

the increased frequency of achondroplasia births in older fathers.

The authors addressed in considerable depth various possible explanations for their findings. Several involve experimental biases or artifacts. For example, fathers of children with sporadic achondroplasia may constitute a subgroup of men with distinct mutation properties that differ from the sperm donor population. There may be unappreciated ascertainment biases with regard to the makeup of donor population or in previous studies. Despite extensive controls, there could have been underreporting of mutations in the PCR assay. These studies may have led to overestimating the magnitude of the paternal age effect.

Two of the possibilities deserve special attention. The first is that there may be an age-dependent increase in germ-line permutations at the G1138A site that are neither converted to a full mutation or repaired before fertilization. One candidate lesion would be an unrepaired G/T mismatch resulting from deamination of 5-methyl cytosine. The cytosine at position 1138 is known to be highly methylated in sperm and therefore a candidate for such a pre-mutation, which might go undetected under conditions of PCR.

Another possibility is that the G1138A mutation gives a selective advantage to sperm that carry it. The authors acknowledge the highly speculative nature of this possibility, but point out that FGFR3 is expressed and presumably active in mature sperm cells. They also caution that invoking this possibility must include an explanation of how a potential selective advantage would increase with age.

Tiemann-Boege et al. *PNAS* 99 2002;14952-57.

Hurst LD, Ellegren H. *Nature* 2002;420:365-66.

**Editor's comment:** *Many observations over the last several years have led to the dogma that FGFR3, especially the site where achondroplasia mutations arise, is extraordinarily mutable during spermatogenesis and that this mutability increases dramatically with age. The idea that DNA is prone to replication or mitotic errors, that there are many more opportunities for such errors to occur during spermatogenesis compared to oogenesis, and these can somehow accumulate with age has been conceptually appealing and is easy to explain during counseling. However, the results reported here cast serious doubt on its validity. Assuming they hold up, which seems highly likely given the considerable lengths to which the authors went to control their experiments and validate their results, the dogma will need to change.*

*The notion of genetic pre-mutation in achondroplasia is not new. It was proposed by John Opitz and others long before mutations of FGFR3 were discovered. It never gained much momentum, probably because it lacked experimental data with regard to a specific locus or mutation; however, the paper by Tiemann-Boege et al may add new life to this concept.*

*The possibility that sperm which harbor activating mutations of FGFR3 have a selective advantage for motility, fertilization or the like, is intriguing. Of note is that activating FGFR3 mutations found in the achondroplasia family of disorders have been detected in several types of cancer, including multiple myeloma and bladder, breast and colon carcinoma. The mechanisms through which the mutations contribute to neoplasia are not well understood. However, they may well give the cancer cells a competitive advantage over the normal cells.*

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## Is Insulin-Like Growth Factor-1 Monitoring Useful in Assessing the Response to Growth Hormone of Growth Hormone-Deficient Children?

In order to assess the relationship between insulin-like growth factor-1 (IGF-1) and the growth hormone (GH) dose utilized to treat GH-deficient children, the IGF-1 response was compared with the changes noticed in height-standard deviation scores (H-SDS) and height velocity during treatment.

The study was carried out in 24 prepubertal GH-deficient patients with a mean age of  $10.5 \pm 1.8$  years and a mean bone age of  $8.4 \pm 2.1$  years. H-SDS for chronological age and bone age before therapy were  $-2.6 \pm 0.8$  and  $-1.2 \pm 0.8$ , whereas height velocity was  $-1.1 \pm 1.5$  cm. Serum IGF-1 and insulin-like-growth factor binding protein-3 (IGFBP-3) levels were measured before, after 6 months and 12 months of GH treatment,

and correlated with the GH dose. IGF-1 increased significantly during the first six months of therapy, but did not increase any further at twelve months, despite the use of higher GH dosages (0.14 vs. 0.1 IU/kg/day), whereas IGFBP-3 increased at both 6 and 12 months. There was no correlation between GH dose and IGF-1 and IGFBP-3 levels. Height velocity as well as height for chronological age and bone age were significantly greater after one year of treatment with GH. The authors concluded that the increment in IGF-1 during therapy did not correlate with the interval height increase and was found to be less useful than height increments in adjusting the GH dose needed to treat prepubertal GH-deficient children.

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

**Editor's Comment:** *The monitoring approach that individualizes therapy and includes both biochemical and auxological determinations to titrate the GH dose utilized to treat GH deficiency is considered standard practice in treatment with GH. A common practice is to monitor height increments and serum IGF-1 and IGFBP-3 concentrations to guide with the treatment of GH-deficient patients. However, in this study IGF-1 and IGFBP-3 levels were not found useful in assessing the response to GH treatment. There are wide variations in IGF-1 levels during the day, as well as different stages throughout time, and even in the same individual. Of great importance is the nutritional status and intake of the patients in relation to the IGF levels. Any one or several of these factors might have played a role in the*

*lack of a clinically relevant, as well as statistically significant, difference in IGF levels found in this small group of patients studied. The reader is advised to read the editorial on this paper published in the same journal by Dr. Barry Bercu<sup>1</sup> entitled "Titration of growth hormone dose using insulin-like growth factor-1 measurements: Is it feasible in children?" This study once again demonstrates that careful measurements of height and the monitoring of growth progression is the most important marker in the assessment of short children with or without GH deficiency, as well as during treatment with GH.*

#### Reference

1. Bercu B. *J Pediatr* 2002;141:601-5.

Fima Lifshitz, MD

## Leptin Measurement in Urine and its Relationship to Other Growth Peptides in Serum and Urine

Leptin is a 167 amino acid product of adipocytes that has multiple physiologic effects including appetite suppression, alteration in energy balance, acceleration of pubertal onset, and both stimulatory and inhibitory effects on bone mineralization. Its role in human physiology other than for appetite suppressive effects and possible hypogonadotropism, is uncertain. The authors have adapted a two-site immunoradiometric assay (IRMA) for measurement of leptin in serum to its determination in urine. In this assay, two mL of urine (unmodified by acidification or dialysis) are incubated initially in a plastic tube coated with antibody (#1) to leptin, followed by incubation with a second, radiolabeled antibody (#2) to leptin with specificity to a different epitope. Free labeled antibody (#2) is removed and radiolabeled bound antibody (#2) quantitated. Leptin in urine (lep/u) is calculated by comparison to standards of leptin similarly prepared. Lep/u was quantitated in timed overnight urine collections in 188 (100 females) children and adolescents 5-19 years of age. Serum and/or urinary levels of growth hormone (GH), insulin-like growth factors (IGF-I and IGF-II), and IGF binding proteins (IGFBP3 and IGFBP-1) were also determined. The IRMA for lep/u was validated by dilution and recovery experiments. In the cross-sectional survey, total lep/u was similar in prepubertal boys and girls (0.2 ng/night). Lep/u values increased to a peak in boys at Tanner genital stage III (0.8 ng) and then declined; in girls, lep/u continued to increase through breast stage V (1.1 ng) and values were significantly higher in adult females than in males. The maturational patterns of lep/u were similar to those described for serum leptin (lep/s) changes. Log transformed values of lep/u and

random lep/s were highly correlated. Lep/u levels were variable related to age, stage of sexual maturation, BMI, IGF-I, and IGF-II. In two adults in whom overnight urines were collected consecutively for more than 30 nights, nocturnal lep/u values varied night-to-night by 42-75%. In a substantial number of specimens (20+%) obtained from both the children and adults, lep/u was not measurable. The authors conclude that measurement of timed overnight lep/u is a feasible method for longitudinal assessment of leptin production in children, adolescents, and adults.

Zaman N, et al. *Clin Endocrinol* 2002;58:78-85.

**Editor's Comment:** *The majority of secreted leptin is catabolized in the kidney to smaller peptides. The investigators relied, in part, upon the specificity of two antibodies directed to different epitopes of leptin to validate the IRMA for lep/u. However, it would have been of interest to examine the physicochemical properties of urinary leptin by size exclusion chromatography and/or mass spectroscopy to determine more accurately the nature of the peptide measured by the IRMA. It would also have been of interest to have measured urinary/serum levels of gonadotropins and sex hormones and to assess their relationships to lep/u and stages of sexual development (perhaps a manuscript already in preparation). Nevertheless, the data are of interest and the described method may be helpful in furthering our understanding of the relationship between growth, sexual maturation, and leptin.*

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