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METABOLIC SCREENING IN THE NEWBORN

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INTRODUCTION

The concept of metabolic screening for the recognition, diagnosis and treatment of inborn errors of metabolism has evolved as new methodology for detection and improved treatment have become available.¹ The diagnosis of metabolic disorders is challenging because of (1) the episodic nature of metabolic illness, (2) the wide range of clinical symptoms that are also associated with more common conditions, (3) the low incidence of these disorders, (4) the consequent lack of experience among the pediatric sub-specialties, and (5) the need for specialty testing. Although the incidence of each disorder is in the range of 1:10⁴ to 1:10⁷, there are thousands of known patients with metabolic disorders. It is probable that collectively, the total incidence exceeds 1:4000. Consequently they certainly account for significant morbidity and mortality in the newborn population.

Without doubt, the most opportune time to diagnose an inborn error of metabolism is at birth. Early recognition

and correct diagnosis enables appropriate treatment, without which tragic outcomes are all too common. Public awareness of metabolic diseases was all but unknown in the United States until 1964; at that time widespread neonatal testing was introduced for phenylketonuria (PKU), a disease resulting from lack of phenylalanine hydroxylase activity and affecting about 1:23,000 newborns. Since then, most states have expanded screening to a handful of additional diseases that fit the "PKU paradigm" – a treatable disease for which an inexpensive screening test is available and that has dire consequences if left untreated.² Currently, most states are screening for at least four disorders: PKU, congenital adrenal hyperplasia of the 21-hydroxylase type, galactosemia because of galactose-1-phosphate uridylyltransferase deficiency, and congenital hypothyroidism due to defects of thyroxine synthesis.

The case of PKU screening exemplifies the benefits of early diagnosis of a metabolic disease to patients, their families and society as a whole. The benefits of finding and treating these patients far outweigh the costs of screening the entire population.

Expanded newborn screening is a very recent development that utilizes tandem mass spectrometry (MS/MS) to screen for more than 20 inborn disorders of metabolism from a single blood spot.¹⁻³ This review explores the development and application of MS/MS as a clinical diagnostic testing method and its impact on newborn screening.^{2,4}

ACYLCARNITINES AND DISORDERS OF FATTY ACID AND AMINO ACID CATABOLISM

The driving force for applying MS/MS in clinical diagnostics was the need to analyze a class of compounds called the acylcarnitines which can accumulate from the defective catabolism of fatty acids and certain amino acids, especially leucine, isoleucine and valine.¹⁻³ These normal metabolic pathways are located in the mitochondria, and are mediated by coenzyme A (CoA) leading to metabolic end-products, such as acetyl-CoA. When there is a metabolic block, abnormal acyl-CoA species accumulate inside the

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mitochondria, and can only escape by biochemical transformation using alternate pathways. One of the most important detoxification pathways is an exchange reaction to form a corresponding acylcarnitine – a biochemical end-product that can cross mitochondrial membranes and exit the cell (Figure 1).

A patient with a defect of fatty acid oxidation typically develops symptoms after several hours of fasting, as may occur during an intercurrent illness. Reserves of glucose are exhausted and the cell switches to the fatty acid and gluconeogenic amino acid oxidative pathways as the primary energy sources. In a defect of fatty acid oxidation, abnormal metabolites can accumulate very rapidly and result in overwhelming cellular dysfunction – causing the symptoms of metabolic decompensation. Depending on the pathway affected, these symptoms can include vomiting, lethargy, respiratory distress, apnea, coma, cardiac arrhythmias, often accompanied by acidosis, ketosis, hypoglycemia and hyperammonemia. It is during such episodes that patients are at high risk for permanent neurological damage. A delay in emergency treatment of a few hours can be fatal. If intravenous glucose is administered on time, the symptoms and the biochemical abnormalities are rapidly ameliorated. The most common defect of fatty acid oxidation is medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. It may present with Reye-like symptoms, or sudden death, yet there can be affected asymptomatic siblings within the family. Severe outcomes are entirely preventable by appropriate treatment.

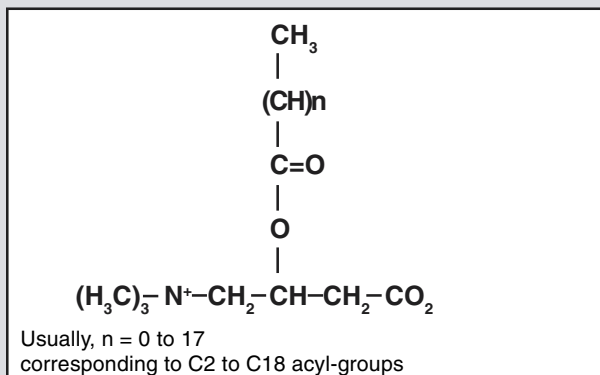
The acylcarnitines in blood reflect the primary accumulating mitochondrial acyl-CoA metabolites in

disorders of fatty acid and amino acid catabolism. Thus, an acylcarnitine “profile” will recognize almost all of the defects in these pathways. While older methods cannot detect acylcarnitines, these metabolites are readily amenable to MS/MS coupled with a “soft” ionization technique such as electrospray (ESI) or fast atom bombardment (FAB).^{1-3,5}

TANDEM MASS SPECTROMETRY AND THE ANALYSIS OF MIXTURES

The tandem mass spectrometer, MS/MS, usually consists of a pair of analytical quadrupole mass analyzers separated by a reaction chamber or collision cell. The triple quadrupole MS/MS is a modern system for analyzing complex mixtures. The mixture to be analyzed undergoes a “soft” ionization to create predominantly quasi-molecular ions, and is injected into the first quadrupole, which separates the molecular ions from each other. The ions then pass in order of mass/charge (m/z , ratio) into the reaction chamber or collision cell, where they are subjected to controlled fragmentation by collisions with an inert gas such as argon or helium. These fragments of the molecular ions then pass into the second analytical quadrupole where they are analyzed according to their m/z ratio. Electrospray ionisation is a ‘soft ionisation’ technique which enables the direct analysis of polar or high molecular weight biological substances like amino acids, acylcarnitines and proteins. These compounds can be detected and quantified directly from the solution without need to volatilize the sample. It offers excellent sensitivity (sub-picomole detection limits). Because separation of compounds in the mixture is by differences in mass spectral behavior instead of by column

Figure 1
Acylcarnitine



Structure of acylcarnitine intermediates in fatty acid oxidation inside the mitochondria. For example, in MCAD deficiency the accumulated acylcarnitine has a side chain containing 8 carbons, such that $n = 7$ as depicted here.

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chromatography, the entire process from sample injection and ionization to mixture analysis and data acquisition by computer takes only seconds.

The acylcarnitine “profile”, generated from a small amount of blood either spotted into filter paper or after coagulation as plasma or serum, can identify more than 20 metabolic defects of fatty acid oxidation and organic acid metabolism, including MCAD deficiency (Table 1). A specimen can be sent to a diagnostic facility by overnight courier and the MS/MS analysis be completed by lunchtime on the day of arrival. MCAD gives a clear diagnostic acylcarnitine pattern as compared with normal controls (Figure 2). This is also true for most of the other disorders of fatty acid and amino acid catabolism. Thus, acylcarnitine analysis has become a valuable front-line diagnostic test for these disorders.

TANDEM MASS SPECTROMETRY AND EXPANDED NEONATAL SCREENING

Five steps are critical to effective newborn screening: screening, follow-up, diagnosis, management, and evaluation.⁴ The following sections discuss the experience with each of these steps in respect to MS/MS newborn screening.

Screening. Table 1 summarizes 2 years of initial experience by the North Carolina State Laboratory of Public Health, when 237,774 babies were screened.

In accordance with other newborn screening programs, MCAD deficiency was detected with the highest

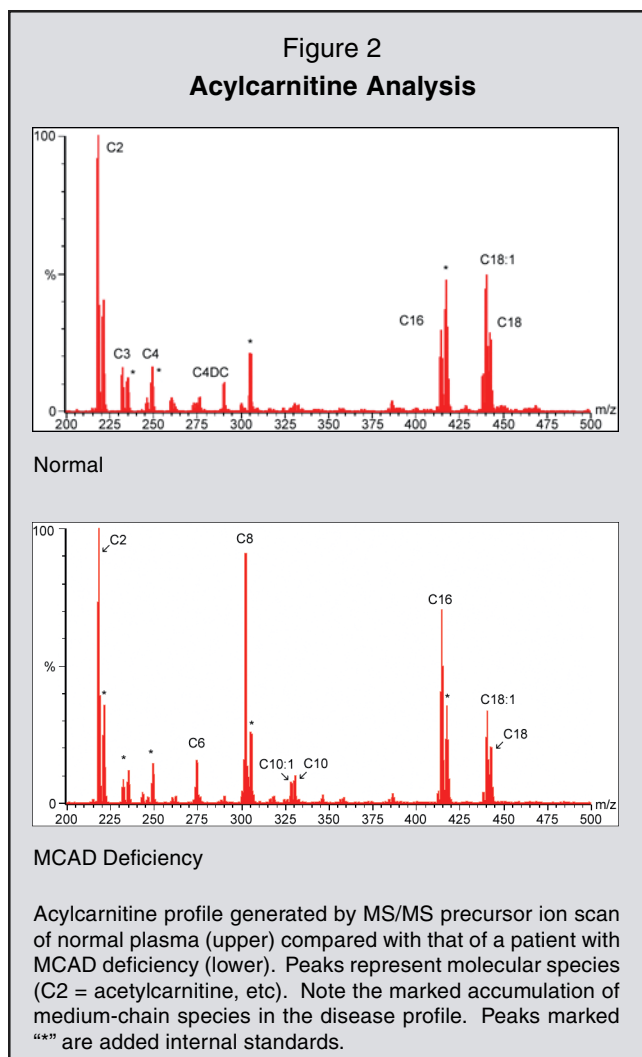


Table 1
Disorders of metabolism detected by MS/MS newborn screening (4/20/99 until 4/15/01)^{6,7}

Fatty acid oxidation	Organic acid metabolism	Amino acid metabolism
<ul style="list-style-type: none"> • MCAD (medium chain acyl-CoA dehydrogenase) deficiency (21) • VLCAD (very long chain acyl-CoA dehydrogenase) deficiency (1) • SCAD (short chain acyl-CoA dehydrogenase) deficiency (3) • GA (glutaric acidemia) type II* • CPT II (carnitine palmitoyl transferase II) Deficiency* • LCHAD/TFP (long chain 3-hydroxyacyl-CoA dehydrogenase) deficiency* 	<ul style="list-style-type: none"> • 3-MCC (3-methyl crotonyl-CoA carboxylase) deficiency (7) • Propionic acidemia (1) • Methylmalonic acidemia (2) • Glutaric acidemia, type I (1) • β-ketothiolase (SKAT or mitochondrial acetoacetyl-CoA thiolase) deficiency (1) • Isobutyryl-CoA dehydrogenase deficiency (1) • 2-methylbutyryl-CoA dehydrogenase deficiency (1) • Isovaleryl-CoA dehydrogenase deficiency (3) • Malonic Acidemia* 	<ul style="list-style-type: none"> • Phenylketonuria (14) • Argininosuccinic acid lyase deficiency* • Citrullinemia (1) • MSUD* (Maple Syrup Urine Disease)

*Cases of these disorders, reported by other screening programs, had not yet been detected in North Carolina. (n)= number of patients. Total number of neonates screened 237,774.

frequency. The incidence of MCAD deficiency was estimated at 1 in 13,600 live births in North Carolina. The overall incidence of disorders of metabolism detected by MS/MS newborn screening was 1 in 4,400 live births.

Beyond implications for the affected infant, newborn screening can have implications for maternal health. An association between the risk of serious complications of pregnancy, especially in the HELPP syndrome (hemolysis, elevated liver function tests and low platelets) with the occurrence of acute fatty liver of pregnancy in the mother and a fetus affected with LCHAD deficiency, was first established 10 years ago. Since then there has been a growing awareness that the presence of other fatty acid oxidation disorders, including MCAD deficiency, can also cause pregnancy complications.

Follow-up. Initial follow-up was directed according to cut-off values for each metabolite, typically set at 4 standard deviations above the mean. In the case of an abnormal value, repeat screening samples were requested. If the initial sample had a higher “alert” value, or if the second sample remained above the cutoff, the infant’s local physician was contacted immediately. The possibility of a metabolic disorder was discussed and recommendations for follow-up were made. Infants were referred directly to a metabolic genetics center. If the elevated metabolite(s) did not signal a specific or life-threatening disorder, blood and urine samples were sent to the centers from the local physicians for follow-up testing.

The importance of appropriate cut-off values and adequate follow-up testing was illustrated by an infant with glutaric acidemia, type I (GA-I), initially detected on the basis of elevated glutaryl carnitine in the bloodspot.⁶ Initial cut-off values for each metabolite are typically set by a statistical determination of 4 standard deviations above the normal mean, but must be adjusted up or down for some metabolites based on experience during newborn screening. Although the patient had an abnormal blood acylcarnitine profile at birth, the repeat specimen was normal; thus, newborn screening ultimately failed to indicate the diagnosis of GA-I. Newborn screening is a powerful tool to potentially diagnose presymptomatic infants; however, it should not be considered a diagnostic test. In order to allow a precise diagnosis and treatment of GA-I, we recommend a complete evaluation, including both a plasma acylcarnitine profile and a urine organic acid analysis of any patient with elevated glutaryl carnitine in a blood spot acylcarnitine profile. The North Carolina State Laboratory has adjusted the cut-off value for glutaryl carnitine to increase the sensitivity of the newborn screening test for GA-I and this is now

suggested as a general recommendation for laboratories screening for GA-I by MS/MS.

Diagnosis. The diagnoses of fatty acid oxidation disorders is established by testing urine organic acids and a plasma acylcarnitine profile; whereas, the diagnoses of organic acid metabolism disorders is confirmed by plasma amino acids +/- urine organic acids. Enzyme analysis is required to diagnose disorders where the elevations of metabolites in blood and urine do not provide a conclusive diagnosis.

Since the addition of MS/MS to the North Carolina Newborn Screening Program, 20 infants with elevated hydroxyl-isovalerylcarnitine (C5OH) levels were evaluated. Eight of these 20 infants had persistent elevations of both 3-hydroxyisovaleric acid and 3-methylcrotonylglycine in their urine, highly suggestive of 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency. Other enzyme deficiencies that could provoke elevated C5OH, including biotinidase and holocarboxylase synthetase deficiency, were eliminated from the differential diagnosis by confirmatory enzyme testing. In 4 of the remaining 12 infants, maternal 3-MCC deficiency was demonstrated. It is likely that the remaining 8 of these 12 infants for whom urine organic acids normalized also represented maternal 3-MCC deficiency; however, follow-up testing was not requested from the mother or she refused to provide her samples in each case. Infants and mothers with 3-MCC deficiency commonly have clinically significant carnitine deficiency, which motivated the detection and treatment of these individuals.

Management. The prompt referral of patients with confirmed or suspected life-threatening disorders of metabolism is critical to fulfill the mission of newborn screening. The successful treatment of inborn errors of metabolism provides justification for MS/MS newborn screening. For example, untreated MCAD deficiency presents as hypoketotic hypoglycemia and is commonly lethal, due to hepatic failure which often mimics Reye syndrome. Since the initiation of MS/MS newborn screening, there have been no deaths among confirmed MCAD deficiency and no cases of missed MCAD deficiency. Treatment consisted of early referral to a metabolic-genetics center, avoidance of fasting, L-carnitine supplementation, and prohibition of formulas containing medium-chain triglyceride (MCT oil). Likewise, nutritional and pharmacologic treatment is available for other disorders detected by MS/MS.

However, the treatment of other potentially detectable disorders of metabolism has been less than optimal, related to issues of detection or delays in detection. While tyrosinemia, type 1, can be effectively treated with

a life-saving enzyme inhibitor, tyrosine levels are not elevated during the newborn period to allow detection of that disorder. More frustrating has been the ineffectiveness of treatment in disorders with severe complications early in life, including glutaric acidemia, type II (GA-II) and maple syrup urine disease (MSUD). GA-II cannot be effectively treated when the presentation is severe, and MSUD can only be effectively treated when a formula lacking branched-chain amino acids is used prior to the onset of symptoms which usually occurs in the first 10 days of life. Although treatment is available for GA-I, MSUD and tyrosinemia, type I, these disorders are quite rare outside selected population isolates (eg. MSUD among the Amish). Consequently, aggressive, earlier detection by more specialized approaches to newborn screening is not practiced.

Evaluation. Newborn screening programs require periodic review and analysis of outcome measures to be successful. Adjustment of cut-off values is one important exercise in MS/MS newborn screening, since the cut-off values determine the likelihood of false positive or false negative results.⁷ False negative results should be assiduously avoided. False positive results can hamstring a program. Specific causes of false positives are listed in Table 2.

Ratios of metabolites are helpful in the interpretation of elevations unrelated to a metabolic disorder, such as the ratio of C8:C10, which is elevated in MCAD deficiency but not in MCT oil supplementation. Age-specific cut-off values could potentially reduce the frequency of false positive results because the majority of spurious elevations are related to prematurity.⁷ Until age-specific cut-off values are available, the newborn screening laboratory typically obtains serial specimens from premature infants until the postconceptual age approaches 40 weeks.

The effectiveness of modifying cut-off values was illustrated by the experience with C5OH. The initial cut-off for C5OH was determined statistically (4 standard

deviations above the mean); the cut-off was increased when the false positive rate was determined to be unacceptably high. Thereafter, the cut-off for C5OH was increased to 5 standard deviations. This adjustment of cut-off values for normal samples has reduced the number of initially elevated samples from 1 in 720 to 1 in 7,400 infants screened, and dramatically reduced the ratio of falsely positive initial screens to a truly positive test in affected infants from 65 to 1 to 3.3 to 1. There was no reduction in the rate of 3-MCC detection observed after the cut-off for C5OH was increased, and no infants with symptomatic 3-MCC deficiency have come to the attention of the North Carolina medical community since the MS/MS screening began.

CONCLUSION

The difference in newborn screening brought about by MS/MS is the ability to detect more than 20 inborn disorders of metabolism from a single blood dot with a single test. The method detects a confirmed disorder in about 1 in 4,000 cases screened. The most common diseases are MCAD deficiency, PKU, and 3-MCC deficiency. Early diagnosis and treatment of these cases is preventing adverse outcomes, and screening programs are reporting a very low incidence of false positives and false negatives. About half of the states are either screening newborns by MS/MS or have made a decision to do so soon. Even so, there is controversy and debate regarding what is perceived to be a paradigm shift, since the testing equipment is expensive and some of the disorders it detects have no effective treatment. However, once a state decides to implement this method it must accept the responsibility of performing the test properly and of treating diagnosed patients. To do so means providing adequate professional support to include dietitians, genetic counselors, biochemical geneticists and appropriate mechanisms in place for follow-up testing. Pediatric Endocrinologists are often called to consult with infants with emergencies due to inborn errors of metabolism, a good review of the subject should be kept at hand.⁸

Table 2

Causes for false positive results in MS/MS newborn screening

Condition	Metabolites affected	False positive
MCT oil supplementation	C8, C10	MCAD deficiency
Prematurity	C4, C5, C8	GA-II & MCAD deficiency
Prematurity	Tyrosine	Tyrosinemia
Carnitine supplementaion	C0, C2, C3, (+ others)	Propionic acidemia & others

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Letter to the Editor:

Dear Dr. Blizzard and Editors of GGH:

I've been reading GGH for years, and have found it so useful. This month's timely release of the intersex review really "hit the mark". I work in a state birth-defects surveillance department. The non-physicians have expressed tremendous interest in the management of ambiguous genitalia, either as an isolated finding, or related to exstrophy. This review will serve as the focal point for our next monthly teaching session to be supplemented by your review (*GGH* Vol. 19, No. 1).

Angela E. Lin, MD
Brigham-Women's Hospital
MA Center Birth Defects Prevention

Dear Dr. Linn: Thank you!

Dear Other Readers:

Please let us know your positive and negative comments – and your agreements or disagreements regarding the abstracts and their comments and the lead articles. Your input is absolutely necessary for us to maintain, upgrade, and disseminate your agreements and disagreements. We encourage you to respond quickly after your thoughts and criticisms come to mind.

Robert M. Blizzard, MD

Abstracts from the Literature

Factors Determining the Pattern of the Variant Creutzfeldt-Jakob Disease (vCJD) Epidemic in Great Britain

Editorial Preface: Growth hormone (GH) extracted from human pituitaries obtained at autopsy was first given to children in 1958. Twenty-seven years later (1985), the first cases of Creutzfeldt Jakob Disease (CJD) resulting from such injections were observed in individuals who had received GH injections 8 to 10 years prior to that time. The fact that no cases of CJD were reported reflects the long latent period between exposure and the onset of symptomatic disease.

The exact number of the pituitary injections that may have been contaminated with the CJD prion is unknown. GH from only one of three laboratories in the U.S. extracting pituitaries has been associated with CJD. All three of the laboratories extracting GH used different procedural techniques. In retrospect, the GH extraction procedure of two of the three laboratories eliminated the active prion from the final product. From 1985 until April 2003 only 26 cases of CJD were recorded among several thousand (7,700) recipients in the U.S. who had received native human growth hormone. All U.S. patients with CJD received GH prior to 1977; afterward a new purification step was added to the GH extraction procedure.

The early symptoms of CJD consist of degenerative neurological function. Death unfortunately follows within a period of 6 to 36 months. The number of catastrophes to date in the United States have been relatively small, particularly in light of the number anticipated in 1985 when the first two deaths were reported within a month of each other. Postulation, with reasonable justification, was that the incubation period and susceptibility to the disease were influenced by the dose of contaminated material, possibly the age of the recipient, and possibly by an individual's genetic susceptibility. The latter was suspected on the basis of a few studies using scrapie disease in sheep as a prototype since CJD, occurring primarily in humans, is similar to scrapie disease in sheep. These diseases produce degenerative neurological alterations; although the histology of the pathological findings in the central nervous system are different. They are known as spongiform cerebral encephalopathies.

Abstract: In 1985 and 1986 a similar but different spongiform encephalopathy manifested itself in England when humans were first diagnosed with "mad cow

disease” or bovine spongiform encephalopathy (BSE). Cows had been infected by the ingestion of commercially prepared food for cows to which had been added a food enforcement consisting of bovine CNS and other organ components that were unmarketable to humans. Cows ingesting these ground up organ components, when the organs were contaminated, developed BSE after a prolonged incubation period. Infected cattle in the presymptomatic stage were often sent to the slaughter house. This meat was sold in the markets and subsequently infected humans. Thus, the mad cow disease was perpetuated and humans developed a variant of CJD (vCJD). The brain pathology of CJD and vCJD are distinguishably different even though both are spongiform encephalopathies. Over one million cows in the UK were believed to be infected. Identification of infected asymptomatic cows is not easy even though the prion accumulates in the lymphoid tissue as well as in the central nervous system.

Spongiform encephalopathies result from a replicating abnormal protein called a prion. The prions proliferate, destroy cell membranes, and accumulate as they are not destroyed themselves. Clinical symptoms develop when the abnormal protein is diffusely spread through the CNS. Transmission from mother to fetus occurs during pregnancy in the cow. It is not known whether prions are transmitted in cow's milk or colostrum. There are no data regarding transmission in humans by placenta, in human milk or colostrum.

At the end of 2001 in the UK there were 113 cases of vCJD, nine of whom were alive at that time. A few cases have occurred in other countries including France and Ireland and two cases in the United States. BSE crosses species barriers and consequently is found in squirrels and other mammals. The disease scrapie has been adapted to mice and genetic predisposition has been studied. Different strains of mice react differently to the exposure of the scrapie prion. Recently a genetic predisposition for susceptibility in humans has been demonstrated. At the time the referenced article was written, all of the human cases tested in the UK (87) shared a common genetic trait, being methionine homozygous (MM) at codon 29 of the prion protein (PrP) gene. Estimates in Caucasian populations are that 40% of the population share this trait. Of the other 60% of the population, 13% are valine homozygous (VV) and the remaining 47% heterozygous for methionine and valine (MV). The authors of the referenced article also refer to a report that there is a decreased risk of CJD in those with HLA-DQ7. This new finding, if correct, suggests complex multi gene determinations of patterns of susceptibility.

The authors discuss extensively the difficulty in predicting the potential magnitude of the UK epidemic.

Of significant importance, the authors believe that even in the worst case scenario in which over 8,000 cases will appear by the year 2080, it is unlikely that a very large increase in case numbers would be expected in the short term (2-5 years).

The epidemiological determinants of the cause of the epidemic which make projections complex include; (a) incubation period distribution, (b) possible age dependent susceptibility to exposure to infection, (c) the effectiveness of the specified bovine ban in the UK, and (d) the genetic susceptibility to infection. For each of these determinants the data used for calculation are nebulous. However the best current estimate (guesstimate) of (a) for mean incubation period is stated with trepidation to be ca. 7 years, (b) the age dependent maximum susceptibility for individuals is 10-20 years of age, (c) for effectiveness of the specified bovine ban, the authors are unable to utilize current data in the calculation, and (d) in respect to utilizing genetic susceptibility, recent studies have indicated that there may be substantial genetic variation in susceptibility, which prevents more than speculation.

The authors conclude that the main priority, in view of all the above stated difficulties, is to develop a diagnostic test that is able to both detect infection early in the incubation period and which can be applied to large population samples in humans, bovine and other species.

Ghani AC, et al. *Proc R Soc Lond B Biol Sci* 2003;270:689-698.

Editor's Comment: *Disease curses continue to befall mankind. These are often of our own making such as in the instance of man promoting “mad cow disease”. Hopefully a test will be designed that permits identification early in the incubation period of the presence of the prions and thus make it possible to identify those animals affected. Much has yet to be learned about the prion and how it might be combated.*

In respect to CJD in humans who received native pituitary growth hormone from autopsied bodies, we have suffered enough, even though only 26 of over 7,000 potentially infected subjects have died. A philosophical point, which hopefully we have learned, is that treatments which physicians prescribe today may not manifest their toxic effects for many years. As the Hippocratic Oath states, and as Lawson Wilkins practiced (Growth, Genetics & Hormones Vol. 19, No. 2) and taught, “do no harm to the patient”. Unfortunately we do not have a crystal ball to assist us with the decisions we must make.

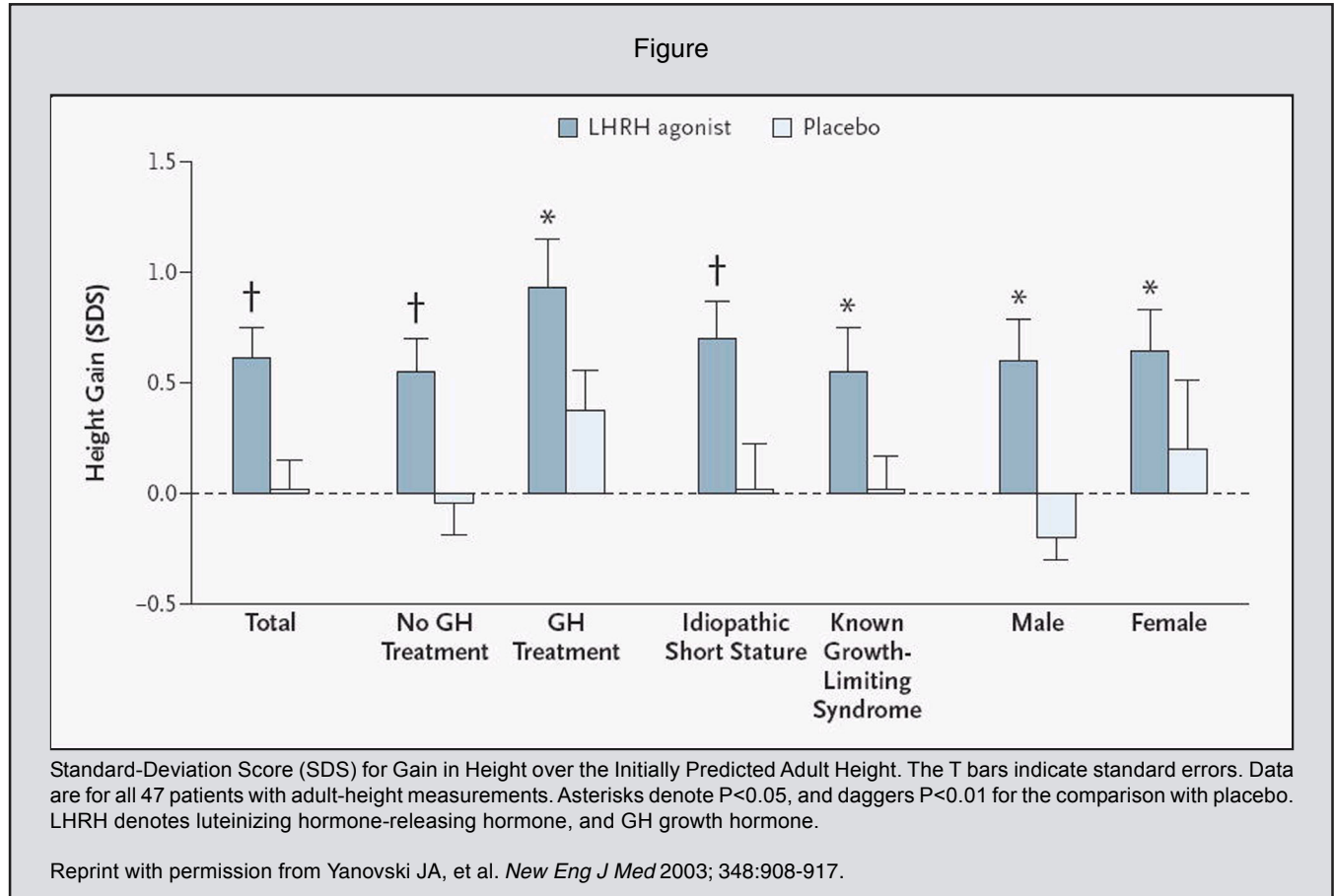
Robert M. Blizzard, MD

Treatment with Lutenizing Hormone-Releasing Hormone Agonist in Adolescents with Short Stature

This study was performed to evaluate whether treatment with a lutenizing hormone-releasing hormone agonist (LHRHa) increases adult height in short adolescents with normally timed puberty. There were 32 girls and 18 boys with a mean predicted adult height of more than 3 SDS below the population mean who were administered an LHRHa or a placebo in a randomized double-blind fashion; 26 subjects received the medication and 24 were given placebo. There were a variety of growth limiting disorders, but principally idiopathic short stature. Three patients were also treated with growth hormone (GH) because they had a peak GH after stimulation of less than 7µg/l. The treatment was started at approximately 12-13 years of age; mean bone age was 11.5-13.2 years, and mean Tanner stages were 2.8 to 3.2 in the two groups, respectively. The mean duration of the LHRHa treatment group was 3.5 years, and that of the placebo group was 2.1 years. Adult height was measured when the bone age exceeded 16 years in girls and 17 years in boys, and when the growth rate was less than 1.5 cm per year. Forty-seven subjects were followed until they attained full adult height.

At the end of the study, those treated with LHRHa were older and taller than those who received placebo (20 vs 18 years of age; and -2.2 vs -3.0 SD below the 50th percentile, respectively). Treatment with LHRHa resulted in a mean increase of 0.6 SDS in height (4.2 cm) over the initial predicted adult height in these short patients. The gain in height among the LHRHa treated group was independent of sex, concomitant GH treatment or presence of growth limiting syndromes (Figure). However, added GH treatment produced an apparent additive effect on growth (+ 0.4 SDS). The principal adverse event of this treatment was a decrease in bone accretion, with reduced bone mineral density below that attained in the placebo group. There were no apparent lasting effects on secondary sexual characteristics. The authors concluded that LHRHa increases adult height, but because of resulting decreased bone mineral density, it should not be routinely employed to augment adult height.

Yanovski JA, et al. *New Eng J Med* 2003; 348:908-917.



First Editor's Comment: This very well controlled study clearly showed that there may be a small increment achieved in adult height (mean of 4.2 cm) with LHRHa treatment of short stature patients. Previous studies have also shown that there is a small gain in adult height with such therapy.^{1,2} However, in this study the medication was given for more prolonged periods (mean 3.5 years) and it resulted in a significant reduction of bone mineral density. This is not surprising, since bone accretion at the time of adolescence is greatly dependent on the presence of adequate pubertal hormones which are suppressed by LHRHa. Of great concern is that this deficit persisted even after the LHRHa treatment ceased. It would have been of interest to ascertain calcium intake and determine if some of these detrimental effects could have been counteracted by an increased ingestion of this mineral. I agree with the authors that LHRHa treatment for augmentation purposes to increase height should not be routinely prescribed. The average cost of such treatment is \$10,000 to \$15,000 per year, and this should also be kept in mind.

Fima Lifshitz, MD

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Second Editor's Comment: While the current study may not be ideal in terms of the present approach to inhibition of hypothalamic-pituitary-gonadal function with LHRHa, it is unlikely that similar investigations will be conducted in the future. Furthermore, the preponderance of girls with intrinsic short stature (32/50) without gonadal dysgenesis is the reverse of that encountered in general pediatric endocrine experience. Thus, present data serve for future recommendations. This writer agrees with the conclusion of the authors and that of the first editor's comment; namely that routine administration of LHRHa is not to be recommended for subjects with intrinsic short stature. It is of interest that the increase in adult height was greatest in patients who received both GH and LHRHa. Nevertheless, in the absence of data demonstrating significant educational, social, and occupational benefit of relatively small increases in adult stature, such efforts cannot be routinely supported.

Allen W. Root, MD

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1. Carel JC, et al. *J Clin Endocrinol Metab* 2002;87:4111-4117.

Do Growth Hormone (GH) Serial Sampling, Insulin-Like Growth Factor-I (IGF-I) or Auxological Measurements Have an Advantage Over GH Stimulation Testing in Predicting the Linear Growth Response to GH Therapy?

Reliable indices that are consistently able to predict the linear growth promoting effects of recombinant human growth hormone (rhGH) in short children have long been sought. The authors analyzed data from a National Cooperative Growth Study of the usefulness of IGF determinations, auxological measurements, and 12-hour serial GH measurements obtained every 20 minutes between 2000 and 0800 hours, in children who were treated with rhGH (0.29 mg/kg/week in 6± weekly injections) for a mean of 3.6-3.8 years. There were 825 prepubertal children with short stature studied (mean height -2.8 SDS; bone age delay ~2.3 years). The children were subdivided into one group of 300 (231 males, 69 females) with isolated GH deficiency (IGHD - peak GH response to provocative stimulation <10 ng/mL by unstated methods) and 525 (404 males, 121 females) with idiopathic short stature (ISS - peak stimulated GH response ≥10 ng/mL). The data were analyzed by the cluster program. In addition, a measurement of the "orderliness" or "regularity" of overnight spontaneous, endogenous GH secretion, which is termed "approximate entropy", was calculated.

As anticipated, mean and maximum spontaneous peak GH levels, pooled mean GH concentrations, and mean area under the GH peaks were significantly lower in subjects with IGHD than in those with ISS. Interestingly, pretreatment IGF-I concentrations were similar in the two groups (120 and 125 ng/mL, respectively). The increment in height SDS after treatment with rhGH was similar in the two groups (+1.2 to 1.3 SDS). Significant but weak correlations ($r < 0.4$) related rhGH-induced height increment to height deficit prior to treatment, duration of treatment, and mid parental height SDS in both groups. Maximum stimulated GH values, spontaneous overnight GH measurements, and pre-treatment IGF-I levels were also inversely related to rhGH-induced growth, but again the r values were low (-0.15 to -0.395). By multiple regression analyses, only the peak GH response to secretagogue was inversely correlated to treatment related height increment; spontaneous GH measurements were not related. When data from children with "severe" IGHD (peak stimulated GH response <5 ng/mL) or "extreme" ISS (height <-3.3 SDS)

were isolated and examined, spontaneous GH measurements were inversely related to treatment induced growth but did not improve calculated height prediction models. Spontaneous GH secretion was more orderly in children with severe IGHD than those with "moderate" IGHD. In the ISS subjects, GH secretion was more orderly in those with "mild" ISS, and IGF-I concentrations were higher than in those with extreme ISS. The authors conclude that in general, serial measurements of spontaneous overnight GH secretion did not provide information helpful in the prediction of the linear growth response to rhGH, thus supporting the conclusion of several earlier studies of this question.

Rogol AD, et al. *Clin Endocrinol* 2003;58:229-237.

First Editor's Comment: Clearly, the diagnosis of IGHD is fraught with difficulty as 40-80 percent of such children will have normal GH secretion as adults. Thus, there must be overlap between the diagnostic categories of IGHD and ISS in this study. In this regard it is of interest that the "disorderliness" of GH secretion was greatest in those subjects with "moderate" IGHD and both "mild" and "extreme" ISS – implying a close relationship between these groups in the regulation of GH secretion. As the investigators suggest, it may be that a defect in the "orderly" secretion of GH is translated into decreased tissue responsiveness to GH even though the absolute amount of GH secreted may be normal. Although several factors were related to height increment on rhGH, none had the high *r* value we seek as an "absolute" predictor of response. Hence, the search goes on!

Allen W. Root, MD

Second Editor's Comment: I feel pressured to comment that a possible reason that many children appear to be GH deficient as children but not as adults is that the sex hormones stimulate GH release. We use the same threshold criteria for GH release to secretagogues in adults as children. How do we know that apparent isolated GHD children are not still partially GHD as adults? Studies are needed in this group of patients when they reach adulthood to evaluate comparison of GH response to secretagogues in adults who were not thought to be GHD as children. In such a study we might find that those diagnosed with GHD as children are still GHD as adults relative to others who were never short as children. The fact that many pediatric endocrinologists used to prime suspect GHD children with testosterone before administering secretagogues for the purpose of exaggerating the GH response in suspect GHD children supports my hypothesis.

Robert M. Blizzard, MD

Third Editor's Comment: In the previous issue of *Growth, Genetics & Hormones* (Vol. 19, No. 2) a paper by Lanes and Jacobowitz was reviewed.¹ These authors also showed that IGF-I and IGFBP3 were not useful in assessing the response to hGH therapy. Careful measurements and monitoring of growth are the gold standards.

Fima Lifshitz, MD

Reference

1. Lanes R, Jacobowitz S. *J Pediatr* 2002;141:606-610.

Is the Growth Hormone/IGF-I Axis Stimulatory or Inhibitory on the Aging Process?

Two recent articles published in *Science* identify the GH/IGF-I axis as playing a major role in the aging process of many species including humans. The data persuasively argue that components of this axis may negatively affect longevity. The majority of the data support the hypothesis that limited secretion of IGF-I promotes long life. A brief synopsis follows.

In yeast, down-regulation of intracellular signaling pathways that are dependent on glucose increases the life span of the organisms up to 300%. In worms (*C. elegans*), loss-of-function mutations of a gene called *Daf-2* encoding an ortholog of the IGF-I receptor extend survival up to 300%. In the fly, inactivation of the gene encoding the insulin receptor increases longevity up to 200%. Mice with homozygous inactivating mutations in *Prop-1*, *Pit-1*, or *Ghr* survive 25%-65% longer than do

wild-type mice. Since mice with a defect in the GH receptor have high serum GH, the decrease in IGF-I signaling probably is the common factor responsible for the extended life of the mutant animals, insects, etc. Partial caloric restriction in rodents and possibly in monkeys also increases life span. Decreased synthesis of IGF-I and lowering of serum concentrations of glucose and insulin occur simultaneously with caloric restriction.

Since IGF-I acts in part by increasing transcription through the mitogen-activated protein kinase pathway (MAPK),¹ which promotes cell division and growth, attenuation of this pathway possibly reduces the potential for lethal errors in this system. Since IGF-I decreases the activities of anti-oxidant enzymes such as superoxide dismutase and catalase, there is reduced ability in the presence of IGF-I to respond to stress and

thus enhanced susceptibility to cellular damage; accordingly, inhibition of this property of IGF-I would be expected to augment the stress response. Caloric restriction also increases the longevity of the *Prop-1* deficient or Ames mouse; thus, the mechanisms by which caloric restriction and IGF-I deficiency act to increase life span may differ. In rodents, partial caloric restriction increases the immune response to infectious agents and attenuates the destructive cellular immune changes of aging. This thereby decreases the incidence of degenerative and inflammatory diseases and tumor formation.

In adult humans, hypopituitarism is associated with abnormal lipid metabolism, atherosclerosis, and early death. Yet, acromegalic subjects with excess GH secretion also have a shortened life span, and critically ill patients who receive exogenous GH have a greater mortality rate than do those with similar illnesses not so treated. Since patients with *PROP-1* deficiency or patients with inactive GH receptors (who do not have ACTH deficiency), do not succumb at an early age and may even live exceedingly long,² Tatar et al suggest that perhaps ACTH rather than GH deficiency is responsible for early death in humans with pan-pituitary dysfunction. The authors further suggest that pharmacological agents designed to reduce IGF-I levels be explored as extenders of life span.

Longo VD, Finch CE. Evolutionary medicine: From dwarf model systems to healthy centenarians? *Science* 2003;299:1342-1346.

Tatar M, et al. The endocrine regulation of aging by insulin-like signals. *Science* 2003;299:1346-1351.

Editor's Comment: *The authors point out that glucose/*

insulin/GH/IGF-I and their signaling pathways may actually decrease rather than prolong life span. While experimental findings cannot always be directly translated into analysis in humans, the authors' conclusions merit consideration when we prescribe GH for our adult patients. These findings also should cause those who claim that GH is an effective anti-aging agent in non-GH deficient elderly adults to reconsider this recommendation. Body weight control combined with an efficient exercise program is likely to be far more effective in lengthening life than is administration of GH to adults without GH deficiency. The need to maintain adequate glucocorticoid replacement therapy in adults with panhypopituitarism must also be emphasized to minimize stress.

Other papers in the Science series are also worth reviewing, particularly one by Hasty et al³ describing aging defects due to genetic abnormalities in genome maintenance (transcription, DNA repair, DNA helicase activity). Bluher et al⁴ recently reported that mice with loss of the insulin receptor only in adipose tissue had extended life span. In addition, an extensive review on caloric restriction and extension of life is available on the internet.⁵

Allen W. Root, MD

References

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2. Rosenfeld RG, et al. *Endocrine Rev* 1994;15:369-390.
3. Hasty P, et al. *Science* 2003;299:1355-1359.
4. Bluher M, et al. *Science* 2003;299:572-574.
5. Masoro EJ. *Science's SAGE KE* 2003; <http://sageke.sciencemag.org/cgi/content/full/sageke;2003/8/re2>

Can Growth Hormone Prevent Aging?

Dr. Vance recently published an article by the above title. She concludes that antiaging therapy with human growth hormone (hGH) has not yet been proven safe or effective. Although not the first investigator to study GH in relation to body composition, Dr. Dan Rudman¹ in 1990 authored the first publication concerning the use of hGH in 12 elderly men. Dr. Vance summarizes the data in that report; the administration of hGH at approximately twice the dose of hGH used in adult growth hormone deficiency (GHD) for six months resulted in a 4.7 kg increase in lean body mass, a 3.5 kg decrease in adipose tissue, and an increase of 0.02 gm/cm in lumbar spine density, and significant increases in BPs and in fasting glucose concentrations. There were no assessments of exercise endurance, muscle strength, or quality of life. Vance points out that the follow-up to this study does not include any substantiation that hGH in elderly men does more than

confirm an increase in lean body mass and a reduction of body fat (with no change generally in total body weight).

Vance appropriately criticizes the proliferation of commercial "antiaging" clinics which promote the sale of inappropriate and ineffective agents such as arginine and other agents to release growth hormone and of hGH itself. Vance chastises those who for monetary gain are so dishonest and potentially destructive of their customer's health.

The use of long-term administration of hGH in adults with no established growth hormone deficiency is appropriately deprecated as it is not known whether the effect of long-term administration of hGH in the elderly is potentially harmful. Cancer of various organs is of particular concern. The work of Chan et al is cited.² In 152 healthy men, the relative risk of the subsequent development of prostate cancer was increased by a

factor of 4:3 among men who had serum concentrations of IGF-I in the highest quartile as compared with those men with concentrations in the lowest quartile.

The author's complete conclusion is that there is no "current" magic bullet medication that retards or reverses aging.

Vance ML. *N Engl J Med* 2003;348:779-780.

Editor's Comment: *This editor agrees with Dr. Vance's conclusions. I concur having initiated in 1982 the first study of the effect of hGH in elderly individuals. I and four other male subjects over the age of 55 received native hGH daily for 2.0 - 2.5 years at a dose that raised IGF-I levels from GHD concentrations to levels above the 50th percentile for young adult males. In myself there was an increase in lean body mass and decrease in free fat mass. The same occurred in two other subjects but not in subjects 4 and 5. Some element of hyperinsulinemia and glucose intolerance occurred but not overt diabetes mellitus. No overt changes in gross body configuration occurred. Subjectively there were no changes in self image, sense of well-being or libido and no changes in psychological mood. There were no*

changes in hair color, the rate of hair or nail growth, or disappearance of wrinkles. The study was stopped in 1985 when native hGH was no longer extracted from human pituitaries because of the development of Creutzfeldt Jakob disease in some GHD patients having received hGH. On the basis of all reports in the literature and my scientific observations among the five normal elderly patients in the study cited, I agree with Dr. Vance and most other pediatric endocrinologists, "to give hGH for purposes of attempting to alter aging in individuals who secrete GH normally for age is unacceptable unless administered under a rigidly controlled double blind study".

Reference to the role of IGF-I in shortening or lengthening life in animals is presented in the abstract immediately preceding this one (page 42). Theoretically longevity can be shortened by the indiscriminate use of GH in mammals.

Robert M. Blizzard, MD

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2. Chan, et al. *Science* 1998;279:563-566.

High Dose Growth Hormone Treatment Induces Acceleration of Skeletal Maturation and an Earlier Onset of Puberty in Children with Idiopathic Short Stature

Kamp et al report on the experience of their multicenter European randomized trial of high dose (0.07mg/kg/week) recombinant growth hormone (GH) in prepubertal idiopathic short stature (ISS) children with baseline heights less than - 2SDS. Forty children (ages 4 -10 years) were recruited and 12 completed 4 years of study while 8 completed 5 years of treatment. Inclusion criteria, in addition to pre-pubertal status and age <8 years for girls and < 10 years for boys, were normal responses to GH stimulation testing (GH >10µg/l). Subjects were measured and Tanner staging performed every three months; bone age determinations were made yearly. During the first year of treatment all subjects randomized to GH treatment participated in a "GH responsiveness" study where GH was administered at two different doses for three months each, separated by three-month washout periods. High dose GH treatment was continued until the first signs of puberty.

In the second and subsequent years of treatment, height SDS for chronological age increased significantly and there was a significant difference in bone age advancement compared to controls. Indeed, height SDS for bone age was not different between the two groups at five years. Eighty-five percent (11/13) of boys in the high dose GH group entered puberty at a median age of 12.2 years during the study, compared with 54% (7/13) of

controls at a median age of 13.9 years. Similar findings for girls included 50% (2/4) of treated children entering puberty at a median age of 10.2 years versus 20% (1/5) of controls at a median age of 9.9 years. The age and sex adjusted relative risk of entering puberty earlier was 4.7.

The authors conclude that there is no evidence that young children with ISS benefit from high dose GH in the pre-pubertal period. They point out that their study differs from previous studies in that they sought to treat younger pre-pubertal children with ISS for a longer period of time with high dose GH, and that they discontinued GH at the onset of puberty so as to separate the influence of GH from that of sex steroids in pubertal growth. They are critical of other studies that did not include randomized ISS control groups, but used reference data for pubertal onset and GH dose.

Kamp GA, et al. *Arch Dis Child* 2002;87:215-220.

Editor's Comment: *This is an interesting and well-conceived study. The use of high dose GH in ISS remains controversial, and well-controlled studies using different GH doses in different age groups are important aids in helping the endocrinologist decide whom to treat and for how long. The data from this manuscript suggest that early high dose GH treatment may improve height*

SDS for CA, but that there may be a price to pay in final height gain by entering puberty earlier. We await the data on final heights of the subjects in this study.

In an accompanying "Commentary", Clayton¹ summarizes and reiterates previous data which demonstrate that the response to GH in ISS, whether short, mid- or long-term is variable, that overall reported gains in final heights range from 3 – 9 cm in various studies, and that pre-pubertal improvements in growth

velocity are dose dependent. He reemphasizes the importance of matched contemporaneous control groups and the current lack of information regarding the dose response for GH in conditions where it is currently being used.

William L. Clarke, MD

Reference

1. Clayton PE. *Arch Dis Child* 2002;87:219-220.

Low Nutrient Intake and Early Growth for Later Insulin Resistance in Adolescents Born Preterm

In this potentially very important paper the investigators study the effects of various diets in the newborn period of premature infants versus the presence at ages 15-16 years of a plasma marker for the development of insulin resistance and non-insulin dependent diabetes in adults. The marker is known as 32/33-SPI (split proinsulin). Plasma concentrations were measured in 216 mid- to late adolescents (13-16 years of age) who had been delivered prematurely (mean gestational age of 31 weeks and mean birth weight of 1.4 kg). Of these preterm infants, 110 had received a low nutrient formula and 106 had received a high nutrient formula.

Not surprising, the preterm newborns fed the lower nutrient formula gained less weight prior to discharge compared to those receiving the higher nutrient formula. The specific formulas were stopped when the infants were discharged from the neonatal unit or had reached a weight of 2000 gms. At 16 years of age the children were re-evaluated and fasting serum concentrations of insulin, proinsulin, and 32/33-SPI were determined in specific assays. As adolescents, the low nutrient group had lower levels of 32/33-SPI than the levels in the high nutrient group. Levels of insulin, proinsulin and glucose were similar in the two groups.

After statistically adjusting for the effects of gender, gestational age, birth weight, neonatal morbidity, and other variables, the relationship between neonatal diet and concentrations of 32/33-SPI remained significant. Further analysis revealed that high rates of weight gain in the neonatal period (basically a surrogate for higher caloric intake) - specifically within the first two weeks after birth - were most closely related to elevated levels of 32/33-SPI in adolescence which were independent of birth weight. There was no association between values of 32/33-SPI and weight gain between two weeks of age and discharge from the nursery, discharge and 18 months, 18 months and 9 years, 9-12, and 13-16 years. Preterm adolescents, fed a low nutrient diet at birth, did not differ in stature, weight, BMI, or sum of skinfold thickness compared with premature infants who

were fed the high nutrient formula or from the control group of adolescents born at term.

The investigators conclude that premature infants who were fed a low nutrient formula (albeit one that impaired neonatal weight gain) for several weeks after birth resulted in lower concentrations of 32/33-SP in adolescence, and by inference these subjects may be less likely to develop insulin resistance. They hypothesize that the risk for developing insulin resistance in low birth weight neonates is not necessarily programmed by the intrauterine environment, but also by the immediate post partum extrauterine environment as exemplified by the high nutrient formula and more rapid weight gain that accompanies this diet. They suggest that altering current feeding practices of preterm infants by lowering their caloric intake and decreasing their early rate of weight gain may prevent later development of insulin resistance, cardiovascular disease, and the dysmetabolic syndrome without adversely affecting their long-term growth.

Singhal A, et al. *Lancet* 2003;361:1089-1097.

Editor's Comment: *Low birth weight infants are at risk for future development of the dysmetabolic syndrome (X) of dyslipidemia, insulin resistance, and type 2 diabetes mellitus.¹ It has been hypothesized that intrauterine factors that affect the fetal response to decreased blood flow or nutrient availability "program" the subsequent development of this syndrome - primarily by inhibiting tissue responsiveness to insulin. However, there is no specific explanation that explains the cellular and molecular mechanisms by which low birth weight predisposes to insulin resistance. The current work is of interest because it points to the possibility that post natal factors, in this instance rapid growth secondary to increased nutritional intake in very early life, contribute to the later development of insulin resistance. Thus, this observation affords the possibility of an intervention that may prevent this long-term complication without*

negatively impacting the overall growth of the low birth weight subject. Considered in the context of the findings is that partial nutrient restriction and growth hormone deficiency extend life in many species^{2,3} including perhaps primates. Since the level of 32/33-SPI is only a marker of insulin resistance, it will be necessary for Singhal et al to continue to follow these subjects and to document the development of insulin resistance and

other adverse events as, and if they occur.

Allen W. Root, MD

References

1. Goran MI, et al. *J Clin Endocrinol Metab* 2003;88:1417-1427.
2. Longo VD, Finch CE. *Science* 2003;299:1342-1346.
3. Tatar M, Bartke A, Antebi A. *Science* 2003;299:1346-1351.

Genetics, Chondrodysplasias and Other: New Potpourri

Skeletal dysplasias are distinguished by what part of the skeleton and/or bone is involved in various types of short stature. Metaphyseal dysplasia (MCD) refers to a group of skeletal disorders in which the diagnostic findings primarily involve the metaphyses of the tubular bones. Other bones are usually normal or only slightly affected. The metaphyseal involvement may be mild (as in Schmid's MCD) or more severe (as in Jansen's MCD). Some MCD syndromes have associated extra-skeletal features (e.g. MCD – McKusick type which is also known as Cartilage Hair Hypoplasia). There appears to be a new type of chondrodysplasia with a distinctive pattern of involvement, as described by Lee et al. An eight-year-old boy with a distinctive form of metaphyseal chondrodysplasia and a previously described family with 4 generations affected are the focus of Lee's report. The child had short stature and the birth weight was 3 kg. Bilateral genu varus and wrist swelling were first noted at 4 years of age. The mother had mild wrist flaring. She was not disproportionate by U/L ratio. At 8 7/12 years the boy's height was -2.9 SD below the mean and his U/L ratio was 1.21 (normal 1.0). No significant differences were noted in the length of the upper versus the lower part of each extremity, the spine, the facial configuration or the hair. Skeletal survey revealed metaphyseal abnormalities affecting proximally and distally the tibias, fibulas, femurs, humeri, radii and ulnar bones and the hands, but the spine was unaffected. The physical and radiological findings did not fit the Schmid, McKusick, or Jansen types of MCD. A very rare autosomal dominant 4 generation affected condition described by Rosenberg and Lohr (*Eur J Pediatr* 1986;145:40-45) has features similar to those of this patient, except the patients in this family reportedly had a wedge deformity and platyspondyly of the spine which Lee et al believed to be within the range of normal variance. No molecular studies were reported in the four patients reported by Rosenberg et al or in this 8-year-old boy.

Lee YS, et al. A distinctive type of metaphyseal chondrodysplasia with characteristic thickening of the distal ulna and radius: Possible MCD-Rosenberg. *Am J Med Genet* 2003;119A:50-56.

Another example of describing a chondrodysplasia by the sites where skeletal abnormalities occur is rhizomelic chondrodysplasia punctata (RCP). This rare autosomal recessive disorder has severe shortening of the proximal long bones (rhizomelia), bilateral cataracts and severe growth and psychosocial delay. White et al report the natural history of rhizomelic chondrodysplasia punctata. Radiographic evidence of stippled epiphyses is present and MRI examination of the cervical spine is often abnormal (kinking without compression of the cord and/or compression of the cord). All children with RCP are born with severe joint contractures that improve with time although not before many of the patients (40-85%) die by one year of age. Less than 10% of the 48 cases described in respect to death were alive by 12 years of age.

Biochemical analysis and complementation studies allowed separation of the 97 patients whose data were tabulated to be differentiated on the basis of peroxisomal enzymes into three types: (Type 1) a spectrum of PEX7 gene mutations, (Type 2) mutations in the acyl-CoA:diOHAcetonePO4 acyltransferase (DHAPAT) gene, and (Type 3) mutations in the ADAPS (alkyl-diOHAcetonePO4 synthesis) gene.

The value of this article by White et al is that there has been a sincere attempt to delineate the natural history of RCP. The authors systematically address health concerns that arise in infants and children with RCP. The intent of White et al is to present evidence-based guidance to care providers so they can better help families understand and cope with this diagnosis. For example, 90% of infants survive for the first year and 50% survive until 6 years. Previously, death was believed to almost always occur early in infancy or childhood. Medical personnel or parents concerned and/or involved with patients with suspect or proven diagnosis of RCP are strongly encouraged to read the complete article.

White A, et al. Natural history of rhizomelic chondrodysplasia punctata. *Am J Med Genet* 2003;118A:332-342.

Other examples of chondrodystrophies are those in the subgroup known as spondylo-epi-metaphyseal dysplasia (SEMDs) which includes a number of disorders each defined by the combination of vertebral, epiphyseal, and metaphyseal anomalies present.

One such entity is the Dyggve-Melchior-Clausen Syndrome (DMCS) which is characterized by short trunk dwarfism (<-4SD) with specific radiological appearances most likely reflecting abnormalities of the growth plates including platyspondyly (flattened peripheral bodies) with notched end plates, metaphyseal irregularities, laterally displaced capital femoral epiphyses, and small iliac wings with lacy iliac crests. Mental retardation is an inherent part of the syndrome. DMCS is progressive and clinical features are reminiscent of a storage disorder, specifically Morquio's disease, but the two conditions can be differentiated by the absence of corneal clouding, deafness, valvular disease and/or mucopolysacchariduria, all of which are characteristic of Morquio's disease.

Ghouzzi et al have used a positional cloning strategy to identify the DMC gene. They detected 7 deleterious mutations within a gene predicted from a human transcript (FLJ20071) in 10 DMC families. The DMC gene transcript is widely distributed but appears abundant in chondrocytes and fetal brain. The authors cannot explain the function of the gene product at this time, but conclude that the DMC syndrome results from loss of function of a gene that they propose to name Dymeclin, which may have a role in the process of intracellular digestion of protein.

Ghouzzi VE, et al. Mutations in a novel gene dymeclin (FLJ20071) are responsible for Dyggve-Melchoir-Clausen syndrome. *Hum Mol Genet* 2003;12:357-364.

A fourth example of types of chondrodysplasia and how they are designated is the entity called acrocapitofemoral dysplasia which is characterized by short stature of variable degrees with short limbs and brachydactyly. It is included in the differential diagnosis of hypochondroplasia. These patients also have large heads and often have pectus deformities. Epiphyseal changes are present at the shoulders, knees, ankles, hands, hips and proximal femurs. The latter are egg shaped with very short femoral necks. Shortened tubular bones characterize the brachydactyly. Congenital anomalies are limited to the skeletal system and intelligence is characteristically unaffected.

Homozygosity mapping by descent was performed in two consanguineous families. The Indian hedgehog gene (IGG) was found to be mutated in affected individuals. The nucleotide changes are seen in the amino terminal signaling domain, which is responsible

for short and long range signaling. Thus, it appears to affect the regulation and proliferation of the hypertrophic chondrocytes in the growth plate. The authors postulate that the mutations cause an increased rate of chondrocyte differentiation by diminished Indian Hedgehog signaling in the growth plate.

Hellemans J, et al. Homozygous mutations in *IHH* cause acrocapitofemoral dysplasia, an autosomal recessive disorder with cone-shaped epiphyses in hands and hips. *Am J Med Genet* 2003;72:1040-1046.

First Editor's Comment: *The genome project has made identification of mutated genes relatively easy to identify. The effects of different mutations of the same gene has been particularly evident among the chondrodystrophies, both in relating two different entities to different mutations of the same gene and differentiating and identifying different gene abnormalities for what used to be thought the same disease entity. Unfortunately descriptive names are often misleading because there is tremendous overlap among these entities. The most recently updated classification of skeletal dysplasias can be found at www.csmc.edu/genetics/skeldys.*

Judith Hall, OC, MD

Second Editor's Comments: *One is struck by the clinical resemblance of acrocapitofemoral dysplasia (ACFD) to achondroplasia. The phenotype is not identical, but the rhizomelic shortening of limbs, large head with prominent forehead, narrow thorax, bowing of the knees and even overgrowth of the proximal fibula on X-ray are similar. The reason for this resemblance may lie in the relationship of *Ihh* to *FGFR3*, which is mutated in achondroplasia, in the growth plate. Both regulate chondrocyte proliferation: *Ihh* positively and *FGFR3* negatively. In ACFD the positive effect on proliferation is lost; however in achondroplasia the mutations are activating in nature so that they enhance the anti-mitotic effects of *FGFR3*. In other, both lead to reduced chondrocyte proliferation. A consequence of the anti-mitotic effects of *FGFR3* mutations in achondroplasia is a reduction in the number of terminally differentiating chondrocytes. Since these cells are the source of *Ihh*, the achondroplasia mutations secondarily reduce the production and local effects of *Ihh*. Thus, these two disorders look alike to clinicians because they involve disturbances of the same regulatory pathways in the growth plate.*

William Horton, MD

Size at Birth and Early Childhood Growth in Relation to Maternal Smoking, Parity & Infant Breast-Feeding: Longitudinal Birth Cohort Study and Analysis

The relationship between maternal smoking, parity and early breast or bottle feeding to size at birth and childhood growth were evaluated. A large representative birth cohort was studied between 0 and 5 years of age. A total of 1335 normal infants had weight, length, height and head circumference measured at birth and subsequently up to ten occasions until they were 5 years of age. Multilevel modeling was used to analyze the longitudinal growth data. Infants of maternal smokers were systematically small at birth when compared with infants of non-smokers. However, these infants showed complete catch-up growth over the first 12 months of life. Infants of primiparous pregnancies were thin at birth and showed dramatic catch-up growth, and were heavier and taller than infants of nonprimiparous pregnancies from 12 months onwards. Breast-fed infants were similar in size at birth to bottle-fed infants, but grew more slowly during infancy; differences in weight and length persisted throughout the study period. Among infants who showed catch-up growth, males caught up more rapidly than females. The authors concluded that early postnatal growth rates are strongly influenced by a drive to compensate for antenatal restraint or enhancement of fetal growth by maternal uterine-factors.

Ong KKL, et al. *Pediatr Res* 2002;52:863-867.

Editor's Comments: *This very interesting paper provides unique longitudinal growth data from a large prospective birth cohort. Some of the factors studied are well known to alter growth, such as maternal smoking which inhibits growth in utero, and/or breast milk which is known to be associated with lower growth rates in infancy as compared with cow-milk*

formula fed children. However, little data existed for long-term measurements of these types of infants up to 5 years of age. This paper contributes significantly with strong data. Although it is reassuring to note that infants born to mothers who smoke during pregnancy exhibit catch-up growth with no long-term consequences in height, the negative effects of smoking should not be overlooked as they transcend growth. These were not studied in this paper.

Of great interest is the long-term growth divergence in breast-fed infants as compared to bottle-fed infants. This difference in growth progression persists after infancy with significant differences throughout the first 5 years of life. Both weight and height were decreased in the breast-fed group as compared to the bottle-fed group. It is now known that the way infants grow in utero, as well as during the first year of life, might have very important consequences for the development of adult-onset disease. Similarly, the rate of weight accretion during infancy and childhood might play a role in the development of obesity later in life. These data provide evidence that human milk feedings are best for feeding infants, allowing them rates of weight gain for the first 5 years of life that may be more compatible with a more appropriate body weight later in life. In light of the current epidemic of obesity, any factor that may contribute to it should be seriously considered. The growth charts for breast-fed infants developed by the CDC (<http://www.cdc.gov/growthcharts/>) and by the Eurogrowth study (www.Eurogrowth.org) are very useful in monitoring the growth of such children, but these do not extend until 5 years of age; such would be highly desirable in light of these data.

Fima Lifshitz, MD

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