difference between the means calculated with and without these additional 17-OHP results added (results not shown). Consequently, these infants were excluded from analysis. Additionally, the possible effect of outliers on the difference between the sexes was evaluated by comparing the mean 17-OHP values for only the infants with a 17-OHP level <55 ng/mL (the current threshold level) and again for infants with a 17-OHP level <100 ng/mL (higher than this level is predictive of salt-wasting CAH). When these outliers were excluded, the difference between the sexes persisted at the same level and the same statistical significance (results not shown). Finally, an analysis that included only the infants who weighed >2200 g yielded the same difference between the sexes and the same statistical significance (results not shown).

Test Characteristics for Each Sex

The results of the analysis of the sensitivity, specificity, PPV, negative-predictive value, prevalence, and natural frequency of the newborn screening for 21-OH-D-CAH in Wisconsin from January 1, 2000, until June 6, 2003, are found for each sex in Table IV. The sensitivity rate of the newborn screening for females was 60%, the sensitivity rate for males was 83%, and the PPV for all infants was 1%.

DISCUSSION

In the past decade, numerous programs have demonstrated that newborn screening enables improved and timely detection and treatment of cases of 21-OH-D-CAH, prevents life-threatening salt-wasting crises, and decreases the number of virilized female infants initially misidentified as males.3–12 The sensitivity of the newborn screening test has ranged from 83% to 100%.5,5,7,10,11 The sensitivity depends on the ability to identify and document infants with false-negative test results, which requires sufficient time for presentation of milder cases. Our study documents an overall sensitivity of the newborn screening test of 73%, but a sensitivity of only 60% in female infants. The sensitivity of newborn screening was higher (83%) in male infants, which is important because male infants are at higher risk of undiagnosed 21-OH-D-CAH and a subsequent salt-wasting crisis. Because several of the infants with false-negative results in this study were identified serendipitously, it is possible that additional unrecognized false-negative results exist, and the sensitivity of newborn screening programs could be even lower than reported.

Other studies have also documented infants with CAH who were not identified with the newborn screening for 21-OH-D-CAH.5,7,11,19,20 A comprehensive analysis of the Swedish newborn screening program revealed 7 infants (4 girls and 3 boys) with false-negative results,8 whereas analyses of the newborn screening programs in New Zealand and the Netherlands report no infants with false-negative results.4,10 All infants with false-negative results had the simple virilizing form of the disorder. Although laboratory evidence of mineralocorticoid deficiency is reported in some missed infants, to our knowledge, there have been no reported infants with false-negative results who had a salt-wasting crisis. Although no individual study revealed a significant difference in the number of girls and boys missed by the screening, pooled information from the aforementioned studies yields 12 girls with false-negative results and 4 boys with false-negative results. All of the 8 infants described here except 1 are female, 6 of 7 female infants had identifiable genital abnormalities, and although none of the infants had a salt-wasting crisis, laboratory evidence of compensated salt-wasting was present in 4 of the 8 infants.

The PPV of the Wisconsin newborn screening program was only 1% for the period of the study. Other analyses have reported PPVs of 1.9%, 2.3%, 16%, and even 50%.4,5,10,11 Pang reported PPVs of 4% in North America, 2% in Europe, and 1.3% in Japan.12 This discrepancy in PPVs is caused primarily by the varying prevalence of CAH and the number of false-positive results the screening program will accept. The PPV of 1% in Wisconsin is primarily caused by the low prevalence of disease during the time of this study, 1.21,345,
which is lower than other states and lower than in past years in Wisconsin.\textsuperscript{13}

A key finding of this study is that female infants have an average 17-OHP concentration that is 2 ng/mL lower than male infants; a report noted a difference between male and female infants in the 17-OHP concentration.\textsuperscript{21} Although sex-specific differences in neonatal adrenal or, more likely, gonadal function at birth might explain higher levels of 17-OHP in male infants, we have not found any published evidence to support this assertion.

What accounts for the marked sex difference in the infants with false-negative results in our study? One possible explanation is that boys who are missed by the newborn screening are not identified as having false-negative results because they have died from a salt-wasting crisis. However, in the 12 years since screening began in Wisconsin, neither the pediatric endocrinologists in the state nor the newborn screening program has learned of any child who has had a salt-wasting crisis. Furthermore, the infants with false-negative results in this study and all previous studies have the simple virilizing form of the disorder, suggesting that the newborn screen successfully identifies children with more severe forms of the disorder.

Another possible explanation of the sex discrepancy is that girls are more likely to be missed more often by the newborn screening because they have an average 17-OHP concentration that is 2 ng/mL lower than male infants. These findings raise the question as to whether the threshold levels for a positive newborn screening result for 21-OH-D-CAH should be adjusted according to the sex of the infant. To answer this question, a female 17-OHP threshold was modeled by using the female mean plus 4.5 SDs (the same number of deviations used to calculate the current threshold), using only the 17-OHP results for female infants weighing >2200 g for the data range from January 2000 to June 2003. The SD for female babies weighing >2200 g is 6.3, and therefore the threshold would be $15.4 \pm 4.5(6.3) = 44$. The effect of applying this hypothetical 17-OHP threshold level showed that only 3 of the 7 female infants with false-negative results would have been reported as positive. Further, there would have been approximately a 60% increase in false-positive results reported. Therefore, efforts to increase the sensitivity of the test by lowering the threshold level for abnormal 17-OHP levels for female infants would yield only marginal gains in sensitivity, with a substantial increase in the number of false-positive test results, which would increase the economic and psychological costs of evaluating infants with false-negative results.

Table II. Summary of infants not initially identified with the Wisconsin newborn screening for CAH (“false negatives”)

<table>
<thead>
<tr>
<th>Infant</th>
<th>17-OHP screening level (ng/mL)</th>
<th>17-OHP level at diagnosis (ng/mL)</th>
<th>Age at diagnosis</th>
<th>Sex (M/F)</th>
<th>Genital abnormality*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>200</td>
<td>12 months</td>
<td>F</td>
<td>Clitoromegaly</td>
</tr>
<tr>
<td>2</td>
<td>55, repeat 46</td>
<td>209</td>
<td>6 weeks</td>
<td>F</td>
<td>Clitoromegaly, posterior vaginal fusion</td>
</tr>
<tr>
<td>3</td>
<td>47</td>
<td>44</td>
<td>Birth</td>
<td>F</td>
<td>Clitoromegaly, full vaginal fusion</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>22</td>
<td>14 months</td>
<td>F</td>
<td>Clitoromegaly, posterior vaginal fusion</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>42</td>
<td>3 months</td>
<td>F</td>
<td>Clitoromegaly, posterior vaginal fusion</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>78</td>
<td>54 months</td>
<td>M</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>43</td>
<td>130</td>
<td>2 weeks</td>
<td>F</td>
<td>Clitoromegaly, full vaginal fusion</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>115</td>
<td>3 months</td>
<td>F</td>
<td>None</td>
</tr>
</tbody>
</table>

*Clitoromegaly is defined as a clitoris $>1.5$ cm.
†Elevated renin level, hyponatremia and hyperkalemia, or both.

A final explanation of the sex discrepancy is that girls are more likely to be missed more often by the newborn screening because they have an average 17-OHP concentration that is 2 ng/mL lower than male infants. These findings raise the question as to whether the threshold levels for a positive newborn screening result for 21-OH-D-CAH should be adjusted according to the sex of the infant. To answer this question, a female 17-OHP threshold was modeled by using the female mean plus 4.5 SDs (the same number of deviations used to calculate the current threshold), using only the 17-OHP results for female infants weighing $>2200$ g for the data range from January 2000 to June 2003. The SD for female babies weighing $>2200$ g is 6.3, and therefore the threshold would be $15.4 \pm 4.5(6.3) = 44$. The effect of applying this hypothetical 17-OHP threshold level showed that only 3 of the 7 female infants with false-negative results would have been reported as positive. Further, there would have been approximately a 60% increase in false-positive results reported. Therefore, efforts to increase the sensitivity of the test by lowering the threshold level for abnormal 17-OHP levels for female infants would yield only marginal gains in sensitivity, with a substantial increase in the number of false-positive test results, which would increase the economic and psychological costs of evaluating infants with false-negative results.

To increase detection of infants with false-negative results, a second screening test at approximately 2 weeks of age has been used in Texas.\textsuperscript{7} However, a cost analysis of the Texas approach found that a single screening was an effective means of detecting infants with the salt-wasting form of CAH, whereas the second screening was less cost-effective and detected primarily infants with simple virilizing CAH.\textsuperscript{22} Even second screening will not detect 100% of the CAH cases,
because 1 infant born in Texas screened negative twice, yet screened positive in Wisconsin several weeks later.

Tandem mass spectrometry and CYP21 genotype analysis can be used as second-tier tests to improve the PPV of the test by reducing the number of false-positive test results. However, these types of second-tier tests are only performed on newborns who screen positive in the primary test. Therefore, unless these techniques are implemented as a primary screening tool or the threshold level for a positive screening result is lowered, they do not have the potential to improve the sensitivity of the test.

The goals of screening neonates for 21-OH-D-CAH in Wisconsin are to prevent a life-threatening salt-wasting crisis in infants with CAH and to avoid male assignment to female infants with CAH. In spite of occasional missed cases, these goals are being met with the use of a single screening test and current thresholds. None of the infants with false-negative results died, had a salt-wasting crisis, or was initially assigned the wrong sex. Therefore, lowering the threshold for positive test results for newborn screening for 21-OH-D-CAH to ascertain a greater number of affected infants is not considered to be a cost-effective strategy.
THE PHENOTYPE OF SHORT STATURE HOMEBOX GENE (SHOX) DEFICIENCY IN CHILDHOOD: CONTRASTING CHILDREN WITH LERI-WEILL DYSCHONDROSTEOSIS AND TURNER SYNDROME

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Objective To evaluate the growth disorder and phenotype in prepubertal children with Leri-Weill dyschondrosteosis (LWD), a dominantly inherited skeletal dysplasia, and to compare the findings from girls with Turner syndrome (TS).

Study design We studied the auxologic and phenotypic characteristics in 34 prepubertal LWD subjects (ages 1 to 10 years; 20 girls, 14 boys) with confirmed short stature homeobox-containing gene (SHOX) abnormalities. For comparative purposes, we evaluated similar physical and growth parameters in 76 girls with TS (ages 1 to 19 years) and 24 girls with LWD (ages 1 to 15 years) by using data collected from the postmarketing observational study, GeNeSIS.

Results In the clinic sample LWD subjects, height standard deviation score ranged from $-2.5$ to $+0.1$ ($-2.2 \pm 1.3$, girls and $-1.8 \pm 0.6$, boys). Wrist changes related to Madelung deformity were present in 18 of 34 (53%) LWD subjects. In comparing the LWD and TS populations in the GeNeSIS sample, Madelung deformity, increased carrying angle, and scoliosis were more prevalent in the LWD population, whereas high arched palate was similarly prevalent in the two populations.

Conclusions Short stature is common in both LWD (girls and boys) and TS (girls). Clinical clues to the diagnosis of SHOX haploinsufficiency in childhood include short stature, short limbs, wrist changes, and tibial bowing. (J Pediatr 2005;147:499-507)

Leri-Weill dyschondrosteosis (LWD; MIM 127300) is a dominantly inherited skeletal dysplasia first described in 1929.\(^1\) LWD affects both sexes and is characterized by short stature, mesomelia, and Madelung wrist deformity.\(^2\) Typically, the phenotype is more severe in females than males, perhaps due to sex differences in estrogen levels.\(^3\) Pubertal development and fertility are generally normal in both sexes with LWD. The molecular basis for LWD\(^4-6\) is haploinsufficiency for the gene \textit{SHOX} (short stature homeobox-containing gene), which is located on the distal part of the X chromosome pseudoautosomal region (PAR1). SHOX deletions or mutations have been detected in 60% to 100% of LWD cases.\(^5,7\) By inference, \textit{SHOX} deficiency is also the major cause of the X chromosome short stature that is nearly universal in 45,X Turner syndrome (TS, monosomy X).\(^4,8\) SHOX is expressed from both sex chromosomes in males and females and is thought to play a role in bone growth and development through regulation of chondrocyte development.\(^9\)

This study involves two databases: one from a single clinic and the other from a large observational study. We studied 34 young children with LWD to characterize the LWD phenotype before pubertal development in this population. We investigated which dyschondrosteosis features are present in the very young that would aid in early diagnosis of LWD as well as the influence of age and sex on these features. In addition, our study included precise molecular description of the SHOX abnormalities. We compared the phenotype of females with LWD versus females with TS (mosomy X, isochromosome X, with or without mosaicism [85%]) to understand the contribution of \textit{SHOX}.

See editorial, p 422.
haploinsufficiency to long bone growth and development in females. The comprehensive evaluation of these very young LWD subjects sheds light on the early role of SHOX in normal bone growth and development.

**METHODS**

**SHOX** deletions were detected either by fluorescence in situ hybridization (FISH), as previously described, or by loss of heterozygosity of a polymorphic microsatellite marker (SHOX-CA) tightly linked to the SHOX locus. SHOX point mutations were detected by a commercial assay that uses denaturing high performance liquid chromatography to screen for heterozygosity (SHOX-DNA-Dx, Esoterix Endocrinology, Calabasas Hills, CA). Mutations were characterized by direct sequencing of genomic PCR products.

Karyotypes were obtained from most families, no karyotype results were available from three families. For the clinical population, cases with an abnormal karyotype were defined as LWD if the karyotype showed nonmosaic deletion of the X chromosome extending no further proximal than band p22.3.

**Subjects**

**CLINICAL LWD POPULATION**. Clinic subjects were generally referred for short stature or the diagnosis of LWD in a parent. In the clinic sample, only LWD subjects with confirmed SHOX abnormalities were included in this study. Results from 13 of 34 patients in this cohort were reported previously. The study was approved by the Human Studies Committees at Thomas Jefferson University and University of Texas Southwestern Medical School, and informed consent/assent was obtained in all cases. None of the LWD patients had received growth hormone treatment.

Clinical assessment included measurements of height, lower segment, arm span, and forearm length. Arm span was measured as the distance from right to left third fingertips, with patients facing the wall, with outstretched arms held parallel to the ground. Circumferences were measured at the upper arm and forearm and at the upper and lower legs at the widest point. Age- and sex-specific height standard deviation score (SDS) were calculated from normative data of the National Center for Health Statistics (NCHS). SDS for ratio of upper to lower segment were calculated from published norms. Karyotypes were obtained from most families (some data not shown). No karyotype results were available from three families. For the clinical population, cases with an abnormal karyotype were defined as LWD if the karyotype showed nonmosaic deletion of the X chromosome extending no further proximal than band p22.3.

**GENESIS POPULATION**. Pretreatment or non-GH treatment data are reported from female subjects with LWD and TS from GeNeSIS (the Genetics and Neuroendocrinology of Short Stature International Study) an open-label, multicenter, multinational, observational study (Eli Lilly and Company). Heights and auxologic measurement from girls with TS and girls with LWD entered in GeNeSIS were compared. Height SDS and weight SDS were calculated by using the year 2000 NCHS standards. Target height SDS was based on the same standards using height for sex at age 18. Birth weight SDS was calculated on the basis of neonatal data from Usher et al. Anthropometric SDS (eg, arm span SDS, upper arm length SDS) were calculated from published norms. Karyotypes of girls with TS were 45,X in 42% and mosaic or isochromosome X (43%) or other (15%) in the remainder. The presence of Madelung deformity was assessed clinically and/or radiographically. Tibial bowing was assessed clinically. Because of the method of data collection, only the presence of phenotypic features in the GeNeSIS population can be clearly determined, since absence of a positive response could mean that the feature was absent, the respondent did not know whether the feature was present, or simply did not respond. Similarly, not all patients underwent radiographic examination, so some results represent only a subset of the patients.

**Statistics**

Continuous variables were compared between males and females in the clinic sample and between LWD and TS patients in the GeNeSIS sample, using unpaired t tests. Dichotomous variables were compared by Fisher exact test. Pearson correlations were calculated to examine the relation between the variables and age. Results with a 2-sided P value of <.05 were considered statistically significant.

**RESULTS**

**Molecular Analysis of SHOX Deletions and Mutations in the Clinical LWD Population**

SHOX abnormalities present in the clinical population of subjects with LWD are listed in Table I. Complete gene deletion was detected by fluorescence in situ hybridization (FISH, Figure 1) in 29 subjects from 26 unrelated families. Eleven patients had karyotypic abnormalities that were the basis for ascertainment for this study. One subject (770) had a partial deletion of the SHOX locus, as evidenced by apparent inheritance from the mother of a null allele of a microsatellite marker (SHOX-CA) tightly linked to the SHOX locus. This putative deletion was not detectable by FISH with a SHOX cosmid probe.

Heterozygous SHOX point mutations were detected in four families (Figure 2). Two mutations (R195X, L154P) were previously described. A novel missense mutation (CGA to CCA) in exon 3 was identified in one family. This mutation changes arginine 147 to proline, altering a conserved amino acid sequence within the homeodomain. This mutation...
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<th>Bone age</th>
<th>Karyotype</th>
<th>SHOX mutation</th>
<th>Madeulung (by X-ray or clinically)</th>
<th>HT SDS</th>
<th>Palate:</th>
<th>Scoliosis:</th>
<th>Carrying angle:</th>
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*TY=too young.

**Deletion not confirmed by FISH, see text for details.

***Unbalanced X: autosome translocation identified by FISH; conventional karyotype not done.

The Phenotype Of Short Stature Homeobox Gene (SHOX) Deficiency In Childhood: Contrasting Children With Léri-Weill Dyschondrosteosis And Turner Syndrome
is predicted by the Polyphen program \(^2\) to be deleterious to protein function. Finally, a novel T to C substitution in exon 6b was identified in one proband and her cousin. This mutation converts the TGA stop codon into an arginine codon (CGA) (Figure 2, X226R), resulting in the addition of 22 residues to the C-terminus of the protein. The same mutation has been identified in 21 probands with idiopathic short stature; in one case the mother, who was also short, was tested and found to carry the same mutation (K. H., unpublished data). None of the \textit{SHOX} point mutations were found in more than 100 control chromosomes.

\textbf{Leri-Weill (LWD) Clinical Population}

We evaluated 20 girls and 14 boys, age range 1 to 10 years (mean [±SD] ages: 5.7 ± 2.6 and 6.3 ± 2.7, respectively, Table I). There were three same-sex sib pairs (241 and 242, 370 and 371, 446 and 448); one pair of subjects (328, male and 629, female) had the same father and different mothers, and two subjects (552, female and 999, male) were first cousins. Twenty-six subjects were white, seven were Hispanic, and one was a native American. All subjects were prepubertal: None had evidence of breast or pubic hair development. All males had prepubertal testicular volumes (<4 mL).

\textbf{Height, Weight, Body Mass Index (BMI), Auxologic Measurements From Clinical LWD Population}

Age- and sex-specific height SDS for the LWD subjects ranged from −5.5 to +0.1 SD (Table I and Table II). Mean (±SD) height SDS was low in both sex groups (−2.3 ± 1.3 [female] and −1.8 ± 0.6 [male], \(P = .18\)), suggesting manifestation of short stature early in childhood in both sexes. The results did not demonstrate any effects of age or sex on height SDS. Eleven of 20 girls (55%) and 5 of 14 boys (36%) had heights below the normal range (<−2 SDS), but this difference was not statistically significant (\(P = .30\), Fisher exact test). There was no significant correlation between height SDS and chronological age (\(r = −0.26\) and \(P = .26\) for females; \(r = −0.39\) and \(P = .17\) for males; \(r = −0.24\) and \(P = .17\) for the group as a whole, Figure 3).

Mean weight SDS values for females and males were −0.9 ± 1.2 and −0.7 ± 1.2, respectively (\(P = .53\)). Mean BMI SDS values for females and males were 1.4 ± 1.7 and 1.1 ± 1.8, respectively (\(P = .63\)), suggesting increased BMI relative to the normal population for age in both sexes. Mean SDS values for the ratio of upper segment to lower segment were 2.4 ± 4.3 and 3.3 ± 2.4 (\(P = .53\)), for females and males, respectively, indicating shortened lower limbs early in childhood. Arm span SDS values were low in females and males (−3.2 ± 1.2 and −2.3 ± 1.0, respectively, \(P = .04\), values not calculated for children <3 years), suggesting early development of mesomelia (disproportionate growth failure) of the arms, more severe in the girls than in boys. Mean arm span minus height values were −5.2 ± 2.7 and −5.0 ± 3.3 cm for females and males, respectively (\(P = .90\)). In normally-growing children, span is slightly is less than height (1 to 2 cm) in early childhood, and by age 10 in males and 12 in females, span equals height.\(^1\) That arm span is substantially shorter than height in these patients suggests disproportionate limb growth.

Mean bone ages were similar between females and males (5.4 ± 2.6 and 5.5 ± 1.8 years, respectively). The difference between bone age and chronological age was −0.3 ± 0.7 (females) and −0.9 ± 1.5 years (males), indicating no significant bone age advancement or delay in females and minimal bone age delay in males.

\textbf{GeNeSIS Results for Girls With LWD Versus TS}

Data were available from the GeNeSIS database for 24 untreated females with LWD and 76 untreated females with

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**Figure 1.** Partial metaphase from subject SW629 shows deletion of \textit{SHOX} gene on one X chromosome. \textit{SHOX}, cosmid probe (see Methods). Xcen, control probe for X chromosome centromeric repeat DXZ1. Available in color online at www.jpeds.com.

**Figure 2.** \textit{SHOX} point mutations identified in this study. The structure of the \textit{SHOX} gene is depicted with the coding region shaded. The homeodomain (residues 117-176) is shown in black. The OAR domain (residues 279-292) is stippled. Mutations are designated by amino acid number and standard single letter amino acid code.
Table II. Auxological measurements (Mean ± SD) in children with Léri-Weill dyschondrosteosis (clinic sample)

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<td>LWD</td>
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<td>N</td>
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<td>Weight SDS</td>
<td>−0.9 ± 1.2</td>
<td>−0.7 ± 1.2</td>
<td>.53</td>
</tr>
<tr>
<td>BMI-SDS</td>
<td>1.4 ± 1.7</td>
<td>1.1 ± 1.8</td>
<td>.63</td>
</tr>
<tr>
<td>Head circumference SDS</td>
<td>−0.3 ± 1.8</td>
<td>0.4 ± 0.9</td>
<td>.18</td>
</tr>
<tr>
<td>Arm span SDS</td>
<td>−3.2 ± 1.2</td>
<td>−2.3 ± 1.0</td>
<td>.04</td>
</tr>
<tr>
<td>Arm span-height (cm)</td>
<td>−5.2 ± 2.7</td>
<td>−5.0 ± 3.3</td>
<td>.90</td>
</tr>
<tr>
<td>Upper segment/lower segment</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>.99</td>
</tr>
</tbody>
</table>

*Not available for children <3 years of age.

Table III. Comparison of patients with TS and LWD from GeNeSIS (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Females-</th>
<th>Females-</th>
<th>P value</th>
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<tbody>
<tr>
<td></td>
<td>TS (n)</td>
<td>LWD (n)</td>
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<tr>
<td>Age (years)</td>
<td>9.4 ± 4.2 (76)</td>
<td>10.3 ± 3.7 (24)</td>
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<tr>
<td>Bone age</td>
<td>7.7 ± 3.6 (53)</td>
<td>8.9 ± 3.7 (21)</td>
<td>.20</td>
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<td>Birthweight SDS</td>
<td>−1.2 ± 1.0 (26)</td>
<td>0.1 ± 1.4 (14)</td>
<td>.001</td>
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<td>Height SDS</td>
<td>−2.7 ± 1.0 (73)</td>
<td>−2.7 ± 1.3 (24)</td>
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<td>Sitting height SDS</td>
<td>−2.5 ± 1.6 (17)</td>
<td>−2.3 ± 1.7 (18)</td>
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<td>Weight SDS</td>
<td>−1.5 ± 1.7 (73)</td>
<td>−0.8 ± 1.2 (24)</td>
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</tr>
<tr>
<td>Head circumference SDS</td>
<td>−0.8 ± 1.0 (23)</td>
<td>−0.8 ± 1.9 (15)</td>
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</tr>
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<td>Arm span SDS</td>
<td>−3.4 ± 2.6 (28)</td>
<td>−4.0 ± 1.9 (17)</td>
<td>.40</td>
</tr>
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<td>Forearm length SDS</td>
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<td>−2.6 ± 1.4 (15)</td>
<td>.35</td>
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<td>−1.5 ± 1.3 (15)</td>
<td>.07</td>
</tr>
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<td>Lower leg circumference SDS</td>
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<td>0.1 ± 2.1 (14)</td>
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<tr>
<td>Forearm circumference SDS</td>
<td>−1.6 ± 1.1 (17)</td>
<td>−1.2 ± 1.7 (14)</td>
<td>.46</td>
</tr>
<tr>
<td>Upper leg circumference SDS</td>
<td>−1.9 ± 1.5 (17)</td>
<td>−0.4 ± 1.2 (14)</td>
<td>.008</td>
</tr>
<tr>
<td>Upper arm circumference SDS</td>
<td>−1.1 ± 1.5 (17)</td>
<td>−0.4 ± 1.2 (14)</td>
<td>.22</td>
</tr>
</tbody>
</table>

Madelung Wrist Deformity and Radiographic Wrist Changes in Clinical LWD and GeNeSIS Populations

Madelung deformity was diagnosed clinically or radiographically (Figure 4A, 4B) in 18/34 (53%) LWD subjects, 13 of 20 (65%) LWD females, and 5 of 14 (36%) LWD males (P = .16) from the clinical population. Wrist radiographs (Figure 4B) were assessed for the presence of radial lucency, which was found in 12/20 (60%) LWD females and 4 of 14 (29%) LWD males (P = .09). Radiographic findings, including radial lucencies, were present in two girls as young as two years of age (Figure 4B). These results indicate that certain features suggesting Madelung deformity such as radial lucencies are present in children before puberty. Wrist abnormalities in patients from the GeNeSIS population were compared for the LWD and TS populations. Altered osseous alignment of the wrist was noted in 7 of 9 (78%) LWD versus 5 of 20 (25%) TS females (P = .01). Bowing of the radius was present in 12 of 12 (100%) of LWD versus 4 of 18 (22%) TS (P < .001), again suggesting that Madelung wrist findings occur more often in LWD than in TS.

Other Physical Features in Clinical LWD and GeNeSIS Populations

In the clinical LWD population, no subject had cardiac or renal abnormalities. One female and one male had mild scoliosis. The frequencies of other TS-associated features, specifically high-arched palate and increased carrying angle in the LWD population (Table I), were 25 of 34 (74%) and 16 of 34 (47%), respectively. High-arched palate was present in 15 of 20 (75%) females and 10 of 14 (71%) males (P = .87). Increased carrying angle of the elbow was present in 13 of 20 (65%) girls versus 3 of 14 (21%) boys (P = .03).

In the GeNeSIS sample, the frequency of high-arched palate was similar in LWD and TS females (15/24 [63%] and 47/76 [62%], P = 1.00); however increased carrying angle at the elbow was observed more frequently in LWD than TS (mean ±SD, ages 10.3 ± 3.7 and 9.4 ± 4.2, respectively, Table III). The mean height deficit in the LWD population (−2.7 ± 1.3 SDS) was similar to that of girls with TS (−2.7 ± 1.0 SDS). Arm span SDS was relatively shorter in the LWD compared with the TS population, but the difference did not attain statistical significance (Table III). There was evidence for mesomelia in the LWD GeNeSIS population, since forearm length SDS was more negative than upper arm length SDS. This was not observed in the Turner syndrome population.
TS (18/24 [75%] versus 34/76 [45%], P = .01). Both scoliosis (4/24 [17%] versus 2/76 [3%], P = .03) and short forearms (13/24 [54%] versus 6/76 [8%], P < 0.001) were reported more frequently in the LWD girls. Tibial bowing was observed in 8 of 20 (40%) females and 4/14 (29%) males with LWD in the clinic sample (P = .72; Figure 5), suggesting that this may be an early finding in children with LWD and that females and males are similarly affected. In the GeNeSIS sample, the frequency of tibial bowing was greater in the LWD than in the TS population (10/24 [42%] versus 2/76 [3%], P < .001).

The appearance of muscle hypertrophy was noted in only one girl and two boys with LWD in the clinic sample (Figure 6), suggesting that when present this phenotypic feature of LWD usually occurs later in childhood or adulthood. The possible presence of muscle hypertrophy was also assessed as part of GeNeSIS (Table III) by measuring circumference of the upper and lower arms and legs. Arm and leg circumference SDS were somewhat greater in the LWD subjects than in the subjects with TS, perhaps reflecting increased muscle mass (Table III) in those with LWD compared with those with TS.

**DISCUSSION**

This study summarizes the genetic and physical findings from a group of 34 prepubertal children with LWD and confirmed SHOX gene deletion or mutation followed in a tertiary referral center. These results are based on a population of referred children subject to ascertainment bias. In addition, pretreatment data from a GH observational study were used to compare aspects of the phenotype of LWD females with those of another SHOX haploinsufficient population, females with TS. Other large studies have reported SHOX haploinsufficiency in most but not all LWD families.4,23-26 In our clinic population, one mutation, X226R, was identified in exon 6b, which is specific to the SHOXb isoform. This mutation ablates the stop codon and elongates the predicted SHOXb protein by 22 amino acids. The same X226R mutation has been identified in identified in 21 patients screened for idiopathic short stature (K.H., unpublished data) but not in any other short stature patients or control subjects, making it unlikely to be a rare polymorphic variant without a phenotype. SHOXb lacks a C-terminal OAR domain27 that is required for SHOXa to activate transcription of an artificial reporter gene, and SHOXb has been proposed to regulate SHOXa transcriptional activity through heterodimerization.28 A recently described SHOX mutation affected the SHOX homeodomain and inhibited SHOX nuclear translocation.29 SHOX is expressed in hypertrophic/apoptotic chondrocytes in the human pubertal growth plate.30 SHOX haploinsufficiency may lead to abnormal bone growth on the basis of atypical proliferation of chondrocytes as well as defective differentiation.9

In this study, short stature (height SDS < −2) was a common childhood finding in LWD in both females and males, as previously reported.11,31,32 Short stature occurs early in childhood in almost half of patients. The mean height deficit in LWD girls (−2.3 SDS clinic sample, −2.7 SDS GeNeSIS sample) was similar to the height deficit generally observed in girls and women with TS (−2.4 SDS)16,33 and observed in the GeNeSIS sample (−2.7 SDS) suggesting that SHOX haploinsufficiency is responsible for most of the height deficit observed in TS in childhood. Age did not significantly correlate with height of LWD patients, indicating early growth failure in this sample. Our results indicate early short stature before puberty, where a previous report indicates that patients with SHOX deficiency develop significant height deficits after puberty.34 The increased BMI may reflect greater weight relative to height in LWD patients or, alternatively, may reflect the altered body proportions of LWD patients (short legs and relatively long trunk evidenced by the increased upper-to-lower segment ratio). The deficit in leg length (height is squared in the formula for calculation of BMI) may minimize the denominator, resulting in artifactually higher BMI.

In our clinic sample, bone alterations including mesomelia (evidenced by reduced arm span) and tibial bowing also.

![Figure 4. A. Clinical Madelung deformity in a child with LWD, age 1.5 years. B. Radiographic abnormalities of radial lucencies in child with LWD (Figure 4B is available in color online at www.jpeds.com).](image-url)
Figure 5. A, Tibial bowing in a toddler with LWD. B, Tibial bowing in a child with LWD. C, Early radiographic findings of tibial bowing in a toddler with LWD.
occurred early in development, prior to pubertal changes and were slightly more prevalent in females than males, as previously reported.26,31

The relatively increased circumference of the upper and lower legs in the GeNeSIS LWD sample versus the TS population (Table III) is a unique finding that may represent an early manifestation of the muscle hypertrophy reported in adults with LWD.11 The cause for apparent muscle hypertrophy may be the shortening of lever length of the long bones.

The ratio of females to males in our population and the GeNeSIS population (data not shown) was 1.4:1, consistent with the previously reported female preponderance of LWD.2,11,26 However, this ratio is less biased toward females than earlier reports (2 to 4:1).2,11,26 Others have suggested that estrogen action is important in the development of dyschondrosteosis8,35 and may account for increased diagnosis in females.

Radiographic abnormalities were commonly seen in our clinic population of prepubertal children with LWD, in agreement with earlier reports.31 Madelung deformity is relatively uncommon (7%) in TS,38 even though virtually all TS females have SHOX haploinsufficiency and short stature. However, girls with TS are reported to have a unique radiographic finding of “distal radio-ulnar phyleal dysplasia,” with the ulna being relatively shorter than the radius, perhaps representing a variant form of the radio-ulnar defect of Madelung deformity.39

The reasons for the relatively low prevalence of Madelung deformity in TS are not clear. One explanation may be that estrogen exposure influences the development of Madelung deformity. Estrogen could have asymmetric effects on the growth plate span by interacting with specific SHOX-deficient areas. In addition, localized apoptosis at the epiphysis in females with LWD may be accelerated by the interaction of estrogen and altered localized SHOX expression.30,36 Last, phenotypic heterogeneity may also be related to variations in SHOX gene expression leading to alterations in functional protein levels.37 TS girls could be protected from developing Madelung deformity by their sex steroid deficiency, whereas LWD females might not have such protection, as they generally have normal ovarian function. Evidence against this hypothesis is the fact that radial lucencies, which appear to be an antecedent of the Madelung deformity, were found in some of our prepubertal female and male LWD patients. Furthermore, the deformity does not appear to develop in TS females treated with estrogen before epiphyseal closure (J.L.R., unpublished observations). Finally, the SHOX gene is expressed in limbs during early fetal development, at which time there is a relatively high circulating estrogen level, but dyschondrosteosis is usually not clinically evident in infancy. For these reasons, a more likely explanation of the paucity of Madelung deformity in TS is that modifying effects of loss of nonpseudoautosomal growth genes ameliorate the asymmetric radial growth abnormality that gives rise to Madelung deformity.8

There are several prevailing notions about LWD phenotypic variability and SHOX deficiency that require further scrutiny. They include (1) LWD is reportedly more common and more severe in females and (2) puberty/adolescence somehow accentuates LWD features such as Madelung deformity. Our data suggest that LWD is somewhat more common in females. However, LWD may be underdiagnosed in males. Careful anthropometric measurements may perhaps permit earlier diagnosis of LWD in males, who share many of the phenotypic features observed in females. Jackson et al41 previously reported that an abnormally low ratio of forearm to upper arm length may be a valuable diagnostic clue, and Binder et al31 reported that decreased extremity-trunk ratio is also an indicator of relative shortness of the limbs and SHOX haploinsufficiency in children with idiopathic short stature. Family history may also allow earlier diagnosis in males, as all the males with SHOX abnormalities in our clinic sample were familial cases.

In summary, SHOX haploinsufficiency affects bone growth and development early in childhood in many girls and boys with LWD, before the onset of puberty. Short stature is an early finding in both LWD (females and males) and TS (females). The diagnosis of SHOX haploinsufficiency, or the more clinically obvious Leri-Weill dyschondrosteosis, should be considered in a young child with any one or more of the following findings: short stature (height below 2 SDS for age and sex, irrespective of parental stature); high-arched palate; increased upper-to-lower segment ratio for age (ie, shorter legs than trunk); reduced arm span for age and also relative to height; increased carrying angle of the elbow; wrist deformity (full-fledged Madelung deformity, bowing of the forearm or radiologic abnormality); tibial bowing; appearance of muscular hypertrophy of the calf. Molecular analysis for defects of the SHOX gene should be considered in a child (male or female) with any of the clinical features listed.
above, particularly when accompanied by a family history of dominant short stature. The roles of SHOX and other X-linked genes in bone growth and development require additional study.

REFERENCES


PREDICTION OF ADULT HEIGHT USING MATURITY-BASED CUMULATIVE HEIGHT VELOCITY CURVES
LAUREN B. SHERAR, MSc, ROBERT L. MIRWALD, PhD, ADAM D. G. BAXTER-JONES, PhD, AND MARTINE THOMIS, PhD

Objective To validate and demonstrate how adult height can be predicted by using reference values obtained from maturity and sex-specific cumulative height velocity curves.

Study design Serial height measurements were taken on 224 boys and 120 girls. Individuals were classified as early, average, or late maturers, depending on their age of peak height velocity. Maturity and sex-specific cumulative height velocity curves were developed for early, average, and late maturers, and the area under these curves were used to develop reference values to predict adult height.

Results This method can predict adult height within ±5.35 cm 95% of the time in boys and ±6.81 cm 95% of the time in girls.

Conclusions The technique is a valid, nonintrusive, inexpensive, and simple method of predicting adult height in adolescent children, free of growth limiting diseases. (J Pediatr 2005;147:508-14)

Growth in stature is known to have a distinct and measurable end point; however, children differ greatly in the rate at which they pass through the various phases of growth. Some children have a rapid tempo of growth and attain adult stature at a relatively early age, whereas others have a slow tempo and finish growing relatively late. Thus, an accurate method of estimating adult height needs to incorporate an indicator of biological maturity.

Previous methods have incorporated measures of secondary sex characteristics and age at menarche. These methods, however, have restricted applicability. Age of menarche is limited to girls who have attained menarche; an event that occurs, on average, fairly late in adolescence. Secondary sex staging techniques lacks precision as an indicator of maturity as the time it takes to move through the stages can be lengthy and vary considerably between individuals.

The most popular predictive equations of adult height are the Bayley and Pinneau method, the Roche-Wainer-Thissen method, and the Tanner-Whitehouse method. These methods all include assessment of skeletal age (or bone age) to account for variation in biological maturity. The assessment of skeletal age, however, is costly and requires exposure to radiation.

In an effort to develop a nonintrusive and inexpensive method of predicting adult height, the modified Roche-Wainer-Thissen method and the Khamis-Roche method were developed. These methods estimate adult stature from current age, stature, weight, and mid-parent stature (adjusted mean height of the parents). However, these nonintrusive methods do not include a measure of biological maturity. Although the inclusion of midparent height has been shown to reduce error in the prediction, the heights of both parents are not always available.

Recently, a method of assessing biological maturity has been developed that requires chronological age of an adolescent and a measurement of height, sitting height, and weight. The timing of leg length velocity and sitting height velocity is used to predict years from peak height velocity (PHV [the adolescent growth spurt in height]), which is an indicator of somatic maturity. This is a nonintrusive, inexpensive and simple way of assessing biological maturity and has the potential to be incorporated into methodologies for predicting adult stature.

It is known that during adolescence, early-maturing individuals of both sexes are closer to their adult height than average and late-maturing individuals of the same chronological age. This is due to their earlier attainment of PHV. This phenomenon is illustrated for male subjects in Figure 1, A. Compared with average and late maturers,
early maturers stop growing in height first. By full maturity, no difference in adult height between maturity groups exist. In addition to the differential timing of PHV among maturity groups, early-maturing individuals also attain a greater magnitude of growth at PHV. This is evidenced when maturity group velocity curves are aligned on the common benchmark of PHV (Figure 1, B). To date, the techniques of predicting adult stature have predominantly used linear regression. We present a novel approach using cumulative height velocity (area under the velocity curve) for early-, average-, and late-maturing male and female subjects to predict the distance an individual has left to grow to adult height. If the present height of an individual is known and an estimation of height left to grow before reaching adult stature is predictable, then adult height can be ascertained. The purpose of this paper is to validate and then demonstrate how a child’s adult height can be predicted by using reference values obtained from maturity and sex-specific cumulative height velocity curves.

**METHODS**

**Subjects**

Subjects were drawn from three longitudinal growth studies: the Saskatchewan Growth and Development Study (SGDS) (1964 to 1973; 1998 and 1999), the Saskatchewan Pediatric Bone Mineral Accrual Study (PBMAS) (1991 to 1998; 2002 to 2004), and the Leuven Longitudinal Twin Study (LLTS) (1985 to 1999). Data from the SGDS and PBMAS were used to develop reference data to predict adult height, and data from the LLTS were used to test the accuracy of the prediction method.

The SGDS used a pure longitudinal design for the boys and a mixed longitudinal design for the girls. The boys were 7 years old at study entry and the girls were 7, 8, or 9 years old at study entry. The PBMAS used a mixed longitudinal design. Boys and girls were incorporated into eight age cohorts. The cohorts were between 8 and 15 years of age at study entry. The LLTS used a pure longitudinal design for the boys and girls, and all participants were 10 years old at study entry. One hundred percent of the SGDS and LLTS participants were white, and more than 98% of the PBMAS participants were white, with Aboriginal, Asian, and African accounting for the remaining 2%.

The studies received approval from the University and Hospital Advisory Committee on Ethics in Human Experimentation. Written informed consent was obtained from parents/guardians and their children. For inclusion in the present analysis, subjects required a measure of adult height and serial measures of stature around the attainment of PHV; 224 boys and 120 girls from the SGDS and the PBMAS fulfilled these requirements. The LLTS subjects required at least one measurement occasion during adolescence and a measurement of adult stature. One member of a subset of same-sexed twin pairs was used, which resulted in data from 28 boys and 24 girls.

**Anthropometric Assessment**

All of the studies longitudinally assessed stretched height (cm). Individuals enrolled in the SGDS were assessed on an annual basis, and individuals enrolled in the PBMAS and LLTS were assessed on a semiannual basis. The LLTS also assessed stretched sitting height (cm) and weight (kg). Sitting height was subtracted from standing height to provide an estimate of leg length in the LLTS sample. Two measurements were taken for standing stature, sitting stature, and body mass. A third measure was required if the two measures differed by more than 4 mm for standing stature and sitting stature and 0.4 kg for body mass. The average of the two closest readings was used.

**Peak Height Velocity**

Peak height velocity was identified in the SGDS and PBMAS samples. Standard whole-year velocity calculations...
were applied to height distance measurements for each individual. The velocity age center for each velocity value represented the mid-point between two measurement occasions. A cubic spline (GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego, Calif) was applied to the individual velocity values and an age of PHV identified. A more detailed discussion of the cubic spline procedure has been published elsewhere.23

Individuals were aligned on their PHV (biological age). This was done by subtracting the chronological age at time of test from the chronological age at PHV. Subtracting years from PHV from age at test gave a predicted age at PHV. The LLTS were categorized as early-, average-, and late-maturing, depending on age of PHV. Early maturers were defined as preceding the average age of PHV (12.0 years of age in girls and 14.0 years of age in boys; for discussion, see Reference 24)24 by 1 year; average maturers were ±1 year from PHV; and late maturers were >1 year after PHV.

Cumulative Height Velocity

Average velocity values around PHV were calculated for the SGDS and PBMAS sample. Each individual velocity curve was aligned on zero, and general mean cumulative velocity curves were calculated for early, average, and late maturers. With the use of using GraphPad Prism, the area under the cumulative velocity curve for early, average, and late maturers was calculated by using the trapezoid rule for each 0.1-year interval.25

Accuracy of Prediction

The accuracy of the area under the cumulative velocity curve tables developed was assessed by predicting the adult height of boys and girls from the LLTS at one time point during adolescence. The testing point used in each individual was selected at random. Predicted years from PHV were used to estimate height left to grow for each individual using the maturity specific cumulative velocity curves. Height left to grow was added to height at time of test to provide a predicted adult height for each individual. Statistical difference between actual and predicted height was estimated using dependent t tests, and correlation coefficients were computed. Statistical significance was set at P < .05. (SPSS version 11.5, SPSS Inc, Chicago, Ill). Predicted adult height was compared against actual adult height according to the procedure described by Bland and Altman.26

Table 1. The average age at peak height velocity (years) and the height velocity (cm/year) of early, average and late maturing boys and girls

<table>
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<th>Variable</th>
<th>Maturity</th>
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<th>Girls</th>
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<td>S.D</td>
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<td>Height Velocity at PHV (cm/year)</td>
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<td>1.33</td>
<td>120</td>
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</table>

Figure 2. Scatterplots and Bland Altman procedure for LLTS in A, boys, and B, girls.
RESULTS

The number of SGDS and PBMAS individuals classified as early, average, and late maturers and the average age of PHV and height velocity for each maturity group are shown in Table I. The early-maturing individuals achieved greater magnitude in height velocity compared with late-maturing individuals.

Figure 1 shows (A) height velocity aligned to chronological age, (B) height velocity aligned to PHV, and (C) cumulative height velocity curves for early-, average-, and
late-maturing boys from the SGDS and PBMAS samples. Figure 1 provides a visual description of the steps taken to produce the cumulative height velocity curves. The 95% confidence intervals in Figure 1, C, indicate a significant difference in cumulative height velocity between early-, average-, and late-maturing male subjects before PHV. Similar steps were taken for girls from the SGDS and PBMAS samples. The area under each of these curves for boys and girls is portrayed in Table II. Table II shows the height left to grow for each fifth of a year from \(24\) years to \(14\) years from PHV for early-, average-, and late maturing-boys and girls.

Figure 2, A and B, illustrates the scatterplots and Bland Altman procedure for the boys and girls from the LLTS sample. Correlations between predicted height and actual height were 0.86 and 0.85 \((P < .05)\) for boys and girls, respectively. In the Bland Altman procedure, the averages of the predicted and actual adult height are plotted against the difference between the two values. The mean difference between the two measurements was 0.91 cm, with a standard deviation of 2.72 cm in boys and 0.92 cm, with a standard deviation of 3.47 cm in girls: neither difference was significant \((P > .05)\). This method can predict final height ±5.35 cm 95% of the time in male subjects and ±6.81 cm 95% of the time in female subjects.

**DISCUSSION**

The method of predicting adult stature presented in this communication is simple to use and, unlike other nonintrusive methods, it takes into account the child's biological maturity status (rate of somatic growth). Popular methods to date have used multiple variables within a regression equation to predict biological maturity. The method displayed in this paper predicts adult height by using the area under cumulative height velocity curves for early-, average-, and late-maturing individuals, and, to the authors' knowledge, this is a novel approach. Results show that this new method predicts adult stature with a reasonable degree of accuracy. Previous methods that used skeletal age reported being able to predict adult height anywhere between 5 cm and 8 cm 95% of the time in boys and anywhere between 2.7 cm and 7.8 cm 95% of the time in girls.\(^2,3,10,27\) The error associated with the prediction method presented in this communication falls within this range. However, it should be noted that to obtain this degree of accuracy, correct protocols of measuring sitting height, standing height,
and weight need to be followed. If accurate measurements are not ensured, there is a chance that an individual could be placed into the wrong maturity category (ie, an average-maturing individual categorized as late maturing).

To facilitate a better understanding of the practical utility of the methods, we show the following example of predicting the adult height of a boy 11.25 years of age. Sitting height, leg length (subtract sitting height from standing height), weight, and chronological age are entered into the sex-specific regression equation\(^1\) to predict years from PHV. An example is shown in Table III. The equation estimates that the boy is \(\pm 2\) years from PHV. Age at PHV is calculated by subtracting years from PHV from the boy's chronological age (11.25 to \(\pm 2.0 = 13.25\) years). For a boy, the average age at PHV is approximately 14.0 years,\(^1,2\) so the boy's age at PHV falls within 1 year of this value and thus is categorized as an average maturer. The calculated years from PHV is now used to determine how much growth the boy has left until he reaches his adult and final height. Using Table II, one can determine that an average-maturing boy at \(\pm 2\) years from PHV has 30.06 cm left to grow. His present height is 149.4 cm, thus his predicted adult height is 179.46 cm (149.4 + 30.06 = 179.46).

Although methods of predicting adult stature that use skeletal age are the gold standard, this new technique could be useful as a noninvasive and inexpensive estimate of a child's final height. Although there is a curiosity in the adult height of normal-growing children, the real interest lies in predicting the adult height of children who are abnormally tall or abnormally small. This is of importance because growth problems can have an impact on the child's physical and psychosocial well-being, interfering with school performance, sports participation, and social integration.\(^28\) Prediction of adult height is a useful tool, both in the diagnosis and in the treatment of abnormal growth; however, we strongly suggest that the method presented in this paper only be used in children free of growth-limiting disease. Caution should be taken when using this equation to predict heights of children with abnormal growth (ie, individuals with hyperthyroidism or hypothyroidism, and so forth), as the reference standards and maturity predictive equation presented in this paper are modeled on a population of normal-growing children. Unfortunately, there are limited longitudinal data that document the natural patterning of growth in abnormally tall or abnormally short children to be able to validate the predictive equation presented in this paper. The lack of longitudinal data is primarily because therapeutic interventions are often used in individuals demonstrating abnormal growth, which alters the destined growth of the child. Furthermore, the patterning of growth may be different, depending on the disease; thus, the prediction technique needs to be disease specific. In addition, this prediction method has been developed and validated on primarily Caucasian boys and girls. Future work would need to validate this method by using data from other ethnic populations. A website (http://www.usask.ca/kinesiology/research_index.php) is available in which a child's adult height can be estimated by using the methodology described in the present paper.

REFERENCES

DEVELOPMENTAL COORDINATION DISORDER, GENERALIZED SELF-EFFICACY TOWARD PHYSICAL ACTIVITY, AND PARTICIPATION IN ORGANIZED AND FREE PLAY ACTIVITIES

JOHN CAIRNEY, PhD, JOHN A. HAY, PhD, BRENT E. FAUGHT, PhD, TERRANCE J. WADE, PhD, LAURIE CORNA, MSc, AND ANDREAS FLOURIS, MSc

Objective To test a theoretical model linking developmental coordination disorder (DCD) to reduced physical activity (PA) through the mediating influence of generalized self-efficacy regarding PA.

Study design This was a cross-sectional investigation of students in grades 4 through 8 from 5 elementary schools in the Niagara region of Ontario, Canada (n = 590). Motor proficiency was evaluated using the short-form Bruininks-Oseretsky Test of Motor Proficiency. Generalized self-efficacy was assessed using the Children’s Self-Perceptions of Adequacy in and Predilection for Physical Activity scale, and PA levels were evaluated using a 61-item Participation Questionnaire. Structural equation modeling was used to test the influence of generalized self-efficacy on the relationship between DCD and PA.

Results In this sample, 7.5% (n = 44) of the children met the requirements for probable DCD. The effect of DCD on PA was mediated by generalized self-efficacy. In this model, 28% of the variance in children’s PA was predicted by generalized self-efficacy and DCD.

Conclusions Our results suggest that children with DCD are less likely to be physically active and that generalized self-efficacy can account for a considerable proportion of this relationship. The implications for appropriate interventions to increase PA among children with DCD are discussed. (J Pediatr 2005;147:515-20)

Developmental coordination disorder (DCD) has become the preferred diagnostic designation for otherwise healthy children with motor skill impairment that significantly interferes with academic performance and/or activities of daily living. The prevalence of DCD is estimated to be between 5% and 9%, and it often co-occurs with attention deficit and hyperactive disorders. Children with DCD suffer both on the playground, where they are subject to ridicule, and in the classroom, where motor difficulties compromise their scholastic performance. Although a causal association has not yet been established, these difficulties may lead to lowered perceptions of personal competence.

Children with DCD are much less likely than their peers to participate in vigorous, active play. However, the potential pathways linking DCD to reduced physical activity (PA) in children with DCD are not well understood, and the psychosocial factors amenable to change that may connect DCD to PA remain unexplored. To develop effective PA interventions for young people with DCD, an understanding of the determinants of their activity levels is essential.

Children with DCD may not participate in PA because they may not perceive themselves to be sufficiently adequate to meet minimum performance expectations. A predilection for sedentary pursuits and an avoidance of structured PA opportunities is likely...
a coping strategy to deal with the risk of failure and humiliation.12 Perceived adequacy and predilection are components of generalized self-efficacy toward PA.13 Although self-efficacy measures are often concerned with single-act criteria, all of these single acts reside within a single larger domain of what Bandura14 defined as generalized self-efficacy. Self-efficacy toward specific acts forms the basis of generalized self-efficacy toward PA. This is a useful perspective, because the activities of children are widely varied and our interest is in those factors that influence overall participation. This study tested a theoretical model linking DCD to reduced PA through the mediating influence of generalized self-efficacy (ie, self-perceptions of adequacy in and predilection for PA, and enjoyment of physical education).

METHODS

The study involved a cross-sectional investigation of students in grades 4 through 8 from 5 elementary schools in the Niagara region of Ontario, Canada. Although schools and students were not randomly selected, particular attention was given to the selection of schools to ensure that the participants represented the socioeconomic, ethnic, and urban/rural mix of the general Canadian population. Eighteen children with preexisting physical limitations were excluded from the study, and 8 children with previously known learning disorders were allowed to take part in the study but were excluded from all analyses. From a total of 929 students, 590 (322 males and 268 females; 63.62%) provided informed consent and participated in the study. After a listwise deletion of cases with missing values, the total sample size was finalized at 564.

Variables and Analysis

Developmental Coordination Disorder (DCD). Motor proficiency was evaluated using the short-form Bruininks-Oseretsky Test of Motor Proficiency (BOTMP-SF). This widely accepted test examines the full scope of motor proficiency (eg, static and dynamic balance, reaction time, bilateral coordination) using selected items from the full scale and takes only 30 minutes to complete, as opposed to 2 hours for the full version. The short form has been validated against the full scale with intercorrelations between .90 and .91 for children in the 8- to 14-year age range.16 Although it does not provide an in-depth analysis of each aspect of motor proficiency, it does provide an excellent assessment of general motor functioning.17,18 The BOTMP-SF was individually administered by a trained investigator to each consenting child in the school’s gymnasium behind a curtained barrier to ensure confidentiality. Furthermore, the BOTMP-SF examiner was blinded to the Children’s Self-Perceptions of Adequacy in and Predilection for Physical Activity (CSAPPA) (see below) scale results. A BOTMP-SF standard score (age-adjusted) below 38, which is at or below the 10th percentile rank on the BOTMP-SF, was required to classify a diagnosis for probable DCD. For all analyses, a binary variable (DCD = 1, no DCD = 0) was used. We use the term “probable DCD” because the BOTMP-SF is a field test administered by trained researchers, not a diagnostic protocol administered by a licensed health care professional (eg, pediatrician or occupational therapist).

Generalized Self-Efficacy Toward Physical Activity. The Children’s Self Perceptions of Adequacy in and Predilection for Physical Activity (CSAPPA) scale is a 20-item scale designed to measure children’s self-perceptions of their adequacy in performing, and their desire to participate in, physical activities.13 Hay13,20 designed the CSAPPA scale for children age 9 to 16 years, and it has demonstrated a high test–retest reliability (r = .84 to .90), as well as strong predictive and construct validity.13,19,21 The CSAPPA scale has 3 imbedded factors: adequacy (confidence in), predilection (preference for), and enjoyment of physical education class. In this study, each of these 3 subscales was used to assess different dimensions of generalized self-efficacy toward PA. In terms of construct validity, the CSAPPA is significantly correlated with aerobic fitness (Leger shuttle run test), PA (energy expenditure and self-reported participation in physical activities), body weight (percentage body fat and body mass index), and motor proficiency.13,22,23

Participation in Organized and Free Play Activities. The Participation Questionnaire (PQ) is a 61-item questionnaire that asks children to report their actual participation levels in the areas of free-time play, seasonal recreational pursuits, school sports, community sports teams and clubs, and sport and dance lessons over the past year. The PQ measures activity units, defined as an active pursuit that is regularly selected in free play/recreational situations and/or enrolment in an organized sport team, club, or lesson. Subtotals are available for unorganized activity (free play) and organized activity (sports teams, lessons). The PQ provides an estimation of a child’s frequency and nature of PA, but does not address overall intensity or duration. The PQ has been demonstrated to have strong construct validity with expected significant gender differences and urban/rural differences present.21 Consistency of the PQ among elementary school children has been established with a test–retest reliability of .81.13

Analysis

First, we conducted an exploratory factor analysis to examine generalized self-efficacy and PA as underlying latent constructs of the CSAPPA and PA. In the second part of the analysis, we used descriptive and bivariate statistics (1-way analysis of variance) to examine whether children with DCD score lower on measures of both perceived self-efficacy and play. In the final section, we used a structural equation modeling (SEM) technique to construct the latent variables and test the proposed model from DCD to self-efficacy to PA.

All SEM analyses were performed using AMOS 5.0.24 Maximum likelihood estimates were used for all analyses. Model fit was assessed using the following goodness-of-fit statistics: x2 goodness-of-fit test (χ2/GoF), normed fit index (NFI), comparative fit index (CFI), goodness-of-fit index (GFI), adjusted goodness-of-fit index (AGFI), and root mean squared error of approximation (RMSEA).25 For the NFI, CFI, GFI, and AGFI, values range from 0 to 1, with higher
numbers indicating greater fit. Generally, values >.90 indicate a good fit between the hypothesized model and the data. For the $\chi^2_{\text{GoF}}$, values closer to 0 indicate better fit (0 is an exact fit). For RMSEA, values <.05 are desirable.

**RESULTS**

There was no evidence of outliers or significant departures from linearity or normality in the data. Table I gives the sample characteristics (DCD, gender, and age) and overall mean scores for PA and self-efficacy. In this sample, 7.5% ($n = 44$) of the children meet the requirements for DCD. Of these children, 57% are girls ($n = 25$) and 43% are boys ($n = 19$). This difference is not statistically different ($\chi^2 = 2.582$; degrees of freedom [df] = 1; $P = .108$).

Table II contains a list of the observed measures for both generalized self-efficacy and PA. A principal components factor analysis using varimax rotation was performed in SPSS version 12 (SPSS, Chicago, Ill). The standardized factor loadings are presented. It is useful to show this preliminary information to give some indication of the reliability and validity of the study measures. Although there are no firm guidelines in the literature, experience suggests it is desirable to have factor loadings in excess of .400. The data in Table II reveal that the variables for self-efficacy and participation in free play and organized activities load cleanly on separate factors. Moreover, the factor loadings for the constructs measured with multiple indicators range from .790 to .877.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Factor 1</th>
<th>Factor 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequacy</td>
<td>.877</td>
<td>.147</td>
</tr>
<tr>
<td>Predilection</td>
<td>.876</td>
<td>.166</td>
</tr>
<tr>
<td>Enjoyment of physical education class</td>
<td>.790</td>
<td>.146</td>
</tr>
<tr>
<td>Organized activities</td>
<td>.081</td>
<td>.854</td>
</tr>
<tr>
<td>Free play</td>
<td>.225</td>
<td>.784</td>
</tr>
</tbody>
</table>

*Standardized factor loadings are shown.*

Taken together, these coefficients suggest very good psychometric properties.

Figures 1 and 2 illustrate the differences in mean scores for PA and self-efficacy between children with DCD and those without. Children with probable DCD report less participation in both organized [$F(1, 562) = 11.68; df = 1; P < .01$] and free play activities [$F(1, 562) = 7.63; df = 1; P < .01$] than children without motoric challenge. Moreover, they also report lower perceived adequacy in [$F(1, 562) = 54.44; df = 1; P < .01$], predilection toward [$F(1, 562) = 63.00; df = 1; P < .01$], and enjoyment of physical education class [$F(1, 562) = 29.04; df = 1; P < .01$].

We used DCD status as a measured variable (exogenous), perceived adequacy, predilection toward PA, and enjoyment of physical education class as indicators of the latent generalized self-efficacy factor (endogenous), and participation in organized activities and free play as indicators of the latent PA factor (endogenous). To test the hypothesis that generalized self-efficacy mediates the relationship between DCD and PA, we tested 2 models. First, we assessed the fit of a model, where we specified that both DCD and self-efficacy would be directly related to PA, but with no paths specified connecting DCD to self-efficacy (mediating effect). In the second model, we included the direct path from DCD to PA, which specified an indirect path from DCD to PA through generalized self-efficacy (mediating effect). Support for the mediating model is obtained if the second model fits the data better than the first model does.

Model Fit. In the first model specifying only the direct paths (not shown), the goodness-of-fit indices indicated that the model did not fit the data well ($\chi^2_{\text{GoF}} = 87.09$; $df = 8$; $P = .000$; NFI = .907; CFI = .914; GFI = .953; AGFI = .878; RMSEA = .133). When we added a mediating pathway from DCD through generalized self-efficacy to PA (see Figure 3), the improvement to the model fit was substantial, resulting in a $\chi^2_{\text{GoF}}$ reduction of 65.3 with 1 df ($\chi^2_{\text{GoF}} = 21.75$; $df = 7$; $P = .003$; NFI = .975; CFI = .979; GFI = .988; AGFI = .940; RMSEA = .091). However, the modification indices still indicated several alterations necessary to improve the overall model fit. Of these, 1 modification made theoretical/conceptual sense: correlating the error terms between predilection for PA and participation in organized activities. Adding this constraint (parameter) resulted in a further significant reduction in $\chi^2_{\text{GoF}}$ (5.73 with 1 df), with
the overall model $\chi^2_{GoF}$ no longer significant, indicating a better fit of the model to the data. This was also supported by improvements in the other model fit statistics ($\chi^2_{GoF} = 11.16; \text{df} = 6; P = .084; \text{NFI} = .988; \text{CFI} = .994; \text{GFI} = .994; \text{AGFI} = .978; \text{RMSEA} = .039$). Because the $P$ value for $\chi^2_{GoF}$ is no longer significant, this indicates that further modifications (i.e., constraining of parameters) to the model are unnecessary.

In this final model, children with DCD had much lower generalized self-efficacy than children without the disorder ($b = -2.4.96; \text{SE} = .609; P < .001$), and higher self-efficacy was associated with greater participation in free play and organized activities ($b = .449; \text{SE} = .655; P < .001$). However, the direct effect of DCD on PA was nonsignificant ($b = -.049; \text{SE} = .655; P > .953$). Rather, the effect of DCD on PA was mediated through generalized self-efficacy. In the model, 28% of the variance in children’s PA can be predicted by generalized self-efficacy and DCD.

**DISCUSSION**

Our results support earlier findings suggesting that children with DCD are less likely to be physically active.\(^8\)\(^-\)\(^10\) One study has examined whether children with DCD report lower generalized self-efficacy with regard to PA.\(^27\) Similar to this work, our results suggest that children with DCD not only perceive themselves to be less competent in basic physical skills,\(^5\)\(^,\)\(^6\)\(^,\)\(^9\)\(^,\)\(^10\)\(^,\)\(^15\) but also perceive themselves to be less adequate in their overall physical abilities, are more likely to select sedentary over active pursuits, and are less likely to enjoy physical education classes. We further investigated whether differences in generalized self-efficacy actually account for the lower participation in PA of children with DCD.

Children with sedentary lifestyles are at increased risk for negative health and psychosocial outcomes,\(^8\) and children with DCD appear significantly more predisposed to a sedentary lifestyle. Unfortunately, DCD, although prevalent, is rarely, if ever, considered a barrier to PA in the general population. Because lower generalized self-efficacy appears to largely account for why children with DCD are less likely to participate in physical activities than other children, we now have a target for intervention to increase PA.

Although therapies to assist children in coping with this impairment exist, the condition itself remains unresolved.\(^28\)\(^,\)\(^29\) Thus the development of psychosocial interventions aimed at improving coping and quality of life seems warranted. One strategy involves modifying the expectations of the child, parents, and teachers with respect to the child’s motor abilities (academic and lifestyle activities), setting appropriate goals that can be achieved by the child, and modifying home and school environments. Hay\(^21\) has argued that simply identifying children with DCD may lead to improvements in these children’s quality of life, because the process of resetting goals and modifying expectations can only occur when children, teachers, and parents recognize the limitations. Too often, children with DCD are simply thought of as clumsy and/or lazy and told to work harder, which reinforces their failure and low self-efficacy. Interventions of the sort described by Hay\(^21\) should increase self-efficacy by altering expectations and minimizing situations where children with DCD cannot succeed. Indeed, Hay\(^21\) found age-typical levels of both PA...
and self-efficacy in children with learning disabilities and poor motor function that appeared to have resulted from appropriate parental and teacher expectations. Further evidence in support of this strategy comes from clinical trials designed to increase motor proficiency in children with DCD. The gains are likely due to increased confidence and willingness to participate in physical tasks, rather than actual improvement in motor skills. Similarly, a more recent study found that a 10-week intervention did not improve actual motor impairments, but did improve perceived self-efficacy toward specific tasks (eg, tying shoelaces) in children with DCD. Therapists and others (parents and teachers) need to carefully consider a child’s motor abilities, assist the child in finding a suitable vigorous activity and environment, and work incrementally toward improving the child’s mastery of the task. Pediatrians may rarely see a child referred for motor coordination problems as a principal concern. Far more likely are referrals as a consequence of academic difficulties not explained by a learning disorder, behavioral problems, coping with bullying, obesity, or poor self-esteem. In each of these cases it may be important for clinicians to consider DCD as a possibility when making a differential diagnosis. Using the CSAPPA as an initial screen, followed by either M-ABC or BOTMP testing, may uncover a factor at the root of a number of associated challenges. Further, as a result of the strong association with attention deficit hyperactivity disorder and attention deficit disorder, clinicians may include motor testing as a practice to provide counseling should DCD also be present.

Our results support interventions designed to improve self-efficacy by demonstrating a direct link between DCD, generalized self-efficacy, and actual participation in physical activities. Although at the present time it seems unlikely that underlying motor proficiency problems can be corrected, developing children’s coping strategies to accommodate their motor difficulties can be accomplished. This is not dissimilar to models for living with chronic pain, where the strategies are to manage and cope with the pain rather than seek its end. Helping children understand and accept their limitations as normal, to set expectations of parents and teachers at a reasonable level, and to find activities in which the child can achieve success and enjoyment might be a fruitful approach. Some limitations of the present study need to be addressed in future work. First, these data are cross-sectional, and as such, we cannot actually test causal ordering between variables. However, our results support both the plausibility of the model and the need for further work with longitudinal data. Second, these data were not derived from a random sample of students in this age range. Future work should be based on probability sampling to ensure that the findings can be generalized back to the population in question. Third, there are many other factors that are associated with children’s participation in play beyond those examined here. However, the limited number of measures included in our dataset and the relatively small number of children with DCD (n = 44) prohibits model building with a large number of covariates. The small number of boys and girls with DCD does not allow for a test of gender differences in the impact of DCD on perceived self-efficacy and PA. Future work should test whether the model proposed here is the same across genders and in different age groups. It is possible that the link between DCD, perceived efficacy, and PA may be stronger for boys than for girls, or that generalized self-efficacy may be a stronger mediator of the relationship between DCD and activity in older children. Maturational differences may affect the relationship between DCD, self-efficacy, and play. The small number of children with DCD prohibited examination of these questions with this sample.

Criterion B of the DSM-IV criteria for DCD was not directly evaluated. Although this is not uncommon in relation to other studies, future work should include an assessment of the degree to which motor coordination problems influence normal activities of daily living. Although some measures currently exist for this purpose, these are parent-reported and thus are not useful when screening large numbers of children. Finally, our measures of participation and perceived self-efficacy are self-reported. Additional work with measures of activity that do not rely solely on the child’s reporting would strengthen the results.

This study contributes to our understanding of the link between perceived generalized self-efficacy and PA in this population, and provides foundational support for designing interventions to enhance generalized self-efficacy in children with DCD.

REFERENCES


CORTICOSTEROIDS VERSUS INTRAVENOUS IMMUNE GLOBULIN FOR THE TREATMENT OF ACUTE IMMUNE THROMBOCYTOPENIC PURPURA IN CHILDREN: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Objective To compare the effectiveness of corticosteroids with intravenous immune globulin (IVIG) for the initial treatment of children with acute immune thrombocytopenic purpura (ITP).

Study design A systematic review and meta-analysis of randomized controlled trials comparing corticosteroids with IVIG. Studies were identified from eight electronic databases, meeting abstracts, expert consultation, and hand-searched reference lists. Two authors independently reviewed potentially eligible studies and extracted data. The number of patients with a platelet count >20,000/mm³, 48 hours after treatment initiation, was the primary outcome. Relative risks (RR) and risk differences were pooled using a random effects model, and numbers needed to treat (NNT) were calculated.

Results A total of 1248 abstracts were reviewed, 55 articles were retrieved, and 10 studies were included. The RR (steroids vs IVIG) of achieving a platelet count >20,000/mm³ at 48 hours was 0.74 (95% CI: 0.65, 0.85), and the NNT was 4.55 (95% CI: 3.23, 7.69).

Conclusion Children treated with corticosteroids for acute ITP are 26% less likely to have a platelet count >20,000/mm³ after 48 hours of therapy, when compared with children treated with IVIG. Given the importance of low platelets in the pathogenesis of intracranial hemorrhage (ICH), this difference may hold important clinical implications. (J Pediatr 2005;147:521-7)

Immune thrombocytopenic purpura (ITP) is an autoimmune disorder, characterized by a decreased platelet count and mucocutaneous bleeding.¹ In children, primary acute ITP is idiopathic in nature and typically occurs in young, previously healthy children following an infectious illness. The rationale for treating ITP is to hasten platelet recovery in an attempt to prevent intracranial hemorrhage (ICH). This life-threatening complication is estimated to occur in 0.2% to 1% of children with ITP, usually in the context of severe thrombocytopenia (platelet count <20,000/mm³).²⁻⁵

Current medical treatment strategies for acute ITP include corticosteroids, intravenous immune globulin (IVIG), anti-Rh (D) immune globulin, and observation alone.¹ Therapeutic recommendations from different countries are not consistent, leading to widespread variation in practice.⁶⁻⁹

In 1996, The American Society of Hematology published a practice guideline for ITP based on a combination of scientific evidence and expert opinion.⁸ This guideline is based on studies published before 1994, does not focus exclusively on treatment for childhood ITP, and is qualitative rather than quantitative in nature. Consequently, the guideline has not been accepted universally.⁶,¹⁰ Two other guidelines—largely based on opinion—also have been published.⁷,¹²

| ICC | Intraclass correlation coefficient |
| ICH | Intracranial hemorrhage |
| ITP | Immune thrombocytopenic purpura |
| IVIG | Intravenous immune globulin |
| NNT | Number needed to treat |
| RR | Relative risk |

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Dr Beck is supported through a studentship, fully or in part, by the Ontario Student Opportunity Trust Fund – Hospital for Sick Children Foundation Student Scholarship Program, and by a Canadian Institute for Health Research Fellowship.

The Paediatric Outcomes Research Team (PORT) is supported by a grant from the Hospital for Sick Children.

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Submitted for publication Sep 14, 2004; last revision received Mar 1, 2005; accepted Apr 14, 2005.

0022-3476/ - see front matter
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10.1016/j.jpeds.2005.04.032
The objective of this research was to conduct a systematic review and meta-analysis of randomized trials comparing corticosteroids with IVIG for the initial treatment of acute ITP in children.

METHODS

Eligible Studies

Study design was limited to randomized controlled trials. Trials were included if patients were between 3 months and 18 years of age, presenting for the first time with primary acute ITP (it is difficult to differentiate ITP from neonatal allo- or autoimmune thrombocytopenia in children younger than 3 months). Studies of children with an underlying disorder, children previously treated for ITP, and patients with chronic ITP also were excluded. Given that a bone marrow examination is only required to diagnose ITP in children with atypical presentations, a diagnosis based on a bone marrow examination was not an absolute requirement for study inclusion. To be eligible for inclusion, studies were required to include at least two arms, comparing systemic corticosteroids (intravenously or orally administered) with IVIG, regardless of the dosage or duration used. Studies also had to report platelet counts over time for the two interventions.

Study Identification

Potentially relevant studies were identified from an electronic search of Medline (1966 to June Week 3 2004), Embase (1980 to 2003 Week 6), CINAHL (1982 to January Week 5 2003), the Cochrane Database of Systematic Reviews (2002 Issue 4), ACP Journal Club (2002 Issue 4), DARE (2002 Issue 4), the Cochrane Central Register of Controlled Trials (4th Quarter 2002), and the Dissertation Abstract Database (February 2003). The search strategy was customized for each database but was of the general format: (ITP or synonyms) AND (IVIG or synonyms) AND (glucocorticoids or synonyms). The searches were carried out without language restriction. Additionally, meeting abstracts from the American Society of Hematology (Science Expanded Index 1945-2003) and from the American Society of Pediatric Hematology/Oncology (1998-2002) conferences were searched. An expert in the field also was consulted (V.B.), and bibliographies from included studies were reviewed.

Two assessors (C.B., P.N.) independently reviewed each potentially eligible study. Only studies considered eligible by both reviewers were included in the final meta-analysis. In the case of disagreement between reviewers, the opinion of a third reviewer (C.M.) was sought.

Quality Assessment

The Jadad scale, a validated tool for assessing the quality of randomized clinical trials, was applied independently by the two reviewers (C.B., P.N.) to the final group of studies. This scale is scored from 0 to 5, with randomization, double blinding, and descriptions of withdrawals and dropouts the key elements. Agreement between reviewers on quality scores was assessed using the intraclass correlation coefficient (ICC). The strength of agreement for ICC point estimates was considered negligible (0.00-0.19), weakly correlated (0.20-0.34), moderately correlated (0.35-0.49), or strongly correlated (0.50-1.00).

Outcomes

Data were extracted independently by the two reviewers (C.B., P.N.). The primary outcome was the number of patients with a platelet count >20,000/mm$^3$ at 48 hours after the initiation of treatment. This outcome was selected because ICH rarely occurs in patients with ITP and a platelet count >20,000/mm$^3$, and because most treatment regimens for ITP are administered for at least 2 days.

Secondary outcomes, established a priori, included the number of patients with platelets >20,000/mm$^3$ at both 24 and 72 hours, the number of patients who developed chronic ITP (persistent thrombocytopenia <150,000/mm$^3$ after 6 months), the presence of ICH, and mortality. Data on side effects reported for each treatment arm also were recorded. Where data for any of these outcomes were unavailable, the authors were contacted by electronic mail or by letter.

Analysis

There was variability across the studies in the dose and duration of treatment regimens for both corticosteroids and IVIG. However, all data, regardless of dose or duration, were combined in the comparison of corticosteroids versus IVIG on the primary and secondary outcomes. Total IVIG dose administered over the first 2 days of treatment was used. Relative risks (RR) and risk differences were calculated for the primary and secondary outcomes (platelets >20,000/mm$^3$ at 24, 48, and 72 hours, yes/no). The number needed to treat (NNT) also was calculated. The NNT is the number of patients who must be treated in order to prevent one adverse event, and is mathematically equivalent to the reciprocal of the risk difference. In this context, the NNT is the number of patients who would have to be treated with the superior regimen (compared with the inferior regimen) in order to prevent one patient from having a persistently low platelet count. Between-study heterogeneity was assessed using a $\chi^2$ test, with significance set at a $P$ value <.05. Results from individual studies were pooled using Review Manager 4.1 (The Cochrane Collaboration, Oxford, England), using a random effects model.

Given the variability in therapeutic regimens across the studies, a subgroup analysis compared the different dosing regimens...
regimens. Three dosing regimens for corticosteroids were identified (methylprednisolone ≥10 mg/kg/day, prednisone 4 mg/kg/day, prednisone <4 mg/kg/day), whereas two dosing regimens were noted for IVIG (2 g/kg, ≤1 g/kg). Data from each study were extracted and combined to estimate the RR of a platelet count >20,000/mm³ at 48 hours comparing the different doses of the same therapy (ie, corticosteroid vs corticosteroid and IVIG vs IVIG), as well as for the different doses of corticosteroid versus the different doses of IVIG.

The proportion of patients who developed chronic ITP in each treatment group was compared using a χ² test. The remaining secondary outcomes (presence of ICH, mortality, side effects) were examined qualitatively.

RESULTS

Description of Studies

Figure 1 diagrams the process of study identification. Of the electronic databases searched, Medline identified all 10 included studies,15-24 whereas all but two23,24 were also identified by Embase. Two studies were supported by grants from the Canadian and Swiss Red Cross and the Physicians’ Services Incorporated Foundation (Ontario, Canada),17,18 and two received their IVIG supply from the Swiss Red Cross and Sandoz Limited.17,22 None of the other studies reported any acknowledgements.

For the included studies, the median quality score on the Jadad scale was 2.0 (range 1.0-3.0). There was strong correlation between reviewer scores (ICC = 0.70). Of the six studies that reported the number of patients with platelet counts >20,000/mm³ at 48 hours,16-19,23,24 the Jadad scores ranged from 1.0 to 2.5. This variation was not sufficient to allow for categorization and analysis by study quality.

Across the interventions used and the outcomes reported (Table I), all studies, however, were statistically homogeneous (Figures 2a-c). One study had three arms, comparing two different IVIG doses with corticosteroid treatment.17 Given homogeneity of results for the two IVIG doses at 48 and 72 hours, data for the two IVIG arms were combined. At the 24-hour time point, however, the two doses of IVIG showed heterogeneous results. Therefore, for the review, the data for IVIG 1 g/kg/day at this time point were used. Another study had four arms, comparing three different corticosteroid regimens with IVIG.23 For all three time points, the corticosteroid results for the different doses were homogeneous and thus were combined in the analysis.

Primary Outcome

In the six studies that reported the primary outcome (N = 401), the RR (steroids vs IVIG) of achieving a platelet count >20,000/mm³ at 48 hours after treatment initiation was 0.74 (95% CI: 0.65, 0.85) (Figure 2a). The NNT was 4.55 patients (95% CI: 3.23, 7.69). In other words, approximately five patients would need to be treated with IVIG (relative to steroids) in order to prevent one patient from having a platelet count <20,000/mm³ at 48 hours.
The same five studies reported platelet counts 72 hours following treatment initiation. The RR (steroids vs IVIG) of having a platelet count >20,000/mm$^3$ 72 hours after treatment initiation was 0.83 (95% CI: 0.76, 0.91) (Figure 2c). The NNT was 6.25 patients (95% CI: 4.17, 11.11).

The number of patients who went on to develop chronic ITP was available from nine studies (N = 576). In total, 25% of patients treated with corticosteroids and 18% of patients treated with IVIG developed chronic ITP (RR, steroids vs IVIG, 1.40, 95% CI: 1.01, 1.93).

Data were available from nine studies as to whether any of the patients developed an ICH (N = 586). Two patients treated with corticosteroids developed an ICH, both of whom improved. Of those treated with IVIG, one patient developed an ICH and subsequently died. The patient was described as having a “florid viral infection and ITP.” This death was the only one reported in the 10 studies.

Side effects of both treatments were transient. Table IV describes the frequency of side effects reported in the included studies.

**DISCUSSION**

Ten studies met the eligibility criteria for the review. Data on the primary outcome were available from six of the 10 studies (a total of 401 patients). Children receiving corticosteroids for primary acute ITP were 26% less likely to achieve a platelet count >20,000/mm$^3$ at 48 hours after treatment initiation compared with children treated with IVIG. Subgroup analyses showed that this finding was independent of the specific corticosteroid or IVIG regimen used. Secondary analyses showed that IVIG was superior to corticosteroids at 24 and 72 hours after initiation of treatment. The NNT was consistent across time periods, with five to six patients needing to be treated with IVIG (relative to steroids) in order to prevent one patient from having a persistently low platelet count.

The impetus for treating acute ITP in children is the prevention of ICH. Therefore, the true significance of these findings depends on the rates of major bleeding in the corticosteroid and IVIG groups. However, ICH is a rare event; although none of the studies in this meta-analysis defined criteria a priori for diagnosing ICH, the combined reported incidence in the included studies was approximately 5 per 1000 (3/586). Therefore, the platelet count was used as an indirect measure of the risk of bleeding. The relevance of the threshold platelet count of 20,000/mm$^3$ is reflected in its use in qualitative practice guidelines. The platelet count was reported for only one of the ICH cases noted in the 10 included studies, and was in keeping with the literature, at 3,000/mm$^3$.

The primary outcome was platelet count at 48 hours. In one study, 62% of clinically important hemorrhages occurred within 48 hours of the presentation of ITP. Reports in the literature, however, are often confounded by the presence of other risk factors for intracranial bleeds.

**Table I. Summary of interventions in the included studies**

<table>
<thead>
<tr>
<th>Reference #</th>
<th>Author/year</th>
<th>Corticosteroid</th>
<th>Corticosteroid (2nd arm)</th>
<th>IVIG</th>
<th>IVIG (2nd arm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Albayrak et al 1994</td>
<td>MP 30 mg/kg/d po ×7d</td>
<td>MP 50 mg/kg/d po ×7d</td>
<td>0.5 g/kg/d ×5d</td>
<td>1 g/kg/d ×1-2d</td>
</tr>
<tr>
<td>16</td>
<td>Ancona et al 2002</td>
<td>MP 30 mg/kg/d IV ×2-3d</td>
<td>1 g/kg/d ×2d</td>
<td>0.8 g/kg/d ×1d</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Blanchette et al 1994</td>
<td>Pred 4 mg/kg/d po ×7d (taper to 21d)</td>
<td>1 g/kg/d ×2d</td>
<td>0.8 g/kg/d ×2d</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Blanchette et al 1993</td>
<td>Pred 4 mg/kg/d po ×7d (taper to 21d)</td>
<td>1 g/kg/d ×2d</td>
<td>0.8 g/kg/d ×2d</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Duru et al 2002</td>
<td>MP 30 mg/kg/d po ×3d then 20 mg/kg/d ×4d</td>
<td>0.4 g/kg/d ×5d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Imbach et al 1985</td>
<td>Pred 60 mg/m$^2$/d po ×21d</td>
<td>0.4 g/kg/d ×5d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Khalifa et al 1993</td>
<td>MP 10 mg/kg/d IV ×5d</td>
<td>0.4 g/kg/d ×5d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Ozzoynlu et al 1993</td>
<td>MP 30 mg/kg/d po ×3d then 20 mg/kg/d ×4d</td>
<td>0.4 g/kg/d ×5d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Fujisawa et al 2000</td>
<td>MP 30 mg/kg/d po ×3d</td>
<td>0.4 g/kg/d ×5d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Erduran et al 2003</td>
<td>Pred 2 mg/kg/d po ×14d (taper to 21d)</td>
<td>1 g/kg/d ×1d</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IV, intravenous; MP, methylprednisolone; PO, orally; Pred, prednisone.
vs IVIG) of platelet counts rising above 20,000/mm³ at the 24, 48, and 72 hour time points steadily increased (0.63, 0.74, 0.83, respectively). There was, however, overlap between the 95% confidence intervals. A more complete understanding of the epidemiology of ICH is needed to better understand the significance of time.

Although the definition of chronic ITP as a platelet count <150,000/mm³ 6 months after diagnosis is well-accepted, in many cases it may not be clinically relevant. For example, it is well established that many children meeting this definition do not require treatment, and their platelet counts spontaneously resolve with time. Thus, there is controversy regarding what constitutes a clinically meaningful definition of chronic ITP. In this systematic review, 21% of patients developed chronic ITP (25% steroids vs 18% IVIG, \( P = .04 \)) based on this traditional definition. This frequency is in keeping with the literature. 1 Therefore, for this secondary outcome, the results of this meta-analysis should be interpreted with caution.

Although this review focused on efficacy, the risks of corticosteroids and IVIG are important to consider in clinical decision making. For corticosteroids, this includes the possibility of missing a diagnosis of leukemia, although this risk is considered to be <1% in the setting of a child with typical features of ITP. 26 IVIG carries a risk of viral transmission, requires intravenous administration, and the presentation of aseptic meningitis in a child at risk for ICH presents a diagnostic dilemma. Finally, although availability and cost issues were not addressed in this review, the cost of IVIG compared with corticosteroids is considerably higher. 27

Strengths of this meta-analysis include a focused and relevant clinical question, and a comprehensive literature search of eight different databases, abstracts from two important meetings, expert consultation, and hand searches of bibliographies from the included studies. Two assessors worked independently at each step and had high correlation coefficients for agreement. Between-study heterogeneity was accounted for using a random effects model, and clinically relevant outcome measures were employed.

Limitations relate to an overall poor quality of studies, with a significant amount of clinical heterogeneity. Additionally, there were too few studies to meaningfully examine publication bias using a funnel plot analysis. Another approach to address this potential bias is to look at the proportion of studies found to be significant. 28 For the primary outcome, four studies favored IVIG, whereas two were not significant. Given that there is no clinical reason to believe that trials favoring corticosteroids are systematically unpublished, publication bias is unlikely to have been a significant issue in this meta-analysis. Another limitation relates to the platelet count being only a proxy measure for the clinically relevant endpoint. Although it is the most practical surrogate marker, experimental studies have shown that corticosteroids may have an additional direct effect on blood vessel integrity, making it possible that a reduction of bleeding risk may not be reflected in the platelet count. 29,30 Finally, despite the inclusion of 10 trials in the systematic review, and attempts to contact all authors, data were only available from six studies for the meta-analysis. A sensitivity analysis, however, showed that the results were robust.

By combining studies of different sample sizes, the results of this meta-analysis provide a more precise estimate of

### Table II. Subgroup analyses by dose

<table>
<thead>
<tr>
<th>Treatment comparison</th>
<th>Relative risk of platelet count &gt;20,000/mm³ (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP 30 mg/kg/d vs Pred 4 mg/kg/d</td>
<td>0.92 (0.73, 1.17)</td>
</tr>
<tr>
<td>MP 30 mg/kg/d vs Pred &lt;4 mg/kg/d</td>
<td>1.27 (0.83, 1.96)</td>
</tr>
<tr>
<td>Pred 4 mg/kg/d vs Pred &lt;4 mg/kg/d</td>
<td>1.38 (0.90, 2.11)</td>
</tr>
<tr>
<td>IVIG 2 g/kg vs IVIG ≤1 g/kg</td>
<td>0.95 (0.83, 1.09)</td>
</tr>
<tr>
<td>MP 30 mg/kg/d vs IVIG 2 g/kg</td>
<td>0.73 (0.61, 0.89)</td>
</tr>
<tr>
<td>MP 30 mg/kg/d vs IVIG ≤1 g/kg</td>
<td>0.70 (0.57, 0.85)</td>
</tr>
<tr>
<td>Pred 4 mg/kg/d vs IVIG 2 g/kg</td>
<td>0.79 (0.66, 0.95)</td>
</tr>
<tr>
<td>Pred 4 mg/kg/d vs IVIG ≤1 g/kg</td>
<td>0.76 (0.63, 0.91)</td>
</tr>
<tr>
<td>Pred &lt;4 mg/kg/d vs IVIG 2 g/kg</td>
<td>0.58 (0.38, 0.86)</td>
</tr>
<tr>
<td>Pred &lt;4 mg/kg/d vs IVIG ≤1 g/kg</td>
<td>0.55 (0.36, 0.83)</td>
</tr>
</tbody>
</table>

MP, methylprednisolone; Pred, prednisone.  *P <.01.

Figure 2 a. Relative risk of platelet count >20,000/mm³ at 48 hours.

Figure 2 b. Relative risk of platelet count >20,000/mm³ at 24 hours.

Figure 2 c. Relative risk of platelet count >20,000/mm³ at 72 hours.
the effectiveness of one treatment relative to the other.31 In children with primary acute ITP, treatment with IVIG results in a higher proportion of patients achieving a platelet count greater than 20,000/mm$^3$ after 48 hours of therapy, when compared with corticosteroids. Given the importance of a low platelet count in the pathogenesis of ICH, this difference may hold important clinical implications. Furthermore, the significant difference between the two treatment arms with respect to the likelihood of developing chronic ITP is an important finding that deserves attention in future studies. Finally, the risks and benefits of each treatment option, including their relative costs, remain to be considered.

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IMPACT OF EXERCISE ON OVERNIGHT GLYCEMIC CONTROL IN CHILDREN WITH TYPE 1 DIABETES MELLITUS

THE DIABETES RESEARCH IN CHILDREN NETWORK (DirecNet) STUDY GROUP*

Objective  To examine the effect of exercise on overnight hypoglycemia in children with type 1 diabetes mellitus (T1DM).

Study design  At 5 clinical sites, 50 subjects with T1DM (age 11 to 17 years) were studied in a clinical research center on 2 separate days. One day included an afternoon exercise session on a treadmill. On both days, frequently sampled blood glucose levels were measured at the DirecNet central laboratory. Insulin doses were similar on both days.

Results  During exercise, plasma glucose levels fell in almost all subjects; 11 (22%) developed hypoglycemia. Mean glucose level from 10 PM to 6 AM was lower on the exercise day than on the sedentary day (131 vs 154 mg/dL; \( P = .003 \)). Hypoglycemia developed overnight more often on the exercise nights than on the sedentary nights (\( P = .009 \)), occurring on the exercise night only in 13 (26%), on the sedentary night only in 3 (6%), on both nights in 11 (22%), and on neither night in 23 (46%). Hypoglycemia was unusual on the sedentary night if the pre-bedtime snack glucose level was > 130 mg/dL.

Conclusions  These findings indicate that overnight hypoglycemia after exercise is common in children with T1DM and support the importance of modifying diabetes management after afternoon exercise to reduce the risk of hypoglycemia. (J Pediatr 2005;147:528-34)

Since the days of Joslin and Allen, exercise has been recommended as 1 of the 3 cornerstones of diabetes management. However, like many aspects of treatment of children with type 1 diabetes mellitus (T1DM), vigorous physical exercise presents clinicians, parents, and patients with a dilemma. On one hand, regular exercise is encouraged in children to enhance psychosocial well-being and cardiovascular health, as well as to achieve and maintain ideal body weight and body composition. On the other hand, prolonged exercise can make regulation of blood glucose levels more difficult both during and after the period of increased physical activity. These difficulties are compounded by the irregular pattern of physical activity that characterizes most youth who are not participating in organized sports or regimented training programs and by traditional methods of diabetes management that feature fixed, inflexible insulin replacement regimens.

The possibility that prolonged periods of aerobic exercise during the day may increase the risk of severe hypoglycemia during the following night is a very common concern. In addressing this issue, investigators in the Diabetes Control and Complications Trial demonstrated that unusual physical activity was more frequent on days with severe hypoglycemic events than on randomly chosen days, but the difference was not statistically significant. Only limited data are available regarding the role of daytime exercise on overnight hypoglycemia in children with T1DM, and none of these studies examined the impact of exercise on asymptomatic, biochemical hypoglycemia during the overnight period using rigorously controlled research protocols.
The Diabetes Research in Children Network (DirecNet) is a multicenter study group whose objectives include the examination of factors that contribute to the risk of or could possibly prevent hypoglycemia in children with T1DM. The present study was undertaken to examine the effect of late afternoon exercise on the frequency of overnight hypoglycemia by comparing glucose data collected on a day of exercise with that collected on a sedentary day in an inpatient clinical research center (CRC) setting. To isolate the effects of exercise per se, meals were similar on both study days, and basal and bolus insulin doses were based on the treatment algorithms that the subject used at home on sedentary days.

METHODS

Subjects

CONSENT PROCEDURES. The DirecNet Data and Safety Monitoring Board and the institutional review boards at each of the DirecNet centers approved the study protocol, consent form, and assent form. A parent or guardian and each of the DirecNet centers approved the study protocol.

ELIGIBILITY CRITERIA AND ASSESSMENT. To be eligible for the study, subjects had to meet the following criteria: (1) age 10 to 18 years; (2) a clinical diagnosis of T1DM of ≥18 months duration; (3) on a stable insulin regimen for at least 1 month, involving either use of an insulin pump or multiple daily injections of Glargine and Lispro insulin or Aspart insulin (5 patients also used NPH in the morning, and 1 patient used both NPH and Ultralente); (4) a hemoglobin A1c (HbA1c) level ≤10.0% measured with the DCA2000+ analyzer (Bayer Diagnostics, Tarrytown, NY); (5) a body mass index (BMI) between the 5th and 95th percentile for age and gender; (6) body weight ≥36.0 kg; (7) normal hematocrit level; and (8) normal thyroid function. Subjects were not eligible if they (1) had asthma that was medically treated in the previous year, (2) were currently using glucocorticoids or beta blockers, (3) had used pseudoephedrine within 48 hours, (4) had experienced severe hypoglycemia (seizure or loss of consciousness) within the previous 2 weeks, (5) had an active infection, (6) anticipated a significant change in exercise regimen between admissions, or (7) had another medical condition or were using a medication that in the judgment of the investigator could affect completion of the exercise protocol.

Study Procedures

The study consisted of 2 inpatient stays in the CRCs at each of the DirecNet sites lasting about 24 hours each and separated by 1 to 4 weeks: 1 stay with a 75-minute exercise session in the late afternoon (“exercise day”) and 1 without the exercise (“sedentary day”). The order of the exercise and sedentary days was determined at random.

Before the first admission, the subject’s daily meal plan and insulin algorithms used at home were recorded. Meals and bedtime snacks of similar caloric and carbohydrate content were consumed on both hospital days. Insulin management on both the exercise day and sedentary day were as similar as possible and followed the same routine that the subject would follow at home on a day without exercise. If the subject used an insulin algorithm with the bedtime snack (or no snack) on a sedentary day at home, then this same pattern was followed on both exercise and sedentary days in the hospital. Subjects using insulin injections were instructed to administer the injection in a site other than the legs on the study days. For subjects using an insulin infusion pump, the basal rate used at home in the afternoon was continued uninterrupted during the exercise session.

On both the sedentary and exercise days, the subject was admitted to the CRC before lunch. An intravenous catheter was inserted in an antecubital vein for blood sample collection. On both days, a standardized protocol was followed for checking the blood glucose level at 2 PM and 3 PM, and either short-acting insulin or an oral carbohydrate was given, if indicated, to titrate the 4 PM blood glucose level to between 100 mg/dL and 200 mg/dL before exercise.

EXERCISE PROCEDURES. On the morning of the exercise day, the subject walked on a motorized treadmill for 5 to 15 minutes to determine the settings needed to achieve a heart rate of 140 beats/minute. This was estimated to be equivalent to the heart rate achieved at 55% maximum effort. These treadmill settings were used for the start of the exercise session, which commenced at about 4 PM. The exercise session consisted of 15 minutes walking on a treadmill at a heart rate of approximately 140 beats/minute, followed by a 5-minute rest period. This cycle was repeated 3 more times, for a total of 4 15-minute exercise periods with 5-minute rest periods in between (75 minutes total). A heart rate monitor was worn throughout the duration of exercise.

Blood glucose measurements were made from venous blood samples both for a central laboratory sample as well as home glucose meter measurement (see below) before starting the exercise session, during each of the 3 rest periods, immediately after the exercise session, and at 15-minute intervals for 1 hour after the completion of exercise. If during exercise the blood glucose level dropped to < 60 mg/dL, then the subject was given 15 to 30 g of carbohydrate and the blood glucose level was rechecked after 5 to 15 minutes. Exercise did not resume until the blood glucose level was > 70 mg/dL.

EVENING AND OVERNIGHT PROCEDURES. Dinner was consumed at about 6:15 PM. After dinner, blood glucose level was checked at 7:00 PM, 8:00 PM, and 9:00 PM. A bedtime snack was given at approximately 9:30 PM if the subject would normally receive one as part of a sedentary day treatment regimen.

The subject was asked to go to sleep at approximately 10:00 PM and was awakened at approximately 7:00 AM. Blood glucose measurements were made using samples from the intravenous catheter every half-hour from 10:00 PM through 6:00 AM (see below).
If at any time the blood glucose reading was < 60 mg/dL, then the subject was given 15 to 30 g of carbohydrate and the blood glucose level was rechecked in 15 minutes. If the blood glucose level was still < 60 mg/dL after 15 minutes, then another 15 to 30 g of carbohydrates was administered. This procedure was repeated at 15-minute intervals until the blood glucose level was > 70 mg/dL.

**Glucose determinations.** Glucose measurements were made with the One-Touch Ultra meter (Lifescan, Milpitas, CA) at the sampling times described earlier. We have previously demonstrated the accuracy of this meter. 6 In addition, blood samples for central laboratory determination of serum glucose levels were obtained from the intravenous catheter during the exercise session and hourly from 10 PM to 6 AM and at other times if the Ultra meter glucose value was < 60 mg/dL. Glucose determinations were made at the DirecNet Central Biochemistry Laboratory at the University of Minnesota using a hexokinase enzymatic method. 7, 8

**Statistical Methods**

Overnight hypoglycemia was considered to have occurred when a central laboratory glucose level was ≤ 60 mg/dL between 10 PM and 6 AM. For the purpose of analysis, unless otherwise stated, the definition of hypoglycemia also included cases in which hypoglycemia treatment was given based on an Ultra meter glucose value but a confirmatory central laboratory glucose value ≥ 60 mg/dL was not present. Analyses using only the central laboratory–confirmed hypoglycemia cases are indicated as such.

The proportions of subjects developing hypoglycemia overnight on the exercise and sedentary nights were compared using generalized estimating equations (GEEs), controlling for a possible period (first vs second visit) effect and repeated measures from the same subject. A hypoglycemia index was calculated for each subject to characterize the cumulative magnitude of hypoglycemia during the period from 10 PM to 6 AM during each admission. At each of the 17 nightly 30-minute measurement points during that interval (9 reference glucose values on the hour and 8 Ultra meter values on the half-hour), the difference between 70 mg/dL and any obtained glucose values < 70 mg/dL was calculated. Glucose values ≥ 70 mg/dL contributed a score of 0 to the index. The mean of the 17 difference scores was computed for each subject for each CRC admission. For cases in which the subject was treated for hypoglycemia, the most recent previous glucose measurement was carried forward 1 hour in the calculation of the hypoglycemia index. Because this hypoglycemia index had a skewed distribution, a permutation test was used to compare exercise results with sedentary results.

Mean overnight glucose values were compared using repeated-measures regression. The association of hypoglycemia with the self-reported number of days with at least 1 hour of physical activity during a typical week (used as a surrogate measure of fitness) was evaluated separately for the exercise and sedentary visits using logistic regression and for the exercise and sedentary visits combined using the GEE regression model described earlier (by adding fitness as an independent variable). Associations of overnight hypoglycemia with hypoglycemia during exercise (binary) and the 9 PM glucose (continuous) were analyzed in a similar manner. An adjusted $r^2$ value was calculated using the generalized coefficient of determination. 9

Multivariate analysis for overnight hypoglycemia included a term for exercise versus sedentary visits and used a stepwise procedure to select among the following factors: gender, age, HbA1c level, insulin route (pump vs multiple daily injections), total daily insulin dose, average bolus dose (carbohydrate to insulin ratio) used in daily insulin regimen, BMI, and self-reported frequency of at-home exercise. The sample size was estimated as 50 subjects to have 90% power with an alpha level of 5% to detect a 3-fold difference in the hypoglycemia index (see the definition earlier) comparing the exercise and sedentary nights and to have approximately 80% power for a comparison of the incidence of hypoglycemia.

**RESULTS**

Fifty subjects participated in the study between June 2004 and November 2004. Their average age was 14.8 ± 1.7 years; 44% were female; and the racial/ethnic distribution was 90% Caucasian, 4% African-American, 2% Hispanic, and 4% Asian. The mean duration of T1DM was 7.0 ± 3.7 years. An insulin pump was used by 54%; multiple daily injections by the other 46%. Mean HbA1c was 7.8 ± 0.8%. Three subjects (6%) reported a severe episode of hypoglycemia (resulting in seizure or loss of consciousness) within the 6 months before the study. Half of the subjects completed the exercise admission first, and the other half completed the sedentary admission first. The median time between the 2 admissions was 14 days (interquartile range, 7 to 16 days; range, 6 to 39 days).

**Exercise Session**

The full exercise session was completed by 46 of the 50 subjects (92%). Three subjects completed the first 3 cycles and part of a fourth, and the remaining subject completed 2 cycles fully and 2 cycles partially. One subject did not reach the target heart rate for 1 of the 4 exercise cycles, and the other 49 subjects achieved the target rate for all 4 cycles. Forty-one subjects (82%) experienced a decrease in pre-exercise glucose level of at least 25%, and 11 subjects (22%) became hypoglycemic (blood glucose level ≤ 60 mg/dL) either during or immediately after the exercise session. An additional 6 subjects were treated for hypoglycemia due to a falling glucose level on the Ultra meter but did not have a confirmatory central laboratory glucose value < 60 mg/dL.

**Postexercise Glucose Levels**

The mean glucose level was similar on the exercise and sedentary days at 4 PM (before the start of exercise). After the completion of the exercise and throughout the night, the glucose levels were significantly lower on the exercise day than...
on the sedentary day (Figure 1). During the overnight period (10 PM to 6 AM), plasma glucose averaged 131 ± 58 mg/dL on the exercise nights and 154 ± 69 mg/dL on the sedentary nights (P = .003).

Between 10 PM and 6 AM, a glucose level ≤60 mg/dL was confirmed by the central laboratory for 21 (42%) of the 50 exercise nights and 8 (16%) of the sedentary nights. On an additional 3 exercise nights and 6 sedentary nights, treatment was given for hypoglycemia based on an Ultra meter glucose value, but either a blood sample was not sent to the central laboratory (n = 5; protocol violation) or the central laboratory value was > 60 mg/dL (n = 4).

As shown in Table I, hypoglycemia (including central laboratory confirmed and unconfirmed cases) occurred more often on the exercise night than on the sedentary night (P = .009 overall and P < .001 in an analysis limited to confirmed cases). Hypoglycemia occurred only on the exercise night in 13 subjects (26%), only on the sedentary night in 3 (6%), on both nights in 11 (22%), and on neither night in 23 (46%). The hypoglycemia index was greater on the exercise nights than on the sedentary nights (mean, 2.3 ± 3.0 mg/dL vs 1.5 ± 3.2 mg/dL, [P = .07 overall] and 2.1 ± 2.9 vs 1.1 ± 2.7 [P = .01] in an analysis limited to central laboratory glucose values).

The overall result of a higher incidence of hypoglycemia on the exercise nights than on the sedentary nights was consistent in subgroups based on gender, age, HbA1c level, insulin route (pump vs multiple daily injections), total daily insulin dose, average bolus dose (carbohydrate to insulin ratio) used in daily insulin regimen, BMI, and frequency of exercise performed at home. Only the latter factor appeared to influence the risk of nocturnal hypoglycemia. As shown in Table II, subjects who exercised more frequently at home were at greater risk for nocturnal hypoglycemia on both the exercise night (P = .02) and the sedentary night (P = .05). On the exercise day, 11 (65%) of the 17 subjects who became hypoglycemic or were treated for low glucose during the exercise session also developed hypoglycemia overnight, compared with 13 (39%) of the 33 subjects who did not become hypoglycemic during the exercise session (P = .14).

### Table I. Hypoglycemia and glucose levels from 10 PM to 6 AM

| Development of hypoglycemia, n (%) | P value  
|-----------------------------------|----------
| Exercise night only                | 13 (26%) |
| Sedentary night only               | 3 (6%)   |
| Neither night                      | 23 (46%) |
| Both nights                        | 11 (22%) |
| Hypoglycemia index, mean ± SD†    | .07      |
| Exercise night                     | 2.3 ± 3.0 |
| Sedentary night                    | 1.5 ± 3.2 |
| Intrasubject difference            | 0.8 ± 3.2 |
| Development of hyperglycemia, n (%)‡ | .008    |
| Exercise night only                | 1 (2%)   |
| Sedentary night only               | 9 (18%)  |
| Neither night                      | 24 (48%) |
| Both nights                        | 16 (32%) |
| Hourly-half hour blood glucose levels, mean ± SD | .003 |
| Exercise night                     | 131 ± 58 |
| Sedentary night                    | 154 ± 69 |
| Intrasubject difference            | -23 ± 52 |

*Hypoglycemia is defined as central laboratory glucose value ≤60 mg/dL or treatment for hypoglycemia given (based on the Ultra glucose value) without a central laboratory glucose value ≤60 mg/dL. In an analysis limited to use of the central laboratory values only, hypoglycemia (≤60 mg/dL) occurred on the exercise night only in 14 (28%), on the sedentary night only in 1 (2%), on neither night in 28 (56%), and on both nights in 7 (14%) (P < .001).

†In an analysis limited to central laboratory values only, mean ± SD of the hypoglycemia index was 2.1 ± 2.9 on the exercise nights and 1.1 ± 2.7 on the sedentary nights (P = .01).

‡Hyperglycemia is defined as central laboratory glucose value or Ultra meter glucose value (if no central laboratory value available) ≥200 mg/dL. In an analysis limited to use of the central laboratory values only, hyperglycemia occurred on the exercise night only in 1 (2%), on the sedentary night only in 9 (18%), on neither night in 25 (50%), and on both nights in 15 (30%) (P = .008).

The presnack blood glucose (9 PM) was predictive of overnight hypoglycemia on both the sedentary day (P = .002; r² = .39) and on the exercise day (P = .04; r² = .12). On the sedentary day, overnight hypoglycemia was unusual if the presnack glucose level was > 130 mg/dL, whereas on the exercise day, hypoglycemia occurred fairly frequently even when the glucose level was higher (Figure 2). On the sedentary day, overnight hypoglycemia developed in 12 (55%) of 22 subjects whose presnack glucose level was ≤130 mg/dL and in only 2 (7%) of 28 subjects whose presnack glucose level was > 130 mg/dL (P = .001). On the exercise day, overnight hypoglycemia developed in 16 (57%) of 28 subjects whose presnack glucose level was ≤130 mg/dL and in 8 (36%) of 22 subjects whose presnack glucose level was > 130 mg/dL (P = .15). Multivariate analysis using stepwise logistic regression (with P < .20 as the inclusion criterion) identified only the 9 PM glucose value (P = .003) and self-reported days per week of exercise (P = .06) for inclusion in the model.
We designed the present study to more carefully define the effect of afternoon exercise on the relative risk of hypoglycemia during the following night in a cohort of 50 children with T1DM who were using an intensive diabetes management regimen involving either insulin pumps or multiple daily insulin injections. A carefully controlled cross-over design that involved a supervised and standardized exercise protocol was used to compare the frequency of overnight hypoglycemia after afternoon exercise with that after a sedentary day in a CRC setting. We specifically chose to have the subjects exercise in the late afternoon, because children and adolescents often are more active at the end of the school day, when different athletic practice and game sessions take place. In addition, the duration and intensity of the exercise regimen was designed to mimic a typical length of time that children are involved in such activities.

It is noteworthy that nocturnal hypoglycemia was very common in our subjects even on the days when they did not exercise. In 28% of the subjects, hypoglycemia developed during the night of the sedentary day even though only 3 of the subjects had experienced a severe hypoglycemic event at home during the 6 months before the study. This may relate to the adverse effects of deep sleep on protective, counterregulatory hormone responses to hypoglycemia.10,11 In adults with T1DM, reduced awakening from sleep during hypoglycemia in comparison with nondiabetic individuals has been observed.12 An even more disturbing finding was that nearly twice as many of our children had a hypoglycemic event on the night after an exercise day than on the night after a sedentary day. Further analysis of the differences between the exercise and sedentary days and nights demonstrated that they were consistent across subgroups, with similar results for older versus younger subjects, those with higher versus lower BMI, and those on insulin pumps versus using multiple daily injections. The level of glycemic control, as measured by HbA1c level, also made no difference on the relative risk of overnight hypoglycemia after exercise. These findings extend those of MacDonald et al,3 who reported in a prospective (though uncontrolled) outpatient case series that 48 of 300 (16%) patients with T1DM (age 4 to 24 years) self-reported at least 1 episode of moderate or severe hypoglycemia occurring 4 or more hours after exercise during a 2-year period. Although a number of studies have evaluated the incidence of hypoglycemia during or immediately after exercise,13-17 we are not aware of a previous study that has systematically evaluated the occurrence of overnight hypoglycemia after exercise in children or in adults.

Glucose level before the bedtime snack was a predictor of overnight hypoglycemia on both the sedentary and exercise days, although more so on the sedentary day. On the sedentary day, overnight hypoglycemia was unusual (occurring in only 7% of subjects) if the presnack glucose level was > 130 mg/dL, and common (55%) when the glucose level was ≤130 mg/dL at bedtime. However, on the exercise day, hypoglycemia occurred fairly frequently whether the presnack glucose level was ≤130 mg/dL (57%) or > 130 mg/dL (36%). These data underscore the usefulness of having somewhat higher target glucose levels at bedtime in children with T1DM, particularly on days of intense exercise.

The study procedures specified the use of similar insulin doses on both the exercise and sedentary days. Specifically,
the subject’s usual routine for a sedentary day was followed on the exercise day even if the subject typically would have lowered his or her overnight basal insulin replacement on days of unusually intense physical activity. This approach allowed us to examine the effect of exercise per se on the risk of nocturnal hypoglycemia and is clinically relevant, because many youngsters on pumps or who receive prebreakfast doses of Glargine insulin do not or cannot adjust their overnight basal insulin.

Although not directly tested in this study, reports indicate that a single bout of exercise can increase glucose transport into skeletal muscle tissue for at least 16 hours postexercise in nondiabetic and diabetic subjects. The molecular signaling mechanisms that lead to increased glucose transport after exercise have not been completely elucidated but appear to involve both insulin-dependent and insulin-independent pathways. Thus the greater frequency and magnitude of hypoglycemia observed in our subjects on the nights after exercise are likely dependent not only on the insulin administered overnight, but also on increased insulin-independent glucose transport activity triggered by intense muscle contractions many hours earlier.

The design of this study did not allow determination of how the insulin sensitivity was changed, whether due to altered pharmacokinetics or to pharmacodynamics. Chronic exercise training in humans results in numerous beneficial effects in skeletal muscle, including an increase in the insulin-sensitive glucose transporter 4 (GLUT4) expression, the rate-limiting step in glucose metabolism in skeletal tissue. The increase in muscle GLUT4 in trained individuals in turn contributes to an increase in the responsiveness of muscle to insulin-stimulated glucose uptake. Increases in overall insulin sensitivity may be one explanation why the subjects who exercised more frequently at home were at greater risk for overnight hypoglycemia after both the sedentary and exercise days in this study.

The findings of this study support the well-recognized clinical observation that exercise has benefit in lowering plasma glucose levels both during and after exercise in children with T1DM. Hyperglycemia was more common during the sedentary night, and lower glucose levels were sustained for many hours after exercise on the exercise day compared with the sedentary day. Our findings also support the use of flexible diabetes management regimens that attempt to adjust food intake and insulin dosing on evenings after exercise to reduce the risk of overnight hypoglycemia. The current FDA-approved continuous glucose sensing systems are not practical for day-to-day use in children and lack sufficient accuracy in the low glucose range to serve as effective guides to overnight glucose control. Consequently, frequent meter glucose testing at bedtime and in the middle of the night remains the only currently effective means of adjusting treatment regimens to minimize the risk of exercise-induced nocturnal hypoglycemia. Our data indicate that such monitoring may be especially important in youth who are about to start or are currently participating in regular exercise, such as high school sports programs. Further carefully controlled studies are needed to systematically examine the most effective methods of adjusting insulin doses and meal composition and quantity to maximize the benefits and safety of exercise in children with T1DM.

*Appreciation is expressed for the work performed by the CRC nurses at the 5 clinical centers.*

**APPENDIX**

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AORTIC VALVULOPLASTY IN THE FETUS: TECHNICAL CHARACTERISTICS OF SUCCESSFUL BALLOON DILATION

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Objectives To describe technical aspects of successful fetal aortic valvuloplasty, with particular attention to balloon size.

Study design We retrospectively reviewed all procedural records and echocardiograms pertaining to 26 attempts at fetal aortic valve dilation performed at a single center over a period of 4 years. We assessed the effect of valvuloplasty as determined by echocardiographic appearance at the time of intervention and in follow-up.

Results In 20 of 26 fetuses who had technically successful aortic valve dilation (median balloon:annulus ratio = 1.1), all had improved antegrade flow and 12 had at least mild regurgitation after dilation. Use of an oversized balloon was associated with the onset of moderate or severe aortic regurgitation, seen in 5 fetuses. This aortic regurgitation was well tolerated and improved through the remainder of gestation.

Conclusions These data imply that fetal aortic valves can be dilated safely with larger balloons than are commonly used for postnatal dilation. The observation of spontaneous improvement in postdilation aortic regurgitation further suggests that fetal valve tissue behaves uniquely. (J Pediatr 2005;147:535-9)

After two decades of progressive technical and clinical refinements, percutaneous balloon valvuloplasty has become a widely accepted treatment modality for congenital aortic valve stenosis, both in children and neonates. In 1984, Lababidi et al1 first described successful balloon dilation of aortic stenosis in children. Shortly thereafter, this group and others reported the results of valvuloplasty in neonates with critical aortic stenosis.2,3 In 1991, Maxwell et al4 introduced an entirely novel adaptation of the procedure, attempting aortic valvuloplasty in a 28-week fetus. Isolated antenatal procedures were attempted during the next decade, and the world experience of 12 fetal aortic valve dilations was collectively reported in 2002.5

Recently, we published the results of fetal aortic valve dilation for the purpose of preserving left ventricular (LV) growth in the setting of evolving hypoplastic left heart syndrome.6 Although our initial attempts at fetal catheterization used tools and techniques similar to those previously described, we have substantially modified the procedure over the course of our 4-year experience. With these modifications, technical success has improved from 25% (the first 4 cases) to 90% (the last 10 cases). The aim of this report is to review the technical aspects of fetal aortic valve dilation in current practice. We also focus on one variable that has been widely discussed in the context of postnatal aortic valve dilation, balloon size.7-9

METHODS

We retrospectively reviewed the procedural records and echocardiograms of all 26 fetuses who underwent attempted aortic valve dilation between March 2000 and August 2004. The written record provided nominal balloon sizes and inflation pressures. Inflated balloon sizes were assigned by consulting manufacturer-provided compliance tables. The aortic annulus was measured echocardiographically, making on-line measurements of the distance between the hinge points of the valve, with gain settings optimized to allow for

<table>
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<th>AR</th>
<th>Aortic regurgitation</th>
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<td>BAR</td>
<td>Balloon:annulus ratio</td>
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<td>LV</td>
<td>Left ventricle (ventricular)</td>
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best resolution (Figure 1). The degree of aortic regurgitation (AR) after valvuloplasty was judged qualitatively, based on echocardiographic appearance, and was graded as none/trivial, mild, moderate, or severe.

Patients

Twenty-six women carrying fetuses between 20 and 31 weeks' gestational age underwent attempted fetal catheterization for the purpose of aortic valve dilation. All of the fetuses had aortic stenosis, as characterized by thickened aortic valve tissue, a severely restricted jet of antegrade flow, and associated left ventricular dysfunction. None had more than trivial AR.

Informed Consent

This study was performed as part of an innovative therapy protocol under the direction of the Committee on Clinical Investigation at the Children’s Hospital, Boston, and the Institutional Review Board at the Brigham and Women’s Hospital. In every case, the parents discussed fetal and maternal risks and benefits with a pediatric cardiologist, an obstetrician, and a fetal surgeon or anesthesiologist before providing written informed consent for the procedure.

Positioning

The mothers were placed under general anesthesia in a supine position with left lateral uterine displacement. Transabdominal ultrasound imaging and external manipulation were used to achieve ideal fetal position. In this position, a line of approach from the anterior abdominal surface traversed the apex of the fetal LV, paralleled the LV outflow tract, and crossed the valve into the ascending aorta. Once in position, the fetus was given intramuscular anesthetic and muscle relaxant before catheterization. After poor positioning resulted in technical failure in 3 of the first 4 cases, we modified our practice. Thereafter, if we were unable to position the fetus using external maneuvers, we performed a limited laparotomy to enable direct uterine manipulation and transuterine imaging.

Catheter Equipment

A low-profile, over-the-wire coronary angioplasty catheter was chosen with a balloon diameter based on the measurement of the aortic annulus. Early in our experience, we chose a balloon size based on the assumption that the fetal valve would behave similarly to the neonatal valve and that a balloon:annulus ratio (BAR) of 0.8 to 1.0 was likely to be safe and effective. After performing 10 successful dilations with variable clinical effect and without evidence of more than mild AR, we began using progressively larger balloons with intended BARs of 1.2.

Over the course of the our first 12 cases, we devised a marking system for the wire and balloon catheter to improve control of their position and minimize intracardiac manipulation time. The balloon catheter was mounted on a floppy-tipped guide wire, with 3 cm of distal wire exposed. We gently precurved the last 5 mm of the wire and then fixed the circular torqueing device to the proximal wire abutting the hub of the catheter. This configuration effectively limited the distal excursion of the wire. The wire/catheter assembly was then advanced through the 19-gauge, 12-cm stainless steel introducer cannula until the balloon emerged. Affixing a visible and palpable marker on the proximal catheter shaft allowed us to reproduce this balloon/cannula relation during the procedure without relying wholly on the ultrasound imaging. After the appropriate markers had been placed, the wire/catheter assembly was removed from the introducer cannula and was replaced with a sharp obturator.

Cardiac Catheterization

The introducer was advanced through the fetal chest wall and to the LV epicardium under ultrasound guidance. Chest wall deformation occurred with passage of the introducer and required reidentification of the ideal line of approach before entering the LV. Once the line had been reviewed, the cannula was advanced further. A palpable change in resistance, associated with recoil of LV wall deformation, signaled entry of the introducer into the LV cavity. The obturator was removed with the tip of the cannula just below the aortic valve (Figure 2). Blood return through the cannula confirmed an intracavitary position.

The wire/catheter assembly was passed through the cannula, and the tip of the wire was identified as it emerged. While maintaining imaging of the aortic valve and ascending aorta, we manipulated the precurved wire to probe for the valve. Maximal wire excursion did not exceed the premarked
3-cm length. Valve passage, confirmed echocardiographically by imaging the wire in the ascending aorta, was followed by catheter insertion to the premarked depth. We inflated the balloon, by hand or by pressure gauge, to a pressure at which it achieved the intended BAR. In most cases, two inflations were performed, with no single inflation lasting longer than 5 seconds. Technical success was defined as a minimum of one balloon inflation across the aortic valve. On completion of the dilation, the entire apparatus was removed from the fetus.

**Statistics**

Data are reported as medians, with ranges in parentheses. The Wilcoxon rank sum test was applied to compare gestational age and aortic annulus size of fetuses having successful interventions to those of fetuses who had unsuccessful attempts. This test was also used to compare procedural times between earlier and later cases. The Fisher exact test was used to determine whether there was an association between use of an oversized balloon and the development of postprocedural AR. A $P$ value of <.05 was considered to be statistically significant.

**RESULTS**

The procedure was technically successful in 20 of 26 cases (77%) performed at a median gestational age of 24 weeks (21 to 31 weeks). Neither gestational age nor annulus size was associated with technical success. In all cases, percutaneous positioning was attempted. Fourteen (54%) of the cases were performed with only percutaneous access. Only 1 of the first 4 cases, before the option of laparotomy, achieved technical success. In the remaining 22 cases, with 12 laparotomies performed because of failure of percutaneous techniques, 19 procedures were successful (19/22, 86%). Among successful procedures, 13 were successful on the first pass of the introducer into the fetus. Although 7 procedures required 2 or 3 needle passes to enter the LV, additional attempts (to a maximum of 5) were not successful.

In 3 of 6 unsuccessful cases, the introducer cannula was never documented in the cavity of the LV, and a wire was not introduced. In the other 3 failed attempts, brisk blood return from the hub of the cannula confirmed intracavitary positioning of the cannula tip. In one of these cases, the first pass of the wire was seen in the pericardial space, presumably secondary to dislodgement of the cannula while loading the wire/catheter assembly. In 2 cases, the cannula remained in the LV, but the course of the cannula repeatedly directed the wire tip at the mitral valve and into the left atrium.

In technically successful cases, with the course of the cannula directed along the LV outflow tract, the wire generally required minimal manipulation to cross the valve. As a result of the catheter preparation and the minimal time spent probing for the valve, median time from LV entry to instrument removal was 5 minutes (range, 3 minutes to 16 minutes, 40 seconds). There was a trend toward shorter instrumentation times in the second half of our experience, after development and application of a catheter marking system (7 minutes, 15 seconds vs 4 minutes; $P = .22$).

For 20 fetuses who underwent technically successful aortic valvuloplasty, the median annulus diameter was 2.9 mm (2.5 to 4.0 mm). Nominal diameters of the balloons used for dilation ranged from 2.5 to 3 mm; larger diameter balloons were incompatible with the introducer cannula. Applying inflating pressures of 8 to 18 atm, we achieved effective dilating diameters of 2.6 to 3.6 mm (median, 3.15 mm). All of the balloons were inflated in excess of nominal diameter. Thus, among the 20 fetuses who had successful aortic valvuloplasty, BAR ranged from 0.83 to 1.37 (with median = 1.09), and more than half (12/20) were dilated with balloons larger than typically used for postnatal valvuloplasty (Figure 3).

In two cases, dilation to pressures in excess of the rated burst pressure resulted in balloon rupture. Balloon rupture resulted in shearing of the distal balloon fragment and retention of this LV foreign body for one fetus, who has been previously reported. In the second case of balloon rupture, the balloon was withdrawn through the LV myocardium without first attempting to resheath the balloon in the introducer. This fetus had a pericardial effusion and died within 72 hours of the procedure. Significant pericardial effusion developed in one additional fetus, though not associated with balloon rupture. The effusion was drained uneventfully.

In every case, technically successful valvuloplasty resulted in improved antegrade flow through the aortic valve, as demonstrated by color Doppler. This improvement in antegrade flow was associated with growth of left heart structures during the remainder of gestation. Of 21 fetuses...
live-born near term, 1 died before surgery, 16 underwent stage I Norwood palliation, and 4 sustained 2 ventricle circulations (2 had neonatal aortic valve dilation, 1 had a coarctation repair, and 1 had no intervention before an aortic valve dilation at 18 months).6

Though none of the fetuses in this series had AR before dilation, postcatheterization echocardiography revealed that 7 fetuses (35%) had mild regurgitation, 3 (15%) had moderate regurgitation, and 2 (10%) had severe regurgitation immediately after dilation (Figure 4). Use of an oversized balloon (BAR ≥1.0) was associated with development of at least moderate AR ($P = .038$). Of 5 fetuses with at least moderate AR, 4 were catheterized in the latter half of our series, and all were dilated with a BAR of 1.1 or greater.

Among the 7 fetuses with mild AR after dilation, there was no increase in regurgitation observed through the remainder of gestation. Serial echocardiography of 5 fetuses with at least moderate postvalvuloplasty AR demonstrated diminishing regurgitation in every case. Spontaneous improvement in AR could be seen as early as 1 day after the procedure, as was the case for 2 of these fetuses. The other 3 fetuses showed gradual change, with moderate or severe AR improving by 1 grade within 4 weeks of the procedure. All had mild AR by 8 weeks after catheterization. All 5 of these fetuses were live-born without hydrops and with only mild AR. Thus, none of the 20 fetuses who underwent technically successful aortic valve dilation had more than mild aortic regurgitation by birth.

**DISCUSSION**

When performing neonatal aortic valvuloplasty, the operator typically chooses an initial balloon with a diameter 80% to 100% of the angiographic annulus diameter.11 The stenotic valve is crossed with a guide wire, and a balloon is positioned across the valve. Resolution of a waist as the balloon is inflated signifies effective dilation. Judicious use of larger balloons may improve results if the initial dilation produces modest gradient relief and does not increase regurgitation.12 By titrating balloon size, based on the appearance of the waist and repeated gradient measurements, neonatal valvuloplasty has been highly effective. However, even with impeccable technique, dilation can infrequently result in moderate or severe AR.7,8

In the case of fetal aortic valvuloplasty, appropriate balloon size is unknown and may be difficult to determine for several reasons: (1) there is no visible waist on the dilating balloon, (2) the transvalvar pressure gradient cannot be directly measured, and (3) reduction in the degree of obstruction may not acutely alter the gradient in the setting of severe LV dysfunction.13 Thus, in contrast to postnatal procedures, we rely primarily on positional cues to determine that the balloon has in fact been inflated across the fetal valve. We also rely on the echocardiographic appearance of improved antegrade flow, and in some cases the finding of new AR, to indicate valve modification.

Our review of 26 fetal aortic valvuloplasty procedures suggests that this procedure can be performed with high rates of technical success, combining transabdominal or transuterine echocardiography with a precise catheter marking system. By performing laparotomy in cases of a failed percutaneous approach, we were able to rapidly and consistently cross the stenotic valve, position the balloon, and perform dilation, as evidenced by improved antegrade flow.

Although BARs were initially chosen to simulate neonatal valvuloplasty, balloon sizes were deliberately
increased over the period of the study, as we attempted to define the appropriate range of BAR for fetal valve dilation. Whether higher BAR yields more effective relief of obstruction remains unknown and will be difficult to determine for the reasons cited above (ie, difficulty in ascertainment and interpretation of gradient measurements). Although recently described animal models of fetal valvuloplasty may offer some insight as to the safety of larger balloons, the question of gradient reduction will remain problematic in the absence of any model of fetal aortic stenosis. In neonatal lambs, BARs in excess of 1.2 appeared to be unsafe, resulting in significant cardiac trauma, such as interventricular septal tears and aortic dissections. In our fetal population, we have not observed any echocardiographic evidence of trauma to the LV outflow tract, despite BARs as high as 1.4. Pathologic examination has not been possible. We have, however, seen clinical complications while attempting to achieve higher BARs. These were primarily related to the technical aspects of inflating balloons in excess of rated burst pressures.

Among these fetuses, use of oversized balloons was associated with a higher incidence of significant AR than is generally seen in the postnatal valvuloplasty population. Remarkably, this iatrogenic AR appeared to behave uniquely. Fetal and neonatal AR have been described in case reports of congenital absence of the aortic valve and have been associated with fetal hydrops and neonatal death. Among 5 fetuses with fetal hydrops and neonatal death, 17-19

Among 5 fetuses in this series who had at least moderate AR after aortic valvuloplasty, none had hydrops and all survived to delivery 12 to 14 weeks after the procedure. Not only was regurgitation well tolerated, but it actually diminished over time, such that none of these fetuses had more than mild AR at birth. The improvement in postdilation AR seen in our series is in distinct contrast to the progressive AR that can be seen after aortic valve dilation in neonates.

We speculate that the regression of prenatal AR after aortic valvuloplasty may be mediated by several factors. Low systemic vascular resistance, unique to the fetal/placental circulation, probably modulates the degree of AR and limits hemodynamic impact. In our selected population with preexisting severe LV dysfunction, elevated LV end-diastolic pressure may further serve to limit regurgitant volume. Second, fetal valve tissue itself may be exceptionally capable of remodeling. Prior experience with fetal surgeries, specifically with regard to wound healing, would support this contention.

Both of these factors have major implications, not only for fetal cardiac interventions but for any therapeutic procedure performed in the fetus. Established techniques and therapies applied to congenital disease in the neonate or infant may have distinctly different outcomes when applied to the fetus. Conversely, procedures that have not been successful or even possible in the neonate may offer therapeutic benefit when performed before birth.

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A 12-year-old boy arrived in the emergency department with a food impaction, unable to tolerate oral secretions. He denied any respiratory symptoms associated with the impaction. Past medical history was significant for asthma and intermittent dysphagia. A coin had become lodged in his esophagus 5 years earlier, requiring endoscopic removal. Family history was only significant for his father having psoriasis. X-rays of the chest and nasopharynx were both unremarkable. The patient was taken to the operating room where an 8-cm food impaction was removed from his proximal esophagus. Following removal of the foreign body, an endoscopic photograph was obtained (Figure), and esophageal biopsies demonstrated >50 eosinophils per high-power field and confirmed the suspected diagnosis of eosinophilic esophagitis (EE).

EE is a disorder that may present clinically in children with dysphagia, food impaction, epigastric pain, nausea, and vomiting. Presenting symptoms are often mistaken for those of gastroesophageal reflux, but treatment with a proton pump inhibitor will provide symptomatic relief in only a minority of patients.1 Patients with EE will often report a family history of asthma, allergies, or atopy. Endoscopic findings in patients with EE may include a corrugated (“ringed”) esophagus,2 mucosal thickening or furrowing, and white exudates.3

Food allergy has been reported in up to 50% of patients with EE.4 If a limited number of food allergies can be identified, these items can be eliminated from the diet in an attempt to improve symptoms. Other therapies include a strictly elemental diet or corticosteroids (topical or systemic). EE is a disease that must be considered in any child presenting with a food impaction. Symptoms of dysphagia or reflux (unresponsive to acid suppression) also should warrant further investigation.

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Supported by NIH T32 DK07727.
0022-3476/$ - see front matter
Copyright © 2005 Elsevier Inc. All rights reserved.
Deletion of a segment of the long arm of chromosome 18 causes characteristic physical features and mental retardation. Autoimmune disorders have been described with this syndrome in a limited number of reports. We describe 2 cases of autoimmune hypothyroidism in children with 18q deletion syndrome. (J Pediatr 2005;147:541-3)

The 18q deletion syndrome is characterized by short stature, hypotonia, mental retardation, and other abnormalities.1 Cases of autoimmune phenomena have been reported with this syndrome.2-9 IgA deficiency is occasionally found in this condition,1 and it is well known that autoimmune diseases are common in patients with selective IgA deficiency.10 However, only some patients described with 18q deletion and autoimmune disorders had IgA deficiency,3,7,9 whereas others had normal IgA levels.4,5,7,8 We describe 2 cases of autoimmune thyroiditis in children with 18q deletion syndrome.

CASE REPORTS

Case 1

A 4-year, 2-month-old male with 18q deletion syndrome was referred for evaluation of an enlarged pituitary gland incidentally noted on magnetic resonance imaging (MRI) of the brain. There were no symptoms of hypothyroidism except for dry skin. Medical history was remarkable for normal birth at term. Nystagmus developed at 2 months of age. Brain MRI showed abnormal myelinization and type 1 Chiari malformation. Karyotype revealed 18q deletion. The patient also had severe developmental delay. There was no family history of endocrine disorder.

Weight was at 5th percentile, and height was <5th percentile. Previous growth data were not available. The patient had microcephaly but did not appear dysmorphic. The thyroid gland could not be palpated. He had normal prepubertal genitalia. He had nystagmus and ptosis bilaterally, flat feet, and clinodactyly of the fifth fingers. The rest of the physical examination was normal.

Laboratory tests revealed thyroid stimulating hormone (TSH) 428 μU/mL (normal 0.55-3.0), total T4 0.4 μg/dL (7.3-15), free T4 0.27 ng/dL (0.9-1.59), and prolactin 35.7 ng/mL (3-14.7). Other pituitary hormones and IgA level were normal. This patient’s severe primary hypothyroidism was believed to be the explanation for enlargement of the pituitary gland. Thyroid scanning showed no significant uptake, indicating either absent or extremely small gland. However, review of newborn screen showed normal T4 and TSH, making congenital hypothyroidism unlikely. Bone age was not delayed, indicating that he had acquired hypothyroidism of relatively recent onset. Thyroid peroxidase and thyroglobulin antibodies were elevated, confirming autoimmune thyroiditis. He was started on levo-thyroxine and had gradual resolution of hypothyroidism, improvement in growth rate during first year of treatment, and normalization of pituitary size.

Case 2

A 15-year-old male with 18q deletion syndrome was referred for evaluation of hypothyroidism, which was detected incidentally on laboratory screening. He had a low energy level and had gained excessive weight during the prior 5 years. However, he had no other specific symptoms of hypothyroidism. Past medical history was remarkable for behavioral problems. He also carried a diagnosis of autism. Family history was notable for hypothyroidism.

Height was at the 5th percentile, and weight was >95th percentile. A previous height measurement 7 years earlier was 50th percentile. He was obese but well appearing. The
thyroid was not enlarged. He had a prominent forehead, flat occiput, high-arched palate, and short 5th fingers bilaterally. The rest of the physical examination was normal.

Laboratory tests revealed TSH 193 μU/mL (0.4-3), free T4 0.4 ng/dL (0.9-1.6), and normal IgA level. Thyroid peroxidase antibody was elevated, confirming autoimmune thyroiditis. He was started on levo-thyroxine and had normalization of the TSH within 3 months. Weight decreased 11 pounds during that time.

DISCUSSION

Since the first description of chromosome 18q deletion in 1964, several groups have reported autoimmune phenomena associated with this syndrome.2-9 These are all case reports; thus it is uncertain whether these represent chance associations or true cause-effect relationships. However, given that only around 100 patients have been described with 18q deletion syndrome, the incidence of autoimmune disease in this group appears to be increased compared with the general population.6 Some have speculated that deletion of critical genes on chromosome 18 (such as IDDM6, located at 18q21) could lead to autoimmune disorders such as type 1 diabetes.2

Several autoimmune disorders have been described with 18q deletion. These include hypothyroidism, type 1 diabetes, hypoparathyroidism, juvenile rheumatoid arthritis, and pernicious anemia (Table). Both of our patients were both found incidentally to have severe autoimmune hypothyroidism, but neither had IgA deficiency. Presently, they have no symptoms of any of the other autoimmune disorders described with 18q deletion syndrome.

Of the 9 previously reported cases of autoimmune disease in 18q deletion, hypothyroidism was present in 5 (56%). In the cases where the TSH value was reported, hypothyroidism was severe. In the case by Stricker et al,9 TSH was >128 μU/mL with a nearly undetectable T4 of 0.6 μg/dL (normal 5-12). In the report by Dacou-Voutetakis et al,2 the TSH was >100 μU/mL. The other cases associated with hypothyroidism do not mention the TSH level.3,4,6 In addition to these case reports, elevated TSH was found in 4% (2/50) of a large cohort of children with 18q deletion syndrome who were being evaluated for growth abnormalities.11 Certainly our 2 patients had marked hypothyroidism and poor linear growth.

The association of IgA deficiency with autoimmunity in these patients should not be overlooked. Autoimmunity is the most prevalent disorder in patients with selective IgA deficiency.10 The cause is believed to be related to lack of antigenic exclusion normally exerted by IgA on mucosal surfaces, allowing penetration of antigens, which may then stimulate autoreactive lymphocytes.10 IgA deficiency is a frequent finding not just in patients with 18q deletion, but in a number of other chromosome 18 abnormalities, including ring chromosome 18 and 18p deletion.12 However, none of the immunoglobulin genes are located on chromosome 18, and there does not appear to be a susceptibility locus for IgA deficiency on chromosome 18.12 Although not all patients with 18q deletion and autoimmunity have IgA deficiency, those who do may be at even higher risk for development of autoimmune disorders.

In conclusion, autoimmune disorders appear to be common in patients with 18q deletion syndrome. Autoimmune hypothyroidism, which can be severe, has been described in several reports and is present in our 2 patients. We therefore recommend screening for hypothyroidism in all patients with chromosome 18 abnormalities with a TSH measurement annually or if there is evidence of poor linear growth.

REFERENCES


This study reports serum lipid levels in 682 children with type 1 diabetes mellitus. We found that 3.5% of the subjects had a high-density lipoprotein (HDL) cholesterol level < 35 mg/dL, 15.4% had a total cholesterol (TC) level > 200 mg/dL, and 18.6% were abnormal for either HDL or TC, compared with prevalences of 5.7%, 11.2%, and 16.3%, respectively, reported in the National Health and Nutrition Examination Survey 2001-02. Hemoglobin A1c value was significantly related to TC and non-HDL cholesterol levels. (J Pediatr 2005;147:544-6)

The literature contains few studies of serum lipids in the general pediatric population and scant recent data in subjects with type 1 diabetes mellitus (T1DM). Clinical guidelines in pediatrics are based largely on expert opinion and extrapolation from the adult literature. The American Diabetes Association (ADA) recognizes that diabetes is a cardiovascular risk equivalent and recommends serum lipid screening in children with T1DM initially at age 12 years and then routinely every 5 years thereafter. Ideally, screening samples should be obtained in the fasting state. However, given the difficulties of obtaining fasting samples, the Adult Treatment Panel III (ATP-III) suggests screening with nonfasting total cholesterol (TC) and high-density lipoprotein (HDL) cholesterol levels, followed by a complete fasting lipoprotein panel if screening results are abnormal. A secondary target in adults is non-HDL cholesterol, particularly among subjects with elevated triglyceride values. This report describes our clinical experience with screening TC and HDL levels in pediatric T1DM subjects.

METHODS

The study subjects were patients age 21 years or younger with T1DM (as defined by ADA criteria) seen at the Barbara Davis Center for Childhood Diabetes between January 1, 2000 and June 30, 2004. Screening lipids were obtained as part of routine diabetes care. Because fasting status was not routinely recorded, only TC, HDL, and calculated non-HDL levels were analyzed.

Overall, 3,101 patients were seen during the study interval. To ensure that only subjects with T1DM were studied and to exclude the lipid abnormalities of untreated diabetes, patients who did not have autoantibody determinations positive for diabetes-associated autoimmunity or who were not specifically physician-diagnosed as having T1DM or who had serum lipid levels measured within the first month of T1DM diagnosis were excluded, leaving 682 subjects for analysis. All subjects eligible for inclusion in the study are reported herein. Age, body mass index (BMI), diabetes duration, hemoglobin A1c (HbA1c), and gender were recorded for each subject.

HDL levels were categorized as either < 35 mg/dL or ≥35 mg/dL, and TC levels were categorized as < 170 mg/dL, 170 to 200 mg/dL, or > 200 mg/dL, based on current ADA and American Heart Association (AHA) guidelines for children. Non-HDL levels were categorized as either < 130 mg/dL or ≥130 mg/dL. Pearson correlation

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ADA American Diabetes Association
AHA American Heart Association
ATP-III Adult Treatment Panel III
BMI Body mass index
CI Confidence interval
HbA1c Hemoglobin A1c
HDL High-density lipoprotein
NHANES National Health and Nutrition Examination Survey
T1DM Type 1 diabetes mellitus
TC Total cholesterol

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Supported by the National Institute of Diabetes and Digestive and Kidney Diseases (grants T32 DK063687-03, T32 DK07446-22, and K12 DK063722-03) and the Diabetes Education Research Center (grant P30 DK57516).
Submitted for publication Nov 23, 2004; last revision received Feb 10, 2005; accepted Apr 27, 2005.
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0022-3476/$ - see front matter
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10.1016/j.jpeds.2005.04.068
coefficients were calculated for TC, HDL, age, duration, BMI, and HbA1c, and those variables with P values ≤ .05 were included in linear regression models for predictors of TC, HDL, and non-HDL levels. TC and HDL were measured in commercial laboratories as part of standard clinical practice. The Colorado Multiple Institutional Review Board approved this study.

RESULTS

Mean TC was 173 ± 36 mg/dL; TC was > 200 mg/dL in 15.4% (95% confidence interval [CI] 12.7% to 18.1%) of the subjects, 170 to 200 mg/dL in 32.8%, and < 170 mg/dL in 51.8% (Figure). Mean HDL was 55 ± 14 mg/dL; 3.4% (95% CI = 2.0% to 4.7%) of subjects had an HDL level < 35 mg/dL. Mean non-HDL was 118 ± 35 mg/dL; 27.7% (95% CI = 24.4% to 31.1%) had a non-HDL level ≥ 130 mg/dL. Of note, 18.6% of subjects (95% CI = 15.7% to 21.5%) had either TC or HDL abnormalities. In comparison, the National Health and Nutrition Examination Survey (NHANES) 2001-02 reported TC levels > 200 mg/dL in 11.2%, HDL levels < 35 mg/dL in 5.7%, and non-HDL levels ≥ 130 mg/dL in 26.4%. Mean serum TC level was higher in females (n = 320) than in males (n = 362) (177 ± 37 mg/dL vs 169 ± 34 mg/dL, respectively; P = .0009), as was mean non-HDL level (122 ± 37 mg/dL vs 114 ± 33 mg/dL; P = .008), but mean HDL level did not differ significantly by gender.

Linear regression models demonstrated that HbA1c was a significant predictor of TC and non-HDL levels and a nearly significant predictor for HDL level. BMI was significantly related to HDL and non-HDL levels and nearly significantly related to TC level (Table), although these models explained only a small amount of the variance. Neither age (mean, 12.6 ± 4.3 years) nor duration of T1DM (mean, 3.9 ± 3.3 years) was a significant predictor of TC and non-HDL levels and a nearly significant predictor of HDL level. BMI is significantly associated with HDL and non-HDL levels, and nearly significantly associated (P = .062) with TC level.

The importance of serum lipids in children has been demonstrated by the Muscatine study,7 in which childhood cholesterol levels were found to be generally predictive of adult levels. Furthermore, autopsy studies have demonstrated that atherosclerosis was already present in adolescence.8,9 Although precursors of adult cardiovascular disease clearly develop in childhood, the optimum treatment approach remains uncertain.

A key limitation of the current report is that fasting status was indeterminate. However, adult data10 support the

Table. Predictors of TC, HDL, and non-HDL in multiple linear regression

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta-coefficient (SE)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of T1DM</td>
<td>0.60 (0.41)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of T1DM</td>
<td>0.16 (0.17)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>0.57 (0.30)</td>
<td>.062</td>
</tr>
<tr>
<td>HbA1c</td>
<td>7.10 (0.73)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Non-HDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.0095 (0.16)</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of T1DM</td>
<td>0.16 (0.17)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.57 (0.16)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.63 (0.32)</td>
<td>.052</td>
</tr>
</tbody>
</table>

Independent variables with significant Pearson correlation coefficients were entered into each model: r² = .13, r² = .04, and r² = .13, respectively. NS, not significant.

DISCUSSION

This study reports the frequency of pediatric T1DM subjects with abnormal TC, HDL, and non-HDL levels in accordance with ADA, AHA, and ATP-III guidelines. Overall, 3.4% of the subjects had abnormal HDL values, 15.4% had abnormal TC values, and 18.6% had abnormal HDL or TC values. In comparison, NHANES 2001-02 reported the following prevalences in subjects age 4 to 21 years: 5.7% abnormal HDL, 11.2% abnormal TC, and 16.3% abnormal for either HDL or TC (Figure). Our data suggest that abnormal TC levels may be more frequent and abnormal HDL levels less frequent in pediatric T1DM subjects than in the general pediatric population. Furthermore, glycemic control is significantly associated with TC and non-HDL levels, and nearly significantly associated (P = .052) with HDL level. BMI is significantly associated with HDL and non-HDL levels, and nearly significantly associated (P = .062) with TC level.

The importance of serum lipids in children has been demonstrated by the Muscatine study,7 in which childhood cholesterol levels were found to be generally predictive of adult levels. Furthermore, autopsy studies have demonstrated that atherosclerosis was already present in adolescence.8,9 Although precursors of adult cardiovascular disease clearly develop in childhood, the optimum treatment approach remains uncertain.

A key limitation of the current report is that fasting status was indeterminate. However, adult data10 support the
ATP-III acceptance of nonfasting TC and HDL levels for screening purposes, followed by a full fasting lipid panel when abnormal values are found. Additionally, data were collected as part of routine clinical care, and only 22% of the patients seen had their lipid levels measured once, introducing a selection bias of uncertain magnitude. Despite these limitations, however, this report provides data on a large pediatric cohort with T1DM.

In summary, our data show that 18.6% of children screened for dyslipidemia were abnormal for TC or HDL, and thus have a second cardiovascular risk factor, supporting the importance of regular screening for dyslipidemia in children with T1DM. In addition, there was a significant relationship between glycemic control and TC and non-HDL levels and a significant relationship between BMI and HDL and non-HDL levels. These data support optimizing glycemic control and lifestyle interventions aimed at obesity as essential components of managing lipid abnormalities in this population. Further data on pharmacologic treatment of dyslipidemia, optimal screening guidelines, and longitudinal data on lipids in children with T1DM are needed.

We thank the Information Technology staff at the Barbara Davis Center for Childhood Diabetes.

REFERENCES

COLORIMETRIC END-TIDAL CARBON DIOXIDE DETECTORS IN THE DELIVERY ROOM: STRENGTHS AND LIMITATIONS. A CASE REPORT
C. Omar F. Kamlin, MRCP, MRCPCH, Colm P. F. O'Donnell, MRCP, MRCPCH, Peter G. Davis, FRACP, MD, and Colin J. Morley, FRCP, FRCPCH, FRACP, MD

Clinical assessment and end-tidal carbon dioxide (ETCO₂) detectors are used to verify tracheal intubation in newborn infants. A case is presented in which an ETCO₂ detector was misleading in determining endotracheal tube (ETT) position but useful in determining the efficacy of ventilation in an extremely preterm infant. (J Pediatr 2005;147:547-8)

Extremely preterm infants are frequently intubated for ventilation. Determining whether the trachea has been intubated may be difficult. End-tidal carbon dioxide (ETCO₂) detectors, which indicate the presence of CO₂ in exhaled gas by a color change, are useful for confirming correct endotracheal tube (ETT) placement in adults, children, and neonates. The manufacturers of these detectors express caution about their use in infants weighing <1000 g and in low cardiac output states. We describe a case in which a ETCO₂ detector was misleading in determining ETT position but useful in determining the efficacy of ventilation in an extremely low birth weight infant.

CASE HISTORY

A 32-year-old primigravida with a monochorionic, monoamniotic twin pregnancy presented at 26 weeks gestation. Cardiotocography indicated that 1 twin had died and the other was severely compromised, prompting immediate delivery by caesarean section under general anaesthetic. Twin boys were delivered. One infant was dead; the other was pale, apneic, and bradycardic and weighed 620 g. He was resuscitated by an experienced pediatrician, with the resuscitation videotaped. Mask ventilation was started using the Neopuff infant resuscitator (Fisher & Paykel, Auckland, New Zealand), a flow-driven, pressure-limited T-piece that provides a consistent operator-selected peak inspiratory pressure (PIP) and positive end-expiratory pressure (PEEP). The flow rate was 8 L/min of 100% oxygen, and the PIP and PEEP were initially set at 30 and 6 cm H₂O, respectively. The infant was ventilated at 60/min. Prolonged inflations were not used. A Masimo Radical pulse oximeter (Masimo, Irvine, Calif) was used to monitor preductal oxygen saturation (SpO₂) and heart rate (HR). At 1 minute of age, the HR was 80 bpm, the SpO₂ was 76%, and chest wall movement was not seen.

The infant was intubated with a 2.5-mm ETT by 2 minutes of age and ventilated using the same pressures. No chest wall movement was seen with inflations, the HR remained < 100 bpm, and SpO₂ was 55%. The infant was then reintubated. His chest wall still did not move, and his condition did not improve with ventilation, so an ETCO₂ detector (Pedi-Cap; Nellcor Puritan, Bennett, Calif) was placed between the ETT and Neopuff to verify the ETT position. No color change was seen, suggesting that the ETT was not in the trachea. At this time, HR was 75 bpm and SpO₂ was 70%. The resuscitator confirmed correct placement of the ETT by direct laryngoscopy, then increased the PIP and PEEP in increments to 70 and 8 cm H₂O, respectively. There was still no chest wall movement with ventilation or color change in the Pedi-Cap.

At age 7 minutes, a 240-mL self-inflating bag (Laerdal, Wappingers Falls, NY) was used with the pop-off valve occluded so that a very high (but unmeasured) PIP was used to inflate the lungs. Within 15 seconds, the Pedi-Cap detected sufficient exhaled CO₂ to cause a color change. The HR then rose above 100 bpm, and chest wall movement was seen.
observed 30 seconds thereafter. Over the next 2 minutes, the SpO2 rose gradually to 85%. The PIP was reduced to produce shallow but obvious chest wall movement. The infant was transferred to the neonatal intensive care unit receiving 100% oxygen, with PIP and PEEP set at 45 and 8 cm H2O, respectively. No epinephrine, volume expansion, or external cardiac massage was used during the resuscitation.

The infant’s initial hemoglobin was 2.8 g/dL, and a diagnosis of twin-to-twin transfusion syndrome was made. He was subsequently ventilated for 20 days without developing air leaks and then treated with nasal continuous positive airway pressure and a low fraction of inspired O2.

**DISCUSSION**

Delivery room intubation is difficult, with success rates of <40% in infants <28 weeks gestational age. Signs of correct ETT placement include direct visualization, chest wall movement with inflation, condensation inside the ETT, auscultation of breath sounds, and improvement in HR and color. These observations are subjective, however, and sometimes can produce false-positive impressions of tracheal ventilation.

The pressures required to inflate the newborn lung are variable and unpredictable. They need to be above the airway critical opening pressure and may be >70 cm H2O. The International Liaison Committee on Resuscitation has stated that visible chest wall expansion is a more reliable sign of appropriate inflation pressures than in-line manometry on the ventilation device. Others suggest that in very preterm infants, visible chest wall movement may represent excessive tidal volumes.

The Pedi-Cap is a colorimetric semiquantitative ETCO2 detector used to verify correct ETT placement. In the presence of even small amounts of expired CO2, the indicator turns yellow, which reverses to purple in inspiration when the measured ETCO2 is <0.5% (4 mm Hg). A persistent purple color is usually due to incorrect ETT placement, but also may be seen in the setting of extremely low pulmonary perfusion. In this case, the ETCO2 detector did not change color because the infant had extremely noncompliant lungs that could not be ventilated until very high inflating pressures were used.

The ETCO2 detector gave the false impression that the ETT was not in the trachea, which disagreed with the impression gained from clinical assessment. Such false-negative results are a matter of concern, because they delay appropriate intervention during resuscitation. Once the resuscitator was confident that the ETT was placed correctly, the inflating pressures were increased until ventilation was achieved. The ETCO2 detector identified gas exchange before the chest wall was seen to move, suggesting that it is more sensitive for assessing adequacy of ventilation. Shortly after exhaled CO2 was detected by the Pedi-Cap, the HR increased. This sequence suggests that the primary problem was inadequate ventilation rather than low cardiac output leading to poor pulmonary perfusion.

Although evidence for the routine use of expired CO2 during neonatal resuscitation is limited, these devices are highly sensitive in detecting tracheal intubation, and there appears to be a low likelihood of causing harm. This case illustrates that clinicians need to be aware that failure of the device to change color may be due to inadequate ventilation through a correctly placed ETT.

**REFERENCES**

ANNUAL INCREASE IN BODY MASS INDEX IN CHILDREN WITH ASTHMA ON HIGHER DOSES OF INHALED STEROIDS

MEGHNA JANI, MB, CHB, SIMON OGSTON, PHD, AND SOMNATH MUKHOPADHYAY, MD, PHD

There is a greater annual increase in body mass index in children with asthma receiving inhaled steroids at a dose $\geq 400 \mu g/day$ (0.5 kg/m$^2$/year; n = 100) compared with those receiving $\leq 200 \mu g/day$ (0.1 kg/m$^2$/year; n = 98) ($P = .0003$). This is consistent with an annual increase in body fat in children with asthma receiving $\geq 400 \mu g/day$ of inhaled steroids. (J Pediatr 2005;147:549-51)

In children, the long-term use of oral corticosteroids is associated with a marked longitudinal increase in body mass index (BMI).\(^1\) This could result from regulation of nutrient ingestion and metabolism that alters total caloric intake and body weight gain over a period of time.\(^2\) Although the use of long-term inhaled corticosteroids in doses $\geq 400 \mu g/day$, as opposed to $\leq 200 \mu g/day$, is associated with systemic effects in children with asthma,\(^3\),\(^4\) the hypothesis that such use leads to a longitudinal increase in BMI in this population has not been tested.

Annual change in BMI has been used to study the long-term effect of another intervention (physical activity) on the caloric nutritional status of children.\(^5\) We addressed the hypothesis that in the asthma clinic setting there is a significantly greater annual change in BMI in the population on high-dose inhaled steroids than in the population on low-dose inhaled steroids. Although we did not exclude patients, we felt that the age distribution (1 to 12 years) in our clinic largely excluded the 2 periods of significant change in BMI with age (<1 year and during puberty), thus allowing a direct comparison of the annual rate of change in BMI between the 2 groups.

METHODS

The Tayside children’s asthma database (age 0 to 18 years) was used to identify 2 groups of patients, those taking inhaled steroids at doses of $\leq 200 \mu g/day$ and $\geq 400 \mu g/day$. We did not analyze the data on children taking doses of $>200 \mu g/day$ to $<400 \mu g/day$. Patients needed to have at least 2 clinic visits with an approximate 12-month (range, 11 to 15 months) interval between the visits. There was no change in the steroid dose in the subjects over the study period. Patients on other regular medications (eg, salmeterol, montelukast) or very high doses of inhaled steroids ($\geq 1000 \mu g/day$) were excluded from the study.

Patient height, weight, and daily dose of inhaled steroids were documented at the start (visit 1) and the end (visit 2) of the 12-month period. For patients with long-term follow-up, only the first 12-month period was included for this study. Age, sex, symptom scores,\(^6\) type of inhaler device, type of inhaled steroid, and deprivation scores\(^7\) were also documented. BMI was calculated using the standard formula (weight in kg/height in m$^2$) for both visits, and the annual change in BMI was calculated as described previously.\(^5\) International age-adjusted standards were used to calculate BMI z-score percentiles for the children.\(^8\) Multiple linear regression using SPSS software (SPSS, Chicago, Ill) was used to study the effects of the foregoing potential confounders, with the rate of change of BMI per year as the dependent variable. The study design was approved by the Tayside Ethics Committee.

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Funded by Scottish Enterprises Tay-side and the Gannochy Trust, Perth, Scotland.

Submitted for publication Nov 2, 2004; last revision received Apr 8, 2005; accepted May 6, 2005.

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0022-3476/$ - see front matter

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10.1016/j.jpeds.2005.05.018

| BMI | Body mass index | CI | Confidence interval |
RESULTS

Table compares the characteristics of the groups at visit 1 and visit 2. There is a significant difference (independent samples t-test; P = .0003) between the annual rate of increase in BMI in the children on ≥200 μg/day as compared to the children on ≥400 μg/day of regular inhaled steroids (0.44 kg/m²/year; 95% confidence interval [CI] = 0.19 to 0.69 kg/m²/year). The difference between the 2 annual rates of change in BMI z-score percentiles (0.28; 95% CI = 0.14 to 0.41) was also significant (P = .000043).

Using multiple regression, the only significant (P < .05) independent variables affecting the rate of increase in BMI in the combined sample (n = 198) were the dose of inhaled steroids and the child’s age. Others variables entered into the model (ie, sex, asthma symptom score, deprivation scores, type of inhaler device, and type of inhaled steroids) produced no significant effect on the annual rate of change in BMI. A regression model fitting age and daily dose of inhaled steroids gave an r² value of 10.4%. Using this method, the age-adjusted difference in rate of change in BMI between the 2 groups of children (inhaled steroid dose ≥200 μg/day versus ≥400 μg/day) was 0.47 ± 0.12 kg/m²/year (P = < .0001).

DISCUSSION

The study demonstrates that the regular administration of ≥400 μg/day of inhaled steroids in children with asthma is...
associated with a significantly higher rate of increase in BMI over a year, compared with the rate observed in children on ≤200 μg/day of inhaled steroids. A significant shift in BMI was seen in the children on regular doses of ≥400 μg/day to a higher z-score percentile level. The shift in mean BMI in the children on higher doses compared to those on lower doses was not accompanied by any significant differences in mean weight or height between the 2 cohorts over the study period (Table).

BMI is a good measure of body fat that partly controls for the influence of height. The observations are thus consistent with a small, but significant, annual increase in body fat between the 2 groups of patients. It is not known whether this effect is transient, levels off over time, or is cumulative year to year, in children who use relatively high doses of inhaled steroids consistently over a number of years.

In addition to the mechanisms proposed here, activity levels and asthma severity can affect BMI in children. Although we were unable to compare activity levels between the 2 groups, asthma severity, as measured by asthma symptom scores, was not significantly different between the 2 groups.

There were no significant differences between the 2 cohorts in any of the variables documented (including BMI) at the start of the comparative study. This could be because the children in the cohort on higher doses of inhaled steroids had not been receiving these doses for a sufficiently long period to lead to a significant increase in BMI. We did not document retrospectively the asthma therapy received by the patients over the year before the study period.

A higher BMI quartile within the population is associated with a greater prevalence of childhood asthma. It is not known, however, whether the children in the higher BMI quartiles were on higher doses of inhaled steroids or whether their asthma was more severe. A cross-sectional study in adults with asthma has not demonstrated a relationship between the intake of asthma medication and BMI. Inhaled steroid use is variable over time, but it may contribute to an increase in population BMI. There is a need for a longitudinal, prospective study comparing the effects on BMI of the long-term use of high-dose versus low-dose inhaled steroids in children with asthma.

We acknowledge the support received from Vicky Alexander, Donald Macgregor, Shirley Binnie, Helen Donald (NHS Tayside), Joanne Elwin, and Anna Crighton (University of Dundee).

REFERENCES
We describe brain lesions in a patient with a monocarboxylate transporter 8 mutation. Imaging showed a high T2 lesion in the left putamen at age 3 and a right putamen lesion at age 6. Cerebrospinal fluid free thyroxine concentrations were low, with normal 3,3',5-triiodothyronine concentrations. (J Pediatr 2005;147:552-4)

Monocarboxylate transporter 8 (MCT8, or SLC16A2), encoded by the MCT8 gene located on Xq13.2, is a protein of 613 amino acids with 12 predicted transmembrane domains. MCT8 is considered a specific and active transporter of thyroid hormones across the cell membrane.

Male patients with various x-linked MCT8 mutations are described as having a combination of abnormal circulating thyroid hormones and characteristic neurologic involvement. Thyroid function tests reveal low blood thyroxine (T4), high 3,3',5-triiodothyronine (T3), and borderline-high thyroid-stimulating hormone (TSH) levels. Patients characteristically exhibit central hypotonia and paroxysmal dystonia. There is an absence of gross motor and mental development, possibly because of a defect in T3 uptake into neurons through MCT8.

We report a 6-year-old Japanese boy with a novel mutation of the MCT8 gene. Brain magnetic resonance imaging (MRI) at age 3 years revealed a T2 high-signal lesion in the left putamen (Figure 1A). The child exhibited poor gaze contact and no verbal communication, and he was quadriplegic with severe peripheral muscle atrophy. At age 6, brain MRI revealed a contralateral lesion in the right putamen and progressive atrophy of the cerebral gray and white matter, thalamus, basal ganglia, and midbrain (Figure 1B). Thyroid function tests demonstrated high free T3 level (6.82 pg/mL; normal, 2.3 to 4.3 pg/mL), low free T4 level (0.56 ng/dL; normal, 0.9 to 1.7 ng/dL), and high TSH level (3.49 mU/L; normal, 0.5 to 5.0 mU/L), compared with the peripheral high level of T3.

CASE REPORT

The patient is the second child born to nonconsanguineous parents. Pregnancy was complicated by polyhydramnios in the third trimester, but delivery was uneventful at 40 weeks gestation. There was no family history of neurologic disease. Birth weight was 3145 g, and occipitofrontal circumference (OFC) was 32 cm. Neonatal screening for congenital hypothyroidism demonstrated a normal TSH level.

At age 5 months, the child was referred to Kanazawa Medical University Hospital because of generalized hypotonia and poor feeding. He had poor weight gain and exhibited a weight of 5770 g (3rd percentile), height of 63.3 cm (50th percentile), and OFC of 43.3 cm (50th percentile). He child had generalized hypotonia with paroxysmal dystonia of the lower limbs. A computed tomography scan of the brain and an electroencephalogram revealed no abnormalities. Feeding problems persisted, necessitating tube feeding. At age 2 years, the boy was not able to sit, crawl, or stand. Brain MRI at age 3 years demonstrated a T2 high-signal lesion in the left putamen (Figure 1A). The child exhibited poor gaze contact and no verbal communication, and he was quadriplegic with severe peripheral muscle atrophy. At age 6, brain MRI revealed a contralateral lesion in the right putamen and progressive atrophy of the cerebral gray and white matter, thalamus, basal ganglia, and midbrain (Figure 1B). Thyroid function tests demonstrated high free T3 level (6.82 pg/mL; normal, 2.3 to 4.3 pg/mL), low free T4 level (0.56 ng/dL; normal, 0.9 to 1.7 ng/dL), and high TSH level (3.49 mU/L; normal, 0.5 to 5.0 mU/L), compared with the peripheral high level of T3.

CSF  Cerebrospinal fluid  T3  3,3',5-triiodothyronine  MCT8  Monocarboxylate transporter 8  T4  Thyroxine  MRI  Magnetic resonance imaging  TSH  Thyroid-stimulating hormone  OFC  Occipitofrontal circumference
Dumitrescu et al\(^2\) and Friesema et al\(^3\) independently reported mutations in the *MCT8* gene in patients with these characteristic neurologic and thyroid abnormalities. Therefore, we conducted mutational analyses of the *MCT8* gene in this patient. Genomic DNA was obtained from blood of the boy and his mother after receiving written informed consent. We identified a c.485T→C transition in exon 1, resulting in a S107P substitution of the protein encoded and a GTG duplication between nucleotide positions 869 and 871 in exon 2. The duplication introduced an extra valine residue between positions 235 and 236 in the MCT8 protein. This mutation, located in the second transmembrane segment, created an Hph-I restriction site. The 325-bp DNA generated by polymerase chain reaction amplification of the mutant allele exon 2 produced 3 bands—84 bp, 119 bp, and 125 bp—when digested with Hph-I. In contrast, DNA amplified from the wild-type allele and digested with the same enzyme produced 2 bands of 119 bp and 206 bp. The boy was hemizygous for the mutation, and the mother demonstrated no mutation; therefore, the novel mutation occurred de novo in the germ line of the mother (Figure 2).

Although the patient had been treated with thyroxine 85 \(\mu\)g/day for 1 year, he still had thyroid function abnormalities, with high serum free \(T_3\) and normal free \(T_4\) and TSH levels, and there was no sign of improvement. While he was receiving thyroxine treatment, CSF free \(T_4\) levels were approximately half the control levels; however, CSF free \(T_3\) and TSH levels were within control range (Table).

![Figure 1](image1.png)

*Figure 1.* Axial (TR: 2500 ms, TE: 90 ms) MRIs at (A) age 3 years showing an abnormally high signal intensity in the left putamen (*arrow*) and (B) age 6 years revealing progression of the left putamen lesion (*arrow*) and emergence of a contralateral lesion in the right putamen (*open arrow*), as well as progressive atrophy of the cerebral gray and white matters, thalami, basal ganglia, and midbrain compared with (A).

![Figure 2](image2.png)

*Figure 2.* Hph-I cleaved the 325-bp polymerase chain reaction product of exon 2 into 2 fragments of 119 bp and 206 bp in the wild-type allele and into 3 fragments of 84 bp, 119 bp, and 125 bp in the mutant allele. Lane 1, the patient; lane 2, the mother; lane 3, size marker.
DISCUSSION

We identified the S107P substitution and valine duplication in our patient. Because the S107P mutation was observed in homozygosity in his mother and was found in 100% of 50 female anonymous Japanese subjects, the mutation is thought to be a single nucleotide polymorphism. Therefore, we infer that the valine duplication was responsible for this disorder.

Our patient had putamen involvement and half the normal CSF level of free T₄, accompanied by normal CSF levels of T₃ and TSH. These observations have not previously been reported with this disorder. It has been suggested that the blood brain barrier (BBB) but not the choroids plexus-CSF barrier is an essential site of T₄ transport into the brain.⁴ Recent expression studies have suggested that organic anion-transporting polypeptide might mediate T₄ transport into the brain through the blood-brain barrier⁵ and that MCT8 might play a crucial roll in T₃ transport into neurons.⁶ However, the basis of the difference in CSF thyroid hormones between the patient and normal controls remains to be elucidated.

Mahant et al⁷ described a patient with focal myoclonic-dystonia of the leg possibly resulting from a lesion of the posterolateral putamen. This lesion of the left putamen is similar to that seen in our patient at age 3 years. Although patients with MCT8 mutations were previously reported to have normal MRI findings, the putamen lesions in our patient appeared to have caused paroxymal dystonia in the lower limbs. In our patient, brain MRI at age 6 years also demonstrated progressive atrophy of the cerebrum, basal ganglia, and midbrain (Figure 1B). Therefore, this disorder seems to be degenerative. Further studies in patients or animal models with MCT8 deficiency are needed to determine whether inadequate T₃ in developing neurons leads to a degenerative process in the brain.

REFERENCES


Table. Results of thyroid function tests in CSF and serum

<table>
<thead>
<tr>
<th></th>
<th>Free T₃ (pg/mL)</th>
<th>Free T₄ (ng/dL)</th>
<th>TSH (mU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>0.44</td>
<td>0.10</td>
<td>0.019</td>
</tr>
<tr>
<td>Controls (n = 5, range)</td>
<td>0.46 ± 0.03 (0.41 to 0.49)</td>
<td>0.19 ± 0.01 (0.17 to 0.21)</td>
<td>0.022 ± 0.004 (0.017 to 0.027)</td>
</tr>
<tr>
<td>Serum levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>7.28</td>
<td>1.04</td>
<td>0.38</td>
</tr>
<tr>
<td>Normal</td>
<td>(2.3 to 4.3)</td>
<td>(0.9 to 1.7)</td>
<td>(0.5 to 5.0)</td>
</tr>
</tbody>
</table>
MUTATION ANALYSIS SHOULD BE PERFORMED TO RULE OUT \( \gamma C \) DEFICIENCY IN CHILDREN WITH FUNCTIONAL SEVERE COMBINED IMMUNE DEFICIENCY DESPITE APPARENTLY NORMAL IMMUNOLOGIC TESTS

RAZ SOMECH, MD, AND CHAIM M. ROIFMAN, MD, FRCP

To study the correlation between genotype and phenotype in X-linked SCID, we have characterized the presentation of 2 unrelated patients. Both had infections suggestive of immunodeficiency, but their immune function and lymphoid tissues were normal. They were found to have an identical R222C mutation in the \( \gamma C \) gene. (J Pediatr 2005;147:555-7)

Severe combined immunodeficiency (SCID) is a primary immune disorder with invariable, profound T-lymphocyte dysfunction. Patients are vulnerable to the onset of serious infections within the first few months of life. The selective block in the differentiation of lymphocytes may vary according to the molecular defect. The ability to predict severity and clinical phenotype based on genotypic analysis—namely, genotype-phenotype correlation—might be of great importance to the management of such patients. Of all types of SCID, a genotype-phenotype correlation was observed only for adenosine deaminase deficiency.

The most common SCID type, accounting for 50% of cases, is linked to the X chromosome, where the interleukin (IL)-2 receptor \( \gamma \) (IL2R\( \gamma \)) receptor is located. The gene encodes for the common \( \gamma \) chain (\( \gamma C \)), a component shared by several cytokine receptors (IL-2, -4, -7, -9, -15, and -21). Aberrations in this gene were first reported in 1993. The vast majority of mutations are unique nucleotide substitutions resulting in missense or nonsense mutations. The typical case of \( \gamma C \)-deficient SCID is characterized by the near-complete absence of T lymphocytes and natural killer (NK) lymphocytes, normal numbers of circulating B lymphocytes, abnormal response to mitogenic stimulation, and a rudimentary dysplastic thymus gland.

We describe 2 unrelated patients with atypical presentation of X-linked SCID, caused by a R222C mutation in the \( \gamma C \) gene.

METHODS

Proliferative Responses and \( \gamma C \) Sequence

Cell surface markers of peripheral blood cells were determined by immunofluorescent staining and flow cytometry (Epics V, Beckman Coulter, Fullerton, Calif), with antibodies purchased from Beckman Coulter. Lymphocyte proliferative responses to mitogens were using standard techniques. Polymerase chain reaction (PCR) and genomic sequencing of \( \gamma C \) was performed as described previously.

RESULTS

Clinical Features

The patients grew and developed normally and exhibited no symptoms suggestive of immunodeficiency until age 6 to 9 months, when they developed progressive respiratory symptoms. Failure to thrive was noticed in parallel or subsequent to diagnosis. Physical

<table>
<thead>
<tr>
<th>BMT</th>
<th>Bone marrow transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>cDNA</td>
<td>complementary DNA</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IL2R( \gamma )</td>
<td>Interleukin-2 receptor ( \gamma )</td>
</tr>
<tr>
<td>MUD</td>
<td>Matched unrelated donor</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>SCID</td>
<td>Severe combined immune deficiency</td>
</tr>
<tr>
<td>( \gamma C )</td>
<td>( \gamma ) chain</td>
</tr>
<tr>
<td>TCR-( \beta )</td>
<td>T-cell receptor V beta region of immunoglobulin</td>
</tr>
</tbody>
</table>
examination findings in both patients were normal, including normal-sized tonsils and cervical lymph nodes (Table I). Both patients suffered from severe life-threatening infections. At age 6 to 9 months, they were diagnosed with interstitial Pneumocystis carinii pneumonia; in addition, patient 1 developed oral thrush and recurrent skin infections. Both patients received matched unrelated bone marrow transplantations (BMTs). Patient 1 is alive and well with full engraftment 4 years after a second matched unrelated donor (MUD) BMT, and patient 2 is alive and well 9 years after a MUD BMT.

**Immunologic Studies**

Both patients had normal peripheral lymphocyte markers, including CD3+ T lymphocytes and the subsets CD4+ and CD8+ cells as well as CD19+ B lymphocytes and CD56+ NK cells (Table II). Maternal T-cell engraftment was ruled out by HLA typing and karyotyping. Examination of T-cell receptor V beta region of immunoglobulin (TCR-Vβ) families using PCR revealed full normal representation that suggested normal thymocyte selection. In vitro T-lymphocyte responses to phytohemagglutinin or to CD3 antibody were consistently comparable with those of control samples. The addition of exogenous IL-2 to CD3 antibody resulted in a significant increase in proliferation of control cells but not in patient 1 or patient 2 cells. This finding was consistent with a defect in IL-2R expression or in a component of its downstream signaling pathway.

Chest radiography (in patients 1 and 2) and ultrasonography (in patient 2) revealed a normal-sized thymus gland. We previously demonstrated that the thymic biopsy of patient 2 contained Hassall corpuscles and had a clear cortico-medullary demarcation.12 Humoral immunity was assessed by measuring serum immunoglobulin levels and detecting specific antibody response after vaccination. Both patients had IgG, IgM, and IgA levels within normal ranges, but specific antibodies to tetanus and polio were absent despite immunization.

**Mutation Analysis**

The familial history of a maternal cousin with SCID combined with the lack of T-lymphocyte response to IL-2 prompted the analysis of the g<sub>c</sub> gene in patient 2.12 Sequencing of patient 2’s cDNA revealed a 1-bp C to T transition at position 664, which predicts an arginine to cysteine substitution at residue 222. This residue is localized in the extracellular domain, close to the transmembrane domain. Based on the identical presentations in both patients, a similar analysis of the g<sub>c</sub> chain was performed on patient 1. An identical homozygous R222C mutation was identified. The mothers of both patients had a similar mutation albeit on 1 allele only (heterozygosity).

**DISCUSSION**

Patients with typical X-linked SCID present at age 6 to 8 months with severe pneumonitis, protracted diarrhea, and failure to thrive. Tonsils and palpable lymph nodes are absent, and the thymus is small and dysplastic.11 The numbers of circulating CD3+ T lymphocytes and NK lymphocytes are markedly reduced or absent, whereas B lymphocytes appear

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**Table I. Clinical presentation and outcome of patients**

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Patient 1</th>
<th>Patient 2</th>
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<tbody>
<tr>
<td>Age at presentation</td>
<td>6 months</td>
<td>9 months</td>
</tr>
<tr>
<td>Growth</td>
<td>10th percentile</td>
<td>25th percentile</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Tonsils</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Thymus imaging</td>
<td>Normal size</td>
<td>Normal size</td>
</tr>
<tr>
<td>Biopsy</td>
<td>Not performed</td>
<td>Normal morphology</td>
</tr>
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<table>
<thead>
<tr>
<th>Infections</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chest</td>
<td>Pneumocystis pneumonia</td>
<td>Pneumocystis pneumonia</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Skin and mucous membranes</td>
<td>Oral thrush, skin infections</td>
<td>None</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Management</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMT</td>
<td>First 6/6 MUD failed; second 6/6 MUD successful</td>
<td>6/6 MUD successful</td>
</tr>
<tr>
<td>Engraftment</td>
<td>&gt;90% donor cells</td>
<td>&gt;90% donor cells</td>
</tr>
<tr>
<td>Outcome</td>
<td>Alive and well 4 years post-BMT</td>
<td>Alive and well 9 years post-BMT</td>
</tr>
</tbody>
</table>

---

**Table II. Studies of humoral and cellular immunity**

<table>
<thead>
<tr>
<th>Serum immunoglobulins (g/L)</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Normal range</th>
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<tbody>
<tr>
<td>IgG</td>
<td>2.3</td>
<td>2.7</td>
<td>2.3 to 14.1</td>
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<td>IgM</td>
<td>0.65</td>
<td>1.1</td>
<td>0.1 to 1.4</td>
</tr>
<tr>
<td>IgA</td>
<td>0.7</td>
<td>0.3</td>
<td>0.1 to 0.8</td>
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<table>
<thead>
<tr>
<th>Specific antibodies</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetanus (U/mL)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&gt;0.04</td>
</tr>
<tr>
<td>Polio</td>
<td>&lt;1.8</td>
<td>&lt;1.8</td>
<td>&gt;1.16</td>
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<table>
<thead>
<tr>
<th>Mitogenic responses*</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Normal range</th>
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<tbody>
<tr>
<td>CD3</td>
<td>96/108</td>
<td>110/108</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>1.6/4.3</td>
<td>0.9/3.1</td>
<td></td>
</tr>
<tr>
<td>CD3 + IL-2</td>
<td>103/194</td>
<td>76/139</td>
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<table>
<thead>
<tr>
<th>Lymphocyte markers (cells/μL)</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Normal range</th>
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<tr>
<td>CD3</td>
<td>2070</td>
<td>3601</td>
<td>2000 to 6900</td>
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<td>CD4</td>
<td>1264</td>
<td>1916</td>
<td>1400 to 5100</td>
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<td>CD8</td>
<td>611</td>
<td>1079</td>
<td>600 to 2200</td>
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<tr>
<td>CD19</td>
<td>647</td>
<td>1319</td>
<td>700 to 2500</td>
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<tr>
<td>CD56</td>
<td>225</td>
<td>339</td>
<td>100 to 1000</td>
</tr>
</tbody>
</table>

*Expressed as stimulation index.
normal. This is the common presentation of X-linked SCID regardless of the mutation detected in the γc gene.13

We have previously reported12 a patient with a remarkably normal-appearing immunologic phenotype but yet a profound susceptibility to infection (patient 2 here). An identical R222C mutation caused a very similar phenotype in an unrelated patient.14 We report here a third patient unrelated to the other 2 and of a different ethnicity in whom a R222C mutation was identified. Together, these cases demonstrate for the first time a consistent correlation between this mutation and a nearly normal-appearing immune evaluation. The arginine to cysteine substitution (R222C) in the extracellular domain of the γc chain appears to elicit this unusual presentation. Other mutations in close by regions lead to an inconsistent phenotype.10,13

It remains unclear why this mutation causes this atypical phenotype. Less than optimal binding of IL-2 or other cytokines may underline this clinical susceptibility to severe infections. Although a rare occurrence, R222C-mutated X-linked SCID may pose a diagnostic challenge. Our report should encourage the consideration of R222C-mutated X-linked SCID on the bases of clinical susceptibility to Pneumocystis pneumonia and respiratory and enteric viruses despite normal circulating lymphocytes and normal-appearing lymphoid glands.

REFERENCES


A 16-year-old girl was referred because of halo nevi (Figure 1). She was well and had no gastrointestinal symptoms. Growth and puberty were normal. She was screened for thyroiditis, Addison disease, and celiac disease. All tests were normal, except she had elevated endomysium antibody levels. A duodenal biopsy was performed, and total villous atrophy was shown (Figure 2). She received a gluten-free diet, and the endomysium antibodies disappeared. She experienced no physical or psychological change, and her weight and halo nevi remained the same. Normal histology with the gluten-free diet was shown with the control biopsy. During the following 6 months, we saw 2 more girls with previously diagnosed celiac disease who were on a strict gluten-free diet, both with halo nevi.

Celiac disease is well known to be associated with certain skin disorders, such as dermatitis herpetiformis, psoriasis, vitiligo, and alopecia areata. The halo nevus is almost always a benign condition, except when found congenitally and not as in adults associated with melanoma. Halo nevi may also be found in autoimmune disorders such as thyroiditis and Mb. In the literature, we have found no association between celiac disease and halo nevi.

In 2001 Lai et al1 published an article questioning whether it was necessary to perform a biopsy when finding halo nevi in childhood. Their question concerned skin biopsy. We want to raise the question with this article whether a biopsy from the duodenum is more appropriate.

REFERENCES

Growth hormone deficiency and HIV infection

To the Editor:

Watson and Counts describe 2 HIV-infected children with growth failure and growth hormone (GH) deficiency and summarize the 2 cases of the only other such children reported (one by us). We want to provide further follow-up to our previous report, which now leads us to question the role of GH therapy for HIV-associated growth failure.

At the time of our publication, our patient was 10 years old, and her growth velocity had doubled from 2.5 cm/year to 5 cm/year with the administration of GH 2 mg 3 times/week (Figure). However, further catch-up growth was not seen, and her skeletal maturation remained unchanged over 12 months. Increasing the frequency of the GH injections did not improve her growth velocity, and GH was discontinued at the child’s request. In spite of eventual suppression of plasma HIV viral RNA to <400 copies/mL (as more antiretroviral agents became available), her growth velocity slowed, and her puberty was delayed. After living for 20 years with her perinatally acquired HIV infection, she discontinued her antiretroviral therapy over a period of months; she died of cryptococcal meningitis at 21 years of age.

![Figure](http://cdc.gov/growthcharts)
Like Watson and Counts’ second patient, our patient had onset of growth failure with aberrant GH secretion in mid-childhood; in contrast, she had neither brain dysfunction nor psychiatric illness confounding the cause of her growth failure. We and others have shown that protease inhibitor-based highly active antiretroviral therapy (PI-HAART) improves height z-scores, as well as weight-z scores. Unfortunately, our patient had likely achieved her final height before PI-HAART became available.

We still believe that growth failure is uncommon in HIV-infected children with successful virologic responses to PI-HAART and that, in these few children, GH deficiency should be considered. However, GH replacement therapy may not always lead to as much growth as expected.

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REFERENCES


Hydroxyurea as secondary prevention for stroke in children with sickle cell anemia

To the Editor:

Ware et al 1 described hydroxyurea as a secondary prevention for stroke in children with sickle cell anemia (SCA). I have a few comments that may provide an alternative viewpoint of the results.

First, I would like to acknowledge the hard work by Dr. Ware and his colleagues. They have systematically addressed an extremely difficult problem, namely, how to manage patients with sickle cell disease after initial strokes who, for a variety of reasons, will not or can not continue on blood transfusion therapy. Rather than haphazardly selecting different therapeutic options, Ware and his group decided to develop a prospective protocol to assess the utility of hydroxyurea administration. Such systematic efforts are far and few between in clinical medicine, and this effort should be applauded.

Ware et al comment that the rate of secondary strokes for persons treated with hydroxyurea and blood transfusion therapy were comparable. However, I think this comment may be slightly misleading. They quote a secondary stroke rate for hydroxyurea of 5.7 per 100 patient years. In addition, the rate of secondary strokes with blood transfusion therapy is quoted as 2.2 to 6.4 events per 100 patient years. However, the secondary stroke rates from these three articles are not directly comparable. In the study that Ware et al performed, patients had previously been on blood transfusion therapy for an average of approximately 4 years and then subsequently were switched over to hydroxyurea. In both the Scothorn and Pegelow articles, the stroke incidence rate with blood transfusion therapy included the entire period while on transfusion. As was noted in Scothorn et al, 2 the highest incidence rate of stroke occurs approximately 2 years after the initial stroke. To compare blood transfusion to hydroxyurea for secondary stroke prevention would require adjustment for the high-risk period of a second stroke.

Further, Ware et al appear to give equal weight to the two studies describing the secondary stroke rate for children receiving blood transfusion therapy, Scothorn et al 2 and Pegelow et al, 3 and only mention the higher of the two rates in the discussion. In the Pegelow 3 study, the secondary stroke rate is derived from 60 subjects followed for 192 patient years with an incidence of 4.2 strokes per 100 patient years (95% confidence interval 1.8 to 8.0 strokes per 100 patient years). In the Scothorn 2 study, 137 patients were followed for 1382 patient years with an incidence of 2.2 strokes per 100 patient years (95% CI 1.5 to 3.2 strokes per 100 patient years).

To mention only the higher of the two rates for stroke is slightly misleading given the more rigorous study design and larger number of patients followed for a longer duration in the Scothorn et al article. In addition, that rate quoted for Pegelow et al was incorrectly stated in the Ware et al article. Pegelow et al quoted a rate of 4.2 strokes per 100 patient years, not 6.4 strokes per 100 patient years.

When the two different methods for secondary prevention of strokes are compared, blood or hydroxyurea, the rate of an adverse outcome (stroke) must be noted. In Evidence Based-Medicine, 4 the common metric that directly compares this tradeoff is referred to as the numbers needed to harm (NNH). 5 Based on these data, if hydroxyurea is used instead of blood, one would anticipate that the NNH would be approximately 25, that is for every 25 patients treated with hydroxyurea, one patient will have a stroke in excess of what would be anticipated if patients were only receiving blood transfusion therapy. Only a formal evaluation of the tradeoffs between stopping blood transfusion therapy and starting hydroxyurea therapy can provide sufficient evidence for the best therapeutic option and determine whether the perceived benefits outweigh the risks. When presented with a choice of therapy, the cost of one additional preventable stroke may not outweigh the benefit of treatment with hydroxyurea.
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All applicants for certifying examinations must complete applications online during the registration periods. The final month of each registration requires payment of a late fee. The requirements for online applications may be found on the ABP Website: www.abp.org or may be obtained by contacting the ABP. Additional information including eligibility requirements and registration dates may also be found on the ABP Website.

General Pediatrics Examination:
- Examination Date: October 23 and October 24, 2006.
- Registration for first-time applicants: December 1, 2005 through May 1, 2006.

Subspecialty Examinations:
- Sports Medicine
- Examination Dates: To be determined by ABFM.
- Registration for first-time applicants: September 15, 2005, through December 15, 2005.

- Pediatric Cardiology - Examination Date: August 16, 2006.
- Registration for first-time applicants: September 15, 2005, through December 15, 2005.
- Pediatric Critical Care Medicine - Examination Date: August 18, 2006.
- Pediatric Pulmonology - Examination Date: August 17, 2006.
- Registration for first-time applicants: September 15, 2005, through December 15, 2005.
- Pediatric Rheumatology - Examination Date: November 10, 2006.
- Developmental-Behavioral Pediatrics - Examination Date: November 15, 2006.
- Pediatric Emergency Medicine - Examination Date: November 15, 2006.
- Pediatric Hematology-Oncology - Examination Date: November 17, 2006.
- Transplant Hepatology - Examination Date: To be determined by ABIM.
- Medical Toxicology - Examination Date: November 14, 2006.
- Registration for re-registrants: March 16, 2006, through June 16, 2006.