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Future Articles

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Molecular Genetics of Peripheral Precocious Puberty
Controversies in the Treatment of Intersex
Agonadia, Germ Cell Failure & Other Multiple
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SOMATOMEDIN HYPOTHESIS: TIME FOR REEXAMINATION

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In 1957, Salmon and Daughaday¹ observed that incorporation of radioactive precursors of cartilage acid mucopolysaccharides could be stimulated in vitro by serum from hypophysectomized rats that had received growth hormone (GH) in vivo. Addition of GH directly to the medium, however, did not enhance precursor incorporation. The authors inferred that GH did not act directly on cartilage; instead, it did so by generation of a factor in the serum that enhanced the incorporation. The serum factor was originally named "sulfation factor", because radioactive sulfate was used as the precursor. The magnitude of the effect was proportional to the volume of serum used, and the factor was originally used as a bioassay for GH activity.² The in vitro incorporation test was discarded when radioimmunoassays of GH became available.³ Subsequently, the sulfation factor was renamed "somatomedin", because it seemed to be the effector by which GH stimulated somatic growth.⁴ Several somatomedins were identified, and the components of the system were designated by letters of the alphabet, as somatomedin A, B, and C.⁵

Before the development of a radioimmunoassay for insulin,⁶ its activity in serum was measured by bioassay of its effects, such as glucose uptake by isolated tissues in vitro.⁷ Radioimmunoassays of serum from fasting animals, however, showed that as little as 10% of the effect on serum glucose was caused by insulin itself.⁸ Furthermore, the insulin-like activities were minimally suppressed by the addition of anti-insulin antibodies to the serum.⁹ The "noninsulin" effects were attributed to the presence in the serum of nonsuppressible insulin-like activities, and a nomenclature was subsequently adopted designating them as insulin-like growth factors (IGF).¹⁰

The amino acid sequences of two nonsuppressible insulin-like activities (IGF-1 and IGF-2) were elucidated by Rinderknecht and Humbel,¹¹ and their tertiary structures were subsequently determined by Blundell et al.¹² They consist of A-domains homologous to the A-chain of insulin, B-domains homologous to the B-chain, C-domains homologous to the C-chain of proinsulin, and D-domains that extend from the C-terminals of the A-chains. Analysis of somatomedin-C, the principal growth factor of the somatomedin family, showed that it had the same amino acid sequence as IGF-1, and the two were considered to be identical.¹³ Because the largest fraction of IGF-1 in the circulation is derived from the liver, where the expression of the gene is regulated by GH,¹⁴ the *somatomedin hypothesis* was developed. It stated that the anabolic effects of GH on cartilage and other tissues were mediated through IGF-1 synthesized in the liver and not by direct action of GH on these original target tissues.⁴ Although the hypothesis has gained widespread acceptance, there is mounting evidence that it may have to be modified or even abandoned. A priori, it would seem unlikely that a factor that *exerts* hypoglycemic effects¹⁵ should be the effector of GH action.¹⁶ Since GH is an insulin counter-regulatory hormone,¹⁷ it seems paradoxical that it should exert its effects through a factor that produces hypoglycemia.

Isaksson et al¹⁸ summarized evidence available in 1985 that GH acts directly on prechondrocytes, epiphyseal plate cartilage, cloned preadipose cells, and myoblasts without the intervention of a mediating factor. GH also has been found to act directly on other tissues in vitro, such as stimulating erythropoiesis in vitro.¹⁹

More recently, additional evidence doubting the somatomedin hypothesis has accumulated. The evidence comes from three different sources. First, Salmon and Burkhalter²⁰ revisited the experiments originally conducted by Salmon and Daughaday¹ that formed the basis for the hypothesis. In these newer studies, they found that in contrast to their earlier experiments, GH added directly to cartilage from hypophysectomized rats did stimulate incorporation of radioactive sulfate into proteoglycans and radioactive

thymidine into DNA. They ascribed their newer findings to the use of a different medium in the more recent experiments; HEPES-buffered amino acid-glucose solution with a low concentration of bovine serum albumin. Amino acids were not added to the medium used in the original experiments, and the authors also speculate that a nondialyzable component of hypophysectomized rat serum may have inhibited the incorporation of sulfate into cartilage.

Secondly, a series of observations that cast doubt on the hypothesis was reported by Yakar et al²¹ who devised an elegant set of experiments to determine if hepatically derived IGF-1 is the circulating mediator of GH effects on postnatal growth and development. Using the Cre/*loxP* recombination system, they deleted the IGF-1 gene exclusively in the livers of mice. Their finding of a 75% reduction in the concentrations of IGF-1 in the serum confirmed that the liver is the primary source of circulating IGF-1. Despite this reduction in circulating IGF-1, there was no evidence of growth impairment when the liver IGF-1-deficient mice were compared with their wild-type litter mates. These experiments have been confirmed by Sjogren et al²² using the model devised by Yakar et al.²¹

A third observation casts doubt on the hypothesis. This concerns the issue of the lipogenic properties of IGF-1. In a report of long-term treatment of European patients with GH insensitivity syndrome, IGF-1 treatment led to accelerated growth, but there was also a substantial gain in fat mass that correlated significantly with the increase in height.²³ Ecuadorian patients with the same syndrome experienced a significant increase in growth rate when treated with IGF-1.²⁴ They also experienced a relative increase in mean body weight for height when they were treated with the higher of two doses of IGF-1.²⁴ It should be noted that not all investigators have reported an increase in fat mass.²⁵ Increased lipogenesis has also been shown to occur in a subject with an IGF-1 deletion who was treated with IGF-1.²⁶ The authors inferred that the lipogenic effects could be ascribed to the reduced concentrations of GH in the serum after IGF-1 treatment. This explanation is untenable, however, because increased lipogenesis was also found in the subjects with GH insensitivity syndrome.^{23,24}

Increased fat mass is inconsistent with the hypothesis that IGF-1 mediates the effects of GH, which is a lipolytic and anabolic hormone.²⁷ It is more in keeping with an insulin-like action, such as that seen in infants of mothers with diabetes in whom hypoglycemia is prevented by placental exchange of glucose despite high concentrations of insulin in the fetal circulation.²⁸ The increased length and fat content of these infants is evidently because of the anabolic and lipogenic effects of insulin secreted by the fetal pancreas.^{29,30} In

considering the role of IGF-1 in growth promotion, distinguishing between the effects of circulating IGF-1 and IGF-1 produced by autocrine/paracrine mechanisms is important. In their experiments, Yakar et al²¹ found that growth was severely restricted in IGF-1 knockout mice in which the gene was deleted from all tissues. There can be little doubt, therefore, that the IGF and their binding proteins are important growth factors when produced locally by autocrine/paracrine mechanisms. Moreover, as pointed out previously, expression of the hepatic gene for IGF-1 is regulated by GH,¹⁴ and plasma concentrations of IGF-1 are uniformly increased in adults with acromegaly and children with gigantism.³¹ Despite earlier findings that plasma IGF-1 and IGF-binding protein-3 concentrations might be useful in the diagnosis of GH deficiency, there are substantial disagreements on this issue.³²⁻³⁴

It is time to take note of the deficiencies in the hypothesis and possibly to abandon it completely. There is a strong body of evidence that liver-generated IGF-1 is unlikely to be responsible for the linear growth effects of GH and that the actions of GH on its target tissues do not require mediation by this factor in the circulation. It is also unlikely that measurement of these growth factors and their binding proteins in the plasma will be useful in assessing the role of GH in growth retardation.

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Abstracts from the Literature

Leptin-Replacement Therapy for Lipodystrophy

Severe lipodystrophy is known to be associated with leptin deficiency, insulin resistance, hypertriglyceridemia and hepatic steatosis. Thus, the authors assessed whether leptin-replacement would ameliorate this condition and its complications. Nine female patients (ages 15 to 42 years; 8 with diabetes mellitus) with lipodystrophy of various types, with serum leptin levels of less than 4 mg/ml, and with high insulin levels received recombinant methionyl human leptin subcutaneously twice a day for four months in escalating dosages (0.03 mg to 0 – 0.4 mg/kg/day) to obtain low, intermediate, and high physiologic serum levels of leptin. During the treatment, the serum leptin levels increased from a mean of 1.3 +/- 0.3 mg per ml to 11.1 +/- 2.5 mg per ml.

The glycosylated hemoglobin values in the diabetic patients decreased, a mean reduction of 1.9%. After four months of therapy, the average triglyceride levels decreased by 60% and the liver volume diminished in size by an average of 28% in all patients. Leptin also led to a discontinuation or a large reduction in the anti-diabetes therapy. The self-reported daily caloric intake also decreased significantly. No major problems or side effects occurred. The authors concluded that leptin replacement improved glycemic control and decreased triglyceride levels in patients with lipodystrophy and leptin deficiency.

Elif AO, et al. *N Engl J Med* 2002;346:570-578.

Editor's Comment: *These investigators demonstrated that leptin deficiency contributes to insulin resistance and other metabolic abnormalities associated with severe lipodystrophy. The reduction of glycosylated hemoglobin associated with leptin therapy is important, reflecting improved diabetic control. This could lead, if the effect persists, to a decrease in the relative risk of retinopathy and/or nephropathy in the diabetic population. The decreased triglyceride levels may reflect a reduced relative risk of adverse cardiovascular events. The alterations that characterize lipodystrophy are known to be refractory to other treatments, and, therefore, this paper reports a novel action of this hormone in addition to its known role in the control of energy homeostasis.*

For those readers wishing more information regarding leptin, consult the article in the last issue (GGH 2002 Vol 18:2), which is entitled "The Endocrine Function of Adipose Tissue" and the article entitled "Molecular Physiology of Leptin and Its Receptor" (GGH 1998 Vol 14:2). Several articles from the literature concerning leptin have been abstracted in GGH since 1998.

Fima Lifshitz, MD

Effect of Growth Hormone Therapy on Height in Children with Idiopathic Short Stature: A Meta-Analysis

The authors reviewed all published (English language) manuscripts and manually searched all issues of the *JAMA*, *Journal of Pediatrics*, *Pediatrics*, and *Acta Paediatrica*, and the meeting abstract books of the *Lawson Wilkins Pediatric Endocrine Society* and the *Endocrine Society* between 1985-2000 for publications (N=761) that reported primary effects of recombinant human growth hormone (rhGH) on the growth of children. From this group, the authors culled those

papers reporting adult stature in more than 5 healthy children with "idiopathic" short stature treated with rhGH whose heights were below the 10th percentile at the initiation of treatment and who had "normal" GH secretion (≥ 10 ng/mL during provocative testing) and in which more than 50% of the starting population completed the study. From this pool, 19 articles describing 10 controlled studies (N=434) and 34 articles reporting 28 uncontrolled studies (N=655) were selected