

GHRELIN A NEW HORMONE IMPLICATED IN THE REGULATION OF GROWTH HORMONE SECRETION AND BODY ENERGY HOMEOSTASIS

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INTRODUCTION

Growth hormone (GH) has a complex regulation with two antagonistic hypothalamic hormones, growth hormone releasing hormone (GHRH) and somatostatin, as well as the liver-derived hormone IGF-I. Perhaps the old name of somatotrophic hormone (STH) is more coherent than GH, as this hormone is tightly regulated by the metabolic milieu; additionally, this regulation appears to be superimposed over the classical regulation by peptide hormones. For example, metabolic signals such as glucose, amino acids, free fatty acids and their by-products, such as keto-acids, as well as the energy balance status regulate the secretion of GH in a very relevant form. In turn, GH causes complex actions on the general metabolism of a given individual.

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From The Editor's Desk

This issue marks the beginning of a new era for *Growth, Genetics & Hormones (GGH)*; Dr. Robert M. Blizzard retired and I became the Editor-in-Chief. This opportunity is an honor and the task is a major challenge, as it will be hard to fill the shoes of my mentor. In this issue you will find some modifications in the format and appearance of the journal and some changes in the editorial board. Hopefully, you will also note and appreciate the continuous high quality of the publication, papers reviewed and editorial comments. The lead article on Ghrelin and the abstracts along with editorial comments are very pertinent and timely, all together we are very pleased with this issue.

Dr. Judith G. Hall who had been with this journal since its inception has retired from *GGH* and will be sorely missed. I bid her farewell and thank her for all of her many contributions. On the other hand, I welcome Adda Grinberg, MD and David E. Sandberg, PhD whose abbreviated biographical sketches are posted on our web site (www.GGHjournal.com). Two years ago we launched *GGH* on the internet and this has allowed us to reach a larger group of colleagues worldwide. Thus, I want to welcome a new group of international consulting editors who will help us project the journal internationally: Yoshikazu Nishi, MD in Japan, Raphael Rappaport, MD in France, and Alfonso Vargas, MD representing Latin America.

The last issue of 2003 included a survey which helped us renew our subscribers list and profile the readership. Over 60% of readers are pediatric endocrinologists and 15% are geneticists. A majority of our readers (78%) reside in the USA. Over two-thirds of the subscribers access the journal via the internet, usually after they are notified via e-mail announcing the publication of a new issue. Sixty percent view the journal and download it to keep as a reference. I thank everyone who responded to the survey and for the wonderful comments received; 81% gave *GGH* a very high/high ranking. The details of the survey are posted at www.GGHjournal.com.

This sets the challenge for the future - to reach more colleagues, to continue to improve the journal and to bring to our readers the most current reviews, updated information and advances in the field along with erudite editorial comments. Only through the internet with the on-line version of *GGH* can this be accomplished; within the budgetary constraints, a printed publication would not allow us to meet these goals. Thus we will continue to limit the printed subscriptions and phase out the printed journal by the end of 2004 while we further expand the readership through the internet.

Finally, I want to express my deep appreciation to our sponsors for the generous unrestricted, no strings attached educational grant for the publication of *GGH*. Please be sure you tell them how much you appreciate *GGH*. We also request that you keep us up-to-date with your current e-mail address so you continue to receive *GGH*. I thank you in advance for your consideration and comments (editor@GGHjournal.com).

Fima Lifshitz, MD, Editor-in-Chief

The upshot of this picture is of one hormone whose actions are implicated in a dual action on somatic growth and in the regulation of general metabolism, and which is in turn, regulated by the energetic homeostasis of the individual.¹ The recently discovered hormone, ghrelin, may well be the bridge connecting somatic growth with general metabolism.

HISTORICAL BACKGROUND

Ghrelin is the result of the so called “reverse pharmacology”, which started with the development of artificial compounds named growth hormone secretagogues (GHS), followed by the cloning of their receptor and finally the identification of the natural hormone. In fact, in the late 1970s the first highly potent GH-releasing hexapeptide (GHRP-6), was developed. This was followed by other GHS compounds such as hexarelin, or MK-0677.² These GHSs were found to be potent releasers of GH *in vitro* and *in vivo*, by acting on specific receptors at the pituitary level not related to GHRH or somatostatin. Furthermore, they were active by any route of administration, including oral, and active in all the species tested. Later GHSs were used for the cloning of the GHS-receptor.³ The GHSs were not discovered, but invented, as no similar compounds existed in nature. Obviously, the new receptor must have a natural endogenous ligand. The orphan-receptor strategy was then employed by the group of Kojima and Kangawa⁴ to screen different tissue extracts. The highest expression of GHS-receptor activating factor was found in the stomach. This endogenous ligand was named ghrelin. Ghrelin was found to be a potent releaser of GH and in addition, actively participate in controlling energy balance and the regulation of food intake.⁵ Reverse pharmacology permitted identification of this natural ligand, ghrelin.

DISTRIBUTION OF GHRELIN-SECRETING CELLS

Two cellular areas in the body were found to be relevant in the production of ghrelin. One was an area in the gastric fundus where ghrelin is predominately expressed and secreted. Specifically, plasma ghrelin originates in the oxyntic gland where A-like cells exist.⁶ Lower concentrations have also been reported in the remainder of the bowel including the colon. Ghrelin positive cells are positioned close to the capillaries and have no contact with the lumen of the oxyntic gland, indicating that secretion occurs into the plasma and not into the intestinal tract.

The second area was found in the central nervous system where neuronal cell groups release ghrelin in a synaptic transmission. Since ghrelin was determined to be implicated in the regulation of appetite, it was not surprising to find abundant ghrelin in the arcuate

nucleus of the hypothalamus which also is a region rich in GHRH neurons.⁴ Elsewhere, in the CNS, ghrelin was also present. Immunoreactive neurons were observed in a continuum filling the internuclear space between the paraventricular, arcuate, ventromedial, and dorsomedial hypothalamic nuclei, the perifornical region, and the ependymal layer of the third ventricle.⁷ Interestingly, these novel cell groups of ghrelin immunoreactive neurons did not overlap with any of the known cell populations implicated in energy homeostasis, thus suggesting new functions. In addition to their role in the regulation of energy balance, whether these neuronal groups also participate in the regulation of GHRH or somatostatin neurons is an open question.

Ghrelin has also been identified in the placenta,⁸ an organ that contains all the main regulatory components of the somatotrope axis, i.e., GH, GHRH, SST, IGF-I, and ghrelin. Although, placental expression of ghrelin changes significantly throughout pregnancy,⁸ and is involved in the decidualization of human endometrial stromal cells,⁹ the physiological function of this new hormone in the placenta is unknown. The pituitary, heart, kidney, endocrine pancreas, gonads, lungs, and lymphocytes all express ghrelin in low amounts.¹⁰⁻¹⁵

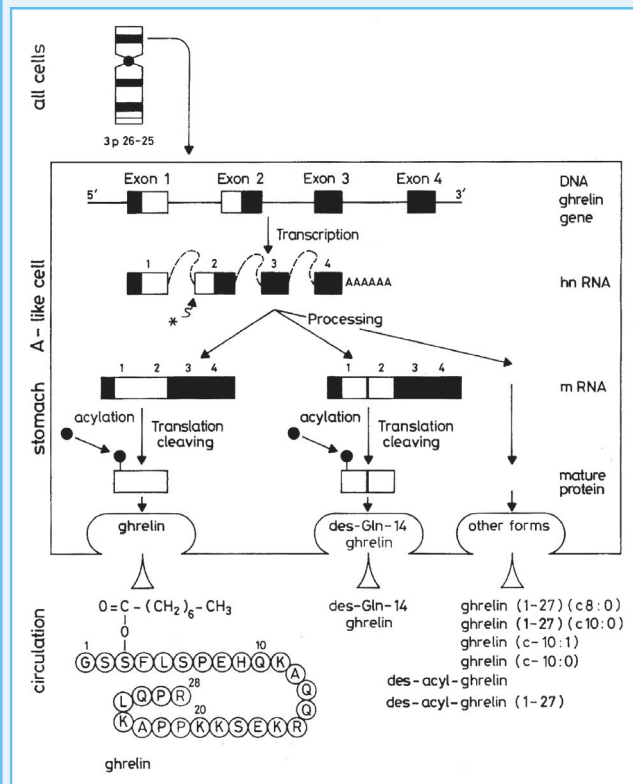
MOLECULAR BIOLOGY

The human ghrelin gene is located in chromosome 3. It is made up of 4 exons and 3 introns. The mature protein is encoded in exons 1 and 2 (Figure 1).¹⁶ The genetic structures of the ghrelin genes in the human and rat are identical and very similar to that gene in the mouse. The 5'-flanking region of the gene contains a non functional TATATAA box, as well as a ghrelin promoter which is activated by glucagon and c-AMP, although no AP1 site or CRE element is present.¹⁷ Some gastric tumor cell lines express the promoter, however others do not, suggesting that human ghrelin promoter may have cell-specific activity. The hnRNA of the gene transcript is processed by alternative splicing to yield two different mature mRNAs; one produces the ghrelin precursor and the second yields des-Gln 14-ghrelin.¹⁸ Ghrelin provides the first example of the production of two different mature biologically active peptides resulting from the alternative splicing of a peptide coding region.

The human ghrelin precursor (prepro-ghrelin) is composed of 117 amino acids, and the ghrelin sequence of 28 amino acids immediately follows the 23-residue signal peptide. Before being secreted, the ghrelin molecule undergoes an enzymatic process at the cytoplasm, an n-octanoyl addition at Ser 3. This esterification by n-octanoic acid, which is essential for the biological activity of ghrelin, yields the finally secreted peptide of 3315 mw. This process of acylation

Figure 1

Operation of ghrelin gene in humans



Ghrelin is encoded in the two first exons, and it is unique in the hnRNA processing that by alternative splicing two mature mRNAs are derived, one for ghrelin and other for des-Gln-14-ghrelin. The asterisk marks the boundary between the first intron and the second exon where the alternative splicing occurs. Before secretion, a n-octanoyl acylation occurs in Ser3 through a novel and still undefined enzymatic mechanism. In addition other related molecules are also secreted in minor quantities. In rodents, a testis specific ghrelin gene-derived transcript is encoded in the third intron.

Reprinted from: Casanueva FF, Dieguez C. *Rev Endocrine Metab Dis* 2002;3:325-338.

has no precedent in cell biology either, being the first example of acylation in a secreted protein.¹⁰

The main product of that original synthesis process is mature ghrelin. The production of des-Gln14-ghrelin is minor. In addition, the human stomach releases small quantities of other related molecules.¹⁹ The active binding core of the molecule consists of the first 4-5 amino acids including the acylated Ser3, short peptides containing this sequence efficiently bind to the GHS receptor, although they are devoid of GH secretory capability.¹⁰ It is interesting to speculate how the fatty acid residue changes the physical properties of ghrelin to facilitate its coupling in the biomembrane-receptor structure.

GHRELIN SECRETION AND ACTION

Ghrelin originates mainly in the stomach, and circulates at plasma concentrations of 200-600 ng/L. However, close to 80% of the total content is deamidated ghrelin, i.e., devoid of biological activity. Current RIAs mostly measure total ghrelin. Precaution is needed in the interpretation of data as bioactive ghrelin does not have a fixed ratio in relation to total ghrelin. Interestingly, the integrated secretion of ghrelin during 24 hours correlates significantly with the values obtained in the basal state²⁰ making it possible to use a single determination in clinical situations. No significant differences occur between serum and plasma concentrations; total ghrelin is resistant to repeated thawing, however warm temperatures for prolonged times should be avoided.²¹

Controversy exists whether ghrelin crosses the blood-brain barrier (BBB) to act as the afferent loop controlling either energy homeostasis or GH secretion. Human ghrelin has been reported to cross the BBB, but rodent

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ghrelin reportedly does it with less effectivity.²² No doubt exists that ghrelin administration activates *fos* and *Egr-1* proteins in neurons of the arcuate, paraventricular and dorsomedial nuclei, and the area postrema of the hypothalamus, while deamidated ghrelin in these studies was devoid of action.²³ The debate is whether peripheral ghrelin acts by directly activating CNS receptors located inside or outside the BBB, or if these actions are mediated peripherally through activation of vagal nervous structures.²⁴ The latter point is of extraordinary interest as several reports state that in rats, vagotomy abolishes ghrelin-induced feeding and GH discharge. This suggests that the gastric vagal nerve is the major afferent pathway conveying ghrelin's signals to the brain.²⁴ Regardless, direct neuronal activation occurs after the activation of the ghrelin receptors, which are located on GHRH and NPY neurons, as well as in additional neurons, as was previously demonstrated for GHRP-6.²³ Somatostatin, cortistatin, thyroid hormones and insulin powerfully reduce gastric ghrelin secretion,^{25,26} while cholecystokinin (CCK) and gastrin stimulate it (Figure 2).²⁷ There is no information on the regulation of ghrelin discharge by hypothalamic neurons.

Ghrelin activates the GH secretagogue receptor called GHSR-1a, a G protein coupled receptor. It activates the phospholipase C signaling route leading to an intracellular Ca²⁺ rise.⁹ An active cross-talk at the somatotrope cell is maintained between the GHRH and the ghrelin receptors in order to coordinate and potentiate the ulterior cell response.²⁸ There is an ongoing controversy about whether the cloned secretagogue receptor is truly the receptor or just one of the receptors for that family of compounds. GHSs have specific receptors in a wide range of endocrine and non-endocrine human tissues. Most probably, different receptor subtypes exist for GHSs, with different tissue distributions.²⁹

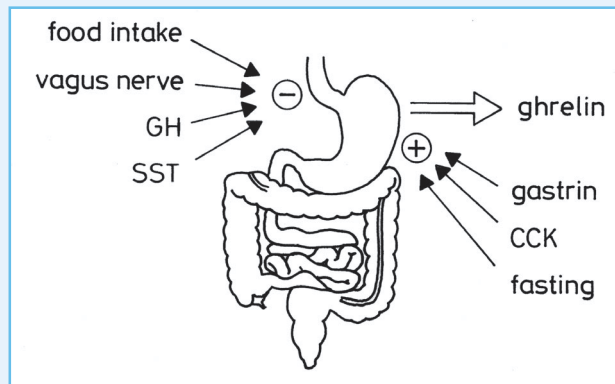
GHRELIN ROLE IN THE REGULATION OF SOMATOTROPE CELL FUNCTION AND GH SECRETION

Ghrelin is a potent GH releaser in humans (Figure 3). No side-effects have been reported after the administration of large doses of this compound.³⁰ The potency of ghrelin as measured by its GH releasing capability is higher than for GHRH and comparable to synthesized GHS.⁹ Thus, for ghrelin to be operative, the normal functioning of the GHRH receptor is necessary, as GHRH antagonists prevent or diminish the GH releasing possibilities of ghrelin.³¹ Ghrelin is able to release GH *in vivo* when administered intravenously (IV), as well as when infused directly via the intracerebroventricular (ICV) route;²⁷ since it is able to enter the CNS from the periphery,²² it is possible that

stomach-derived ghrelin may physiologically participate in GH regulation, although this has not yet been demonstrated. An important point is that ghrelin's mechanism of action is route dependent, as the vagus nerve and the arcuate nucleus are in the loop when

Figure 2

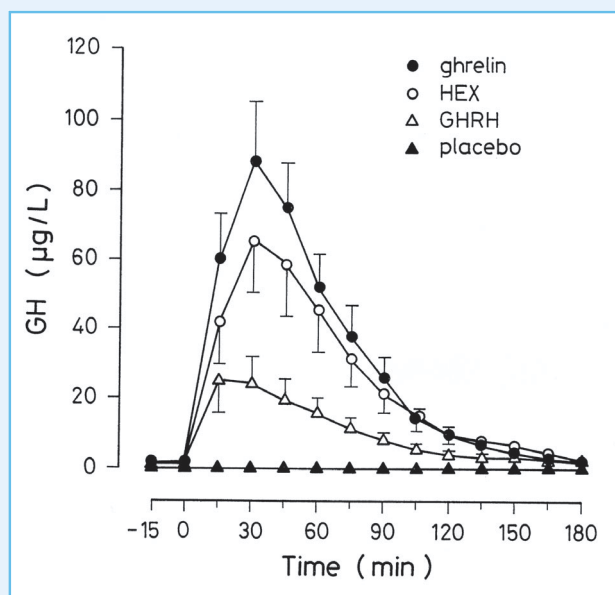
Regulation of gastric-derived ghrelin by different signals



SST= somatostatin; CCK = cholecystokinin.

Figure 3

GH secretion



GH secretion in normal subjects after the administration of ghrelin, the GHS hexarelin, and GHRH (all at 1 µg/Kg intravenously).

Redrawn from: Arvat E, et al. *J Clin Endocrinol Metab* 2001;86:1169-1174. Reprinted from: Casanueva FF, Dieguez C. *Rev Endocrine Metab Dis* 2002;3:325-338.

ghrelin is administered peripherally, but not when administered ICV.²⁴ Ghrelin-mediated GH secretion is partially insensitive to the inhibitory action of somatostatin and of metabolic compounds such as glucose or free fatty acids.²⁵ Ghrelin and GHRH showed a strong potentiation of their GH secretory capability when injected together in humans.³⁰ This peculiar activity occurs due to a simultaneous ghrelin activation of pituitary and hypothalamic structures.³¹ There is some evidence suggesting that hypothalamic ghrelin may participate in the physiological regulation of pulsatile GH secretion.³² Contrasted with the *in vitro* data, ghrelin *in vivo*, administered in what were probably pharmacological doses, induced a significant secretion of prolactin and ACTH/cortisol without altering the secretion of LH, FSH or TSH.^{9,30} It remains to be determined what happens in respect to these responses when more physiological ghrelin doses and long-term administration are tested.

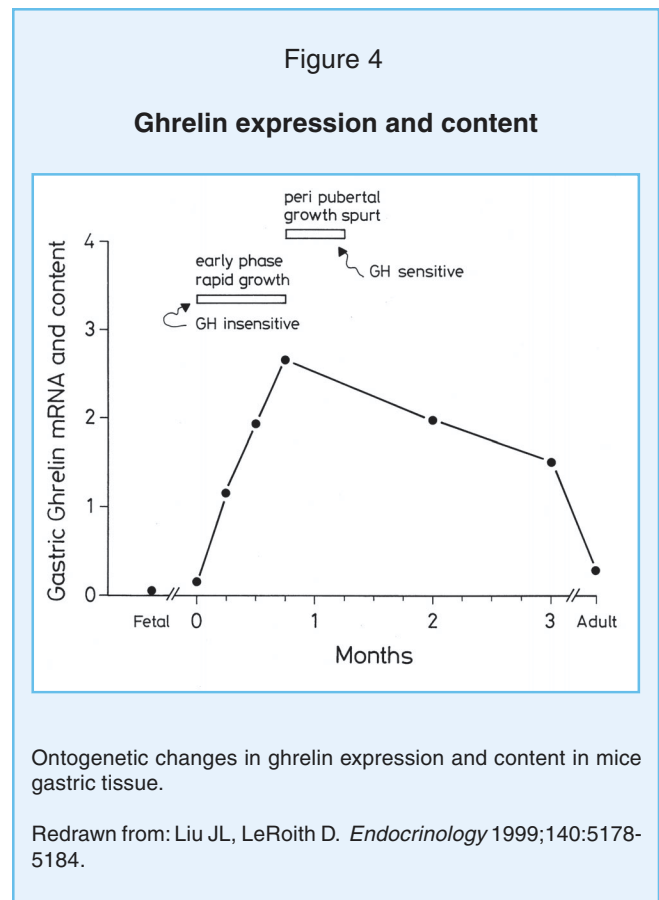
To show that IV pharmacological doses of ghrelin raise GH levels suggests, but is not proof, that ghrelin participates in the physiologic regulation of GH. A negative point is that rodents with knockout of the GHSR-1a did not show significant alterations in somatic growth, although a compensatory mechanism during fetal development may explain the lack of such results. Inferential evidence favoring a regulatory role for ghrelin, are from one side, the report of a simultaneous increase in GH and ghrelin in states of negative energy balance, and from the other the simultaneous decrease in GH and ghrelin in states of positive energy balance and obesity.⁹ In the fetus, ghrelin mRNA is undetectable, but starts rising progressively after delivery to reach a peak at 3 weeks post-partum and it decreases thereafter.³³ The general pattern of ghrelin changes reminds one of similar patterns of growth rate, and GH and IGF-I secretion. Furthermore, ghrelin mRNA level increases rapidly during the early phase of rapid growth (in the 2-3 first weeks of life), a phase which is GH insensitive,³⁴ and a high level is maintained prior to and during the pubertal growth spurt which is GH sensitive (Figure 4).

In trying to understand the participation of this new hormone in the regulation of the somatotrope axis, it is worth mentioning that adult patients with GH deficiency or GH excess (i.e. acromegaly) have ghrelin levels similar to control subjects.^{35,36} However, it may be that ghrelin plays a contributing role in the gender based differences in the pattern of GH secretion, as women in the late follicular stage have higher ghrelin levels than men.³⁶ In addition to its regulatory role on GH secretion, ghrelin has recently been reported to activate *pit-1* expression in anterior pituitary cells, an action that appears to be developmentally regulated as it is observed only in infant rats but not in adult rats.³⁷

GHRELIN AND THE REGULATION OF ENERGY HOMEOSTASIS

Ghrelin administration in humans powerfully induces a sensation of hunger in 75% of the subjects tested.³⁰ In rodents, ghrelin stimulates food intake while reducing fat utilization by a metabolic switch that increases the consumption of carbohydrates.³⁸ Different mechanisms than those involved in GH regulation³⁸⁻⁴⁰ control the activity of ghrelin over food intake. Its action seems to be the exact opposite of leptin. Ghrelin is the most powerful appetite stimulant of all the known peptides; it is the unique gastrointestinal peptide that stimulates food intake. All other peptides affecting appetite are anorexigenic. Ghrelin also stimulates food intake in rodents when administered either centrally or peripherally. Other orexigenic peptides are devoid of action with peripheral administration. CNS peptides such as NPY, orexin, and agouti-related protein (AGRP) partially mediate the ghrelin action.^{41,42}

Relevant changes in plasma levels of ghrelin appear to endorse the hypothesis that *gastric derived circulating ghrelin* regulates central appetite mechanisms. For example in rodents, ghrelin mRNA in stomach and ghrelin levels in plasma are increased by fasting and reduced by feeding, actions unrelated to gastric volume



changes.^{38,43} Passive immunoneutralization with ICV ghrelin antibodies inhibited starvation-induced as well as natural food intake in rodents, clearly indicating a tonic ghrelin action at hypothalamic receptors.⁴⁴ However, as blockade of the vagus nerve inhibits ghrelin-induced feeding in rodents,²⁴ perhaps peripheral ghrelin does not need to cross the BBB to activate central structures. These data do not preclude that the CNS neuronal groups secreting ghrelin may play a role, perhaps one even more relevant in the physiological regulation of appetite.

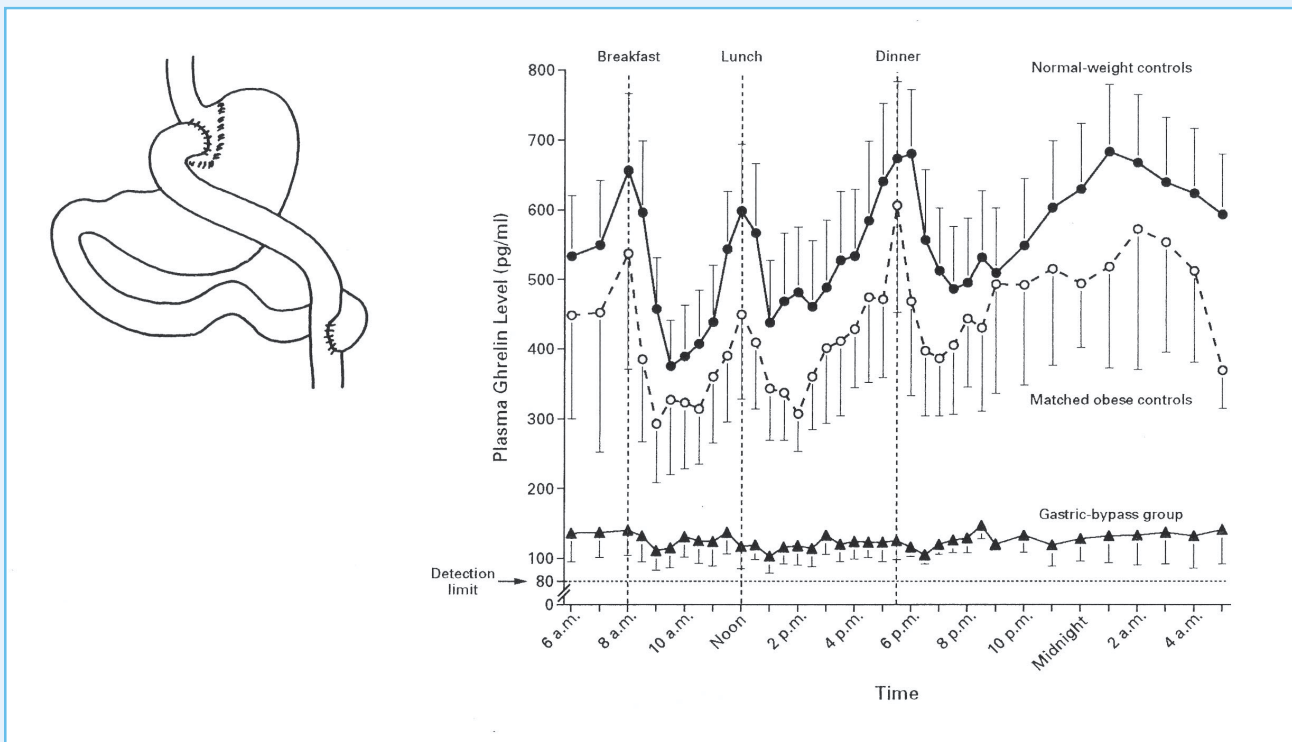
Ghrelin levels are decreased in obese subjects while elevated in states of malnutrition such as cachexia and anorexia nervosa. In the latter, weight recovery normalizes ghrelin plasma values.⁴⁵ In respect to the etiology of human obesity, no solid information supports its association with polymorphisms in the ghrelin gene. Circulating ghrelin undergoes relevant changes in relation to food intake, it is elevated before and decreased after feeding in a reciprocal pattern with insulin, and with intermeal changes that are in phase

with leptin.²⁰ Such results suggest that the preprandial ghrelin rise has a role in initiating meal consumption in humans. Interestingly, obese subjects who lose weight show an increase in plasma ghrelin. This fact may explain the facility of obese individuals to recover weight after dieting on the classic low-calorie diets.⁴⁶ Patients who have undergone bariatric surgery as treatment for obesity show a reduced ghrelin level, probably due to the absence of direct food stimulation on the gastric fundus (Figure 5).⁴⁶ It is a well known fact that bypass bariatric surgery is more effective over the long-term than other techniques, and that patients often refer to an absence of appetite after the surgical intervention.

Although they need to be replicated by different groups, the above results open new ways of understanding the regulation of energy homeostasis. Furthermore, the linear correlation in humans between hunger sensation and ghrelin levels, and the supranormal levels of plasma ghrelin in patients with uncontrolled hunger, such in Prader-Willy patients,⁴⁷ directly links ghrelin with hunger control.

Figure 5

Circulating ghrelin levels in controls and in obese subjects



The action of gastric bypass surgery decreases ghrelin levels.

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GHRELIN ACTION ON OTHER HORMONAL SYSTEMS AND NON ENDOCRINE STRUCTURES

Ghrelin may also be involved in the neuroendocrine and behavioral response to stress,⁴⁸ and in reducing LH secretion.⁴⁹ Ghrelin and its functional receptor have been shown in testicular tissue to inhibit testosterone secretion, as well as in both the rat and human ovary, suggesting that ghrelin may be responsible in part for the energy homeostasis associated with control of reproduction.^{17,50}

Ghrelin mRNA and ghrelin receptor mRNAs are expressed in gastric, thyroid, breast and lung neoplasias.^{15,51} This opens potential new routes of treatment. Also recent data suggests that ghrelin may be an endogenous factor to promote sleep.⁵²

In a totally different perspective, a most promising report is that both ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells.⁵³ These data support the protective actions of ghrelin on the cardiovascular system, and possibly more importantly, that there may be biological actions for the deacylated molecule.

SUMMARY AND SPECULATION

As ghrelin anticipates the initiation of meals and releases GH, one could share the teleological view that ghrelin integrates anabolic changes in the body. In catabolic situations, raised ghrelin levels may induce a combination of enhanced food intake, increased gastric emptying and food assimilation coupled with GH levels which promote a prompt nutrient incorporation into muscles and to fat. These actions of ghrelin are the opposite of leptin which reduces food intake and selectively eliminates fat mass. Thus, both peptides may act as physiological regulators of energy balance. Interestingly, each comes from a peripheral organ (stomach and white adipose tissue, respectively). Furthermore, with conceptual incorporation of ghrelin into the group of physiological regulators of GH (i.e., GHRH, somatostatin, IGF-I), we may be on the verge of understanding better aspects of the regulation of secretion of GH that previously were not understood.

The clarification of these and other speculations are eagerly awaited. For example, it is not known if ghrelin participates in a physiological way in regulating GH secretion and energy homeostasis. If it does, it needs to be clarified whether stomach-derived circulating ghrelin and/or neuron secreted ghrelin regulate CNS food intake and GH secretion. Similarly, it is unknown whether circulating ghrelin acts after crossing the BBB, or alternatively through an unexpected mechanism related to the structure of the vagus nerve. Finally, the

part played by the scattered neuronal systems which secrete ghrelin at both hypothalamic and extrahypothalamic sites have been largely ignored for both food intake and regulation of GH secretion. Such studies will provide better knowledge of the intricate regulation of GH secretion and appetite. It can be foreseen that important new physiological insights and contributions will be provided in the future.

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Letter to the Editor: Preterm Birth Weight and Insulin Resistance at Adolescence

In the September issue of *GGH* (Vol 19, No 3) you reviewed an interesting publication by Singhal et al¹ who studied the relation between infant feeding, early growth and insulin resistance at age 13-16 years in individuals with a birth weight below 1,850 grams (which the authors labeled preterm). In their study, insulin resistance was not associated with birth weight but with growth in the first two weeks of postnatal life; thus, they concluded that Barker's hypothesis "can be reinterpreted as a postnatal event". In our opinion, their data should be interpreted more cautiously, for the following reasons.

The first point is that selection bias is quite likely. The application of birth weight instead of gestational age as inclusion criterion (< 1,850 grams) suggests that severely growth-retarded individuals born at term are also included. Another point of our concern is that in both experimental groups there were considerable numbers lost to follow-up (65-68%).

Secondly, conclusions with respect to insulin resistance in later life were drawn from a population aged 13-16 years. In this age period there is a wide variation in pubertal stages, and during pubertal development insulin sensitivity is decreased.² Moreover, girls born small for gestational age have a tendency towards early and rapid progression of puberty,³ and hyperandrogenism,⁴ which is accompanied by decreased insulin sensitivity. It is likely that many infants in the experimental groups had a low weight for gestational age at term; thus, it is conceivable that they may have shown abnormalities in pubertal onset and tempo, as well as in androgen metabolism.

Thirdly, although an earlier study of this research group (in the same population at age 7.5-8 years) suggested that suboptimal nutrition, which may result in poor early postnatal growth, adversely affects neurodevelopmental outcomes, little emphasis is put on the possible beneficial effects of nutrient-enriched preterm formulas.⁵ This suggests that discouraging early postnatal catch-up growth by restricted food intake in infants with a birth weight below 1,850 grams is hard to justify.

The last and major point is that Singhal and colleagues have extrapolated their findings in individuals born preterm to conclusions about the general population. The first two postnatal weeks of the experimental groups took place halfway into the third trimester. In our opinion it cannot be automatically assumed that postnatal growth taking place at an age that is normally spent *in utero*, can be considered equivalent to postnatal growth of a term infant.

In conclusion, this interesting study has shown that slow early postnatal growth of preterm infants is associated with low insulin resistance at adolescence. In our view it is uncertain whether these effects persist into adulthood and whether early postnatal catch-up growth predisposes to insulin resistance only in preterm infants or also in those born at term.

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Response: *The comments of Dr. Finken and colleagues are welcome as they point to several possible methodologic and interpretive flaws in the work of Singhal et al. Although we do not know the exact number of small for gestational age neonates included in the cohort of subjects reported, it is likely that the majority were preterm and appropriate for gestational age. If there is a potential way in which to prevent the development of insulin resistance and the dysmetabolic syndrome, it should be explored. However, clearly, one would not want to jeopardize optimal neural development under any circumstances.*

Allen W. Root, MD

ABSTRACTS

Low-Carbohydrate Diet, Weight Loss and Cardiovascular Risk

The prevalence of childhood obesity continues to rise to epidemic proportions, with adolescents beginning to show significant signs of developing cardiovascular risk factors. A variety of weight-loss diets have been tested in adult populations, but the assessment of these diets in children, especially those with decreased carbohydrate (CHO) or fat remains limited. Sondike and colleagues report on the use of a low carbohydrate (LC) versus a low fat (LF) diet in a group of adolescents (ages 12-18) with a BMI >95th percentile. Thirty-nine adolescents participated in the 12-week randomized controlled study. The LC diet consisted of a daily CHO intake of <20g/d for the initial 2 weeks and then up to 40g/d. There were no restrictions on protein, fat or calories. The control group was assigned to a LF diet (< 30% energy from fat, <40g/d) with 5 servings of starch (15g CHO each serving) daily. There were no restrictions on calories. Thirty minutes of exercise 3 times a week was encouraged, but not monitored. Subjects were weighed every 2 weeks and dietary adherence was monitored at those visits by a dietitian who reviewed 3-day food records. Lipid profiles including fasting total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol were measured along with electrolytes and liver function studies at baseline and at 12 weeks. Ketouria was monitored and recorded by the subjects daily.

Thirty subjects completed the study (LC=16, LF=14). Subjects in the LC group lost significantly more weight than those in the LF group (9.9±9.3kg vs 4.1±4.9kg, p<0.04) despite having consumed more daily average calories (1830±615 vs 1100±297, p<0.03). BMI improvement was significantly greater in the LC vs LF group as well (p<0.05). LF group subjects had significantly lower LDL cholesterol levels at 12 weeks than at baseline, whereas there was no change in these levels in the LC group. HDL cholesterol rose significantly in both groups and triglycerides fell significantly in the LC group. The authors state their results were consistent with those from previous weight-loss studies employing strict calorie control (protein-sparing modified fasts). Their de-emphasis on calorie control may reduce the concern for the effects of dieting on linear growth velocity. The authors also suggest that the LC diet may not be appropriate for adolescents with significant baseline elevations in LDL cholesterol. The palatability of the LC diet may be one reason that 8 of the LC subjects voluntarily remained on the diet for a year.

Sondike SB, et al. Effects of low-carbohydrate diet on weight loss and cardiovascular risk factors in overweight adolescents. *J Pediatr* 2003;142:253-258.

First Editor's Comment: *This is an important study and hopefully it is but the first in a series of weight-loss studies designed to improve fitness and cardiovascular risk among obese children. The authors refrained from overstating their findings. As pointed out in an accompanying editorial by Daniels,¹ the long-term effects of LC diets on bone density, body composition, insulin resistance, and glucose metabolism remain to be defined. Sondike and colleagues do not, and because of the short 12-week duration of their study, could not address these issues. But these will need to be addressed, as will the metabolic and pathophysiologic abnormalities associated with obesity; none of these are trivial. It is anticipated that pieces of this complex "bio-psycho-behavioral" disorder will become more evident over the next few years as more and more investigators begin to study obesity and develop effective treatment regimens.*

William L. Clarke, MD

Second Editor's Comment: *The first low-carbohydrate diet for weight loss was described in 1863² and was popularized by Dr. Atkins^{3,4} in the modern era. However, the efficacy and safety of such diets are still being debated. A systematic review of 107 articles recently concluded that there is insufficient evidence to make recommendations for or against its use.⁵ The first randomized trial conducted for up to 12 months of such dietary therapy showed that LC diets initially induced more weight loss than the low-calorie high-carbohydrate LF diets. However at the end of one year the differences were no longer evident.⁶ Differences in weight loss were principally associated with energy intake.⁷ A calorie is a calorie no matter its source.*

Fima Lifshitz, MD

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Interactions in Gene Encoding Mutations Leading to Cortisone Reductase Deficiency

Draper et al studied a virilized 6-year-old boy with gonadotropin-independent isosexual precocious puberty and two adult women with polycystic ovarian syndrome (PCOS); subjects had low ratios of urinary tetrahydrocortisol to tetrahydrocortisone excretion. These findings were consistent with an autosomal recessive deficiency of cortisone reductase - the enzyme complex that interconverts cortisone (E) and cortisol (F). Cortisone reductase has dual dehydrogenase and oxo-reductase activities depending on the availability of a cofactor - NADP/NADPH. There are two isozymes of 11 β -hydroxysteroid dehydrogenase (11 β HSD) - hepatic (and adipose tissue) type 1 (E \rightarrow F) and renal type 2 (F \rightarrow E). In previous studies, as in the present patients, the nucleotide sequence of the 6 exons of 11 β HSD1 (chromosome 1q32-q41, OMIM 604931) was normal. However, in the three subjects in this report, mutations were found in intron 3 of 11 β HSD1. One woman with PCOS was homozygous for double mutations - insA @ NT 83557 and T \rightarrow G substitution @ NT 83597, while the second woman and the virilized boy were heterozygous for these mutations. Heterozygous carriers (parents, siblings, general population) of these linked mutations were clinically and biochemically normal. Further examination of the importance of these mutants (or polymorphic variants) revealed that the linked mutations impaired expression of 11 β HSD1 and biologic activity of the enzyme product. Thus, the investigators concluded that intron 3 of 11 β HSD1 served as an "intronic enhancer" of the expression of its gene.

Because the activity of 11 β HSD1 requires a co-factor (NADPH) the authors examined NADPH generating systems and identified two mutations in the gene (*H6PD*, chromosome 1pter-p36.13, OMIM 138090) encoding the enzyme - hexose-6-phosphate dehydrogenase - that is the principle generator of NADPH in the endoplasmic reticulum in which 11 β HSD1 is located. One mutation in *H6PD* - heterozygous 29 bp insertion between NTs 620 and 621 was present in the woman who was homozygous for the double mutation in 11 β HSD1; a homozygous mutation - Arg453Gln - was present in the other woman with PCOS and the virilized youth. Both mutations resulted in products with substantially decreased H6PD functional activity.

The investigators concluded that inactivating mutations in both 11 β HSD1 and *H6PD* (a total of 3 mutated alleles) must be present in order to result in sufficiently decreased 11 β HSD1 activity to lead to the syndrome of cortisone reductase deficiency. Thus, this disorder is another example of a digenic-triallelic pattern of inheritance as are some forms of the Bardet-Biedl syndrome (OMIM 209000).^{1,2}

Draper N, et al. Mutations in the genes encoding 11 β -hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. *Nature Genet* 2003;34:434-439.

Editor's Comment: *This manuscript presents yet another cause of gonadotropin-independent pseudoisosexual precocity in boys - cortisone reductase deficiency - indicating the need to measure cortisol, cortisone, and their urinary metabolites in patients with otherwise unexplained hyperandrogenic states. One wonders why females with a similar enzymatic defect do not manifest signs of hyperandrogenism until adulthood. Might there be yet another factor (gene product?) present/absent in young females that preclude early disease expression? It has been suggested that enhanced reductase activity in visceral adipose and perhaps other tissues, with consequent local hypercortisolism, might be associated with the development of visceral obesity and the "dysmetabolic syndrome".^{3,4} Loss-of-function mutations in the gene 11 β HSD2 (chromosome 16q12, OMIM 218030) encoding renal 11 β HSD2 lead to hypertension in the presence of subnormal mineralocorticoid values (the syndrome of "apparent mineralocorticoid excess") because unmetabolized cortisol occupies and activates the mineralocorticoid receptor leading to renal tubular reabsorption of sodium and water and hypervolemia.*

Allen W. Root, MD

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Anorectic Effects of PYY in Obesity

The gut hormone fragment peptide YY₃₋₃₆ (PYY) is known to reduce appetite and food intake when given to subjects of normal weight as well as to rodents. The authors investigated whether obese subjects were also sensitive to the anorectic effects of PYY. They compared the effects of this peptide by infusing it into 12 obese

and 12 lean subjects in a double-blind, placebo-control, crossover study, and measured the effects on appetite, food intake as well as plasma levels of PYY, ghrelin, leptin and insulin. Caloric intake during a buffet lunch two hours after the infusion of PYY was significantly decreased by 30% in the obese and by 31% in the lean

subjects. PYY infusion also caused a significant decrease in the cumulative 24-hour calorie intake in both obese and lean subjects. The average decrease in the food ingestion was about one-third of the calories, as compared to the amount consumed the day prior to the infusion. However, food intake from 0-12 hours following PYY administration was more markedly reduced than that ingested from 12-24 hours after the infusion. The administration of PYY also reduced plasma levels of the appetite stimulatory hormone, ghrelin. Endogenous fasting and postprandial levels of PYY were significantly lower in obese subjects as compared to the non-obese group. Furthermore, the fasting PYY levels correlated negatively with BMI. The authors concluded that obese subjects were not resistant to the anorectic effects of PYY and suggested that a deficiency of PYY may contribute to the pathogenesis of obesity in humans.

Batterham RL, et al. Inhibition of food intake in obese subjects by peptide YY₃₋₃₆. *N Engl J Med* 2003;349:941-948.

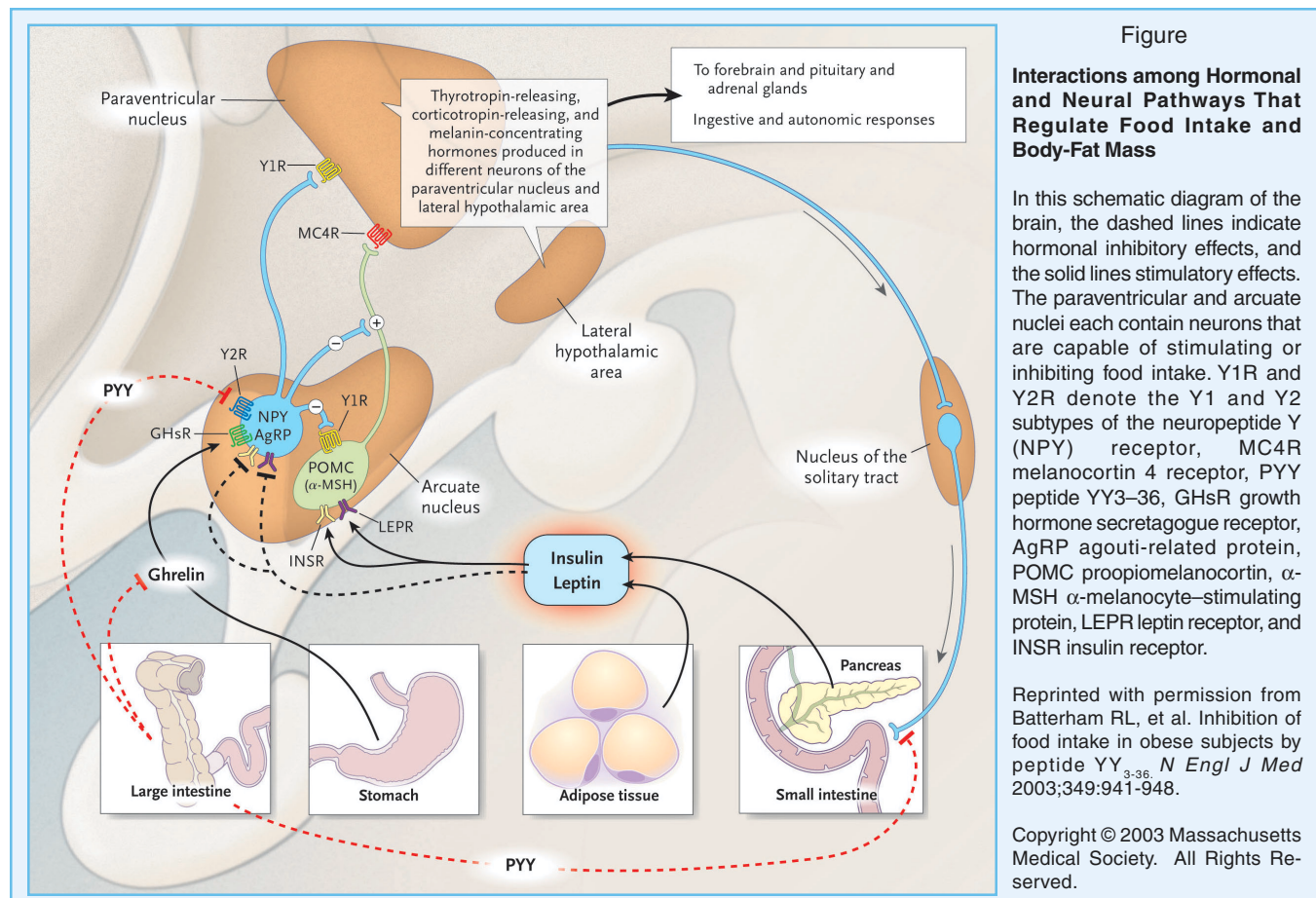
Editor's Comment: PYY is secreted postprandially, in proportion to the calories ingested, by endocrine L cells lining the distal small bowel and colon. PYY leads to a decrease food intake by inhibiting gut motility and increasing satiety. In this study, PYY infusion reduced hunger in both the obese and the lean individuals. These effects were directly related to the action of PYY, as there were no effects on the palatability of meals, feelings of

well being, or the presence of nausea. This peptide is one of the many signals that have been recently identified providing short-term information to the hindbrain and hypothalamus regarding hunger and satiety. Other gut hormones, such as cholecystokinin and ghrelin, also play a role in communicating with the hypothalamus and brain stem to stimulate or reduce the appetite. In this issue of *GGH* there is a review of ghrelin, the hunger hormone, acting on growth hormone secretagogue receptors and its pathophysiologic role in obesity related diseases.¹ However, the regulatory controls of food intake are more complex and involve other endocrine functions of adipose tissue, principally leptin, and appetite controlling genes, as previously reviewed.² However, PYY signal in satiety appears to play a role in obesity in humans and could be thought of as a therapeutic agent; a hope that was not realized by leptin, as in obesity there is marked resistance to the actions of this hormone. A graphic depicting the complex interactions among hormonal and neural pathways that regulate food intake and body fat mass³ is shown below (Figure).

Fima Lifshitz, MD

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A Gene Regulator of Puberty

While evaluating a Saudi family with several first cousin marriages in which many offspring had “idiopathic hypogonadotropic hypogonadism” transmitted as an autosomal recessive trait, the authors identified a locus on chromosome 19p13.3.^{1,2} This locus had a homozygous mutation of *GPR54* (chromosome 19p13.3, OMIM 604161, encoding an orphan G-protein receptor termed GPR54) at codon 148 in which serine was substituted for leucine (Leu148Ser). An unrelated patient was demonstrated to be a compound heterozygote with mutations in both alleles of *GPR54* - Arg331Stop leading to a truncated product and Stop399Arg - the latter resulting in an elongated protein product. *In vitro*, all mutations were found to decrease signal transduction through phospholipase C in response to the natural ligand of this receptor - kisspeptin-1 - sequence 112-121 (encoded by *KISS1*, chromosome 1q32, OMIM 603286). Kisspeptin-1 [sequence 68-121] suppresses metastases of melanoma and breast carcinoma experimentally. This 54 amino acid peptide, termed metastatin, is secreted by the placenta. In the compound heterozygotic subject, there were low basal concentrations of LH and testosterone that increased during pulsatile administration of exogenous GnRH; interestingly, this patient was more sensitive to the gonadotropin stimulating effects of GnRH than were comparable patients with hypogonadotropic hypogonadism without this specific genetic mutation.

The investigators extended these studies by developing a “knock-out” mouse model of *GPR54*^{-/-} that reproduced the clinical picture. The *GPR54*^{+/-} heterozygous mice had normal growth and fertility. The *GPR54*^{-/-} deficient animals of both genders were hypogonadotropic with small gonads, hypotrophic internal genitalia, and absence of secondary sexual characteristics. Interestingly, the adrenal glands of the *GPR54*^{-/-} animals were immature as well. Serum gonadotropin and sex hormone levels were low in *GPR54*^{-/-} animals, but LH and FSH values increased following administration of exogenous GnRH, but the hypothalamic concentrations of GnRH were normal. The authors conclude that the kisspeptin-GPR54 system is

important in the regulation of GnRH processing or secretion in the hypothalamus rather than in the movement of GnRH secreting neurons from their embryologic site of origin in the olfactory placode (the error in Kallmann syndrome) or in the synthesis of GnRH itself.

Seminara SB, et al. The *GPR54* gene as a regulator of puberty *N Engl J Med* 2003;349:1614-1627.

Editor’s Comment: *This exciting report exemplifies the best of clinical investigation employing the most up-to-date technology in a multi-institutional collaborative that should serve as a model for future studies. The identification of a G-protein receptor (and its aptly named endogenous ligand - kisspeptin) that are involved in the regulation of GnRH release opens an entirely new control system of the reproductive endocrine axis,³ a finding analogous in importance to the discovery of the role of ghrelin in the regulation of growth hormone secretion⁴ and energy metabolism. Elucidation of the mechanism(s) by which this unit regulates GnRH secretion is eagerly anticipated. One can envision many future studies of the kisspeptin-GPR54 axis. Perhaps it is involved in the development of normal puberty. Might polymorphisms of its component genes or signal transduction system account for variations in the early or delayed onset of adolescence? Are gain-of-function mutations in *GPR54* present in some children with idiopathic central precocious puberty? Does the development of gonadotropin secreting tumors involve this pathway? Since metastatin is secreted by the placenta, this suggests that it has a physiologic role during gestation - possibly in regulation of fetal gonadotropin secretion. Future studies are eagerly and impatiently awaited.*

Allen W. Root, MD

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Growth Hormone Effects on Quality of Life of Young Adults

The investigators’ goals were to document changes in quality of life (QoL) over the course of the first year post-growth hormone (GH) withdrawal, and to subsequently assess the psychological effects of reinstating GH. Participants in the GH discontinuation study were recruited from a Dutch outpatient clinic and comprised of 14 males, 8 females (ages 15 to 22 years, mean = 19 years), 11 with isolated GH deficiency (IGHD), and 11 with multiple pituitary hormone deficits (MPHD). All had

achieved adult height and were receiving adequate replacement of other hormones. Although all tested GH deficient (GHD) as children, 8 of 11 IGHD retested GH-sufficient as young adults. In contrast, all MPHD patients retested as GHD in early adulthood.

During the first six months of discontinuation of GH, a statistically significant increase in psychiatric symptoms (assessed by Hopkins Symptom Checklist) was observed, with no further increases between 6 and

12 months. There were no differences in symptoms between IGHD and MPHD, or between GHD and non-GHD. These findings corresponded temporally with a decline in IGF-I. IGF-I concentrations did not differentiate the MPHD and IGHD groups. Depressive symptoms, assessed by the Profile of Mood States (POMS), increased in both IGHD and MPHD groups by 6 months of GH discontinuation and thereafter increased further for the IGHD, but decreased within the MPHD group. The opposite pattern was observed for the POMS Tension scale, which increased across the 12 months for the MPHD group, but declined for those with IGHD. Lower IGF-I concentrations were associated with more negative mood states and somatic complaints for the combined group, whereas higher IGF-I was associated with greater 'vigor'.

Nine of 14 patients (64%; 4 males, 5 females; 2 with IGHD and 7 with MPHD) from the GH discontinuation study who remained GHD when retested as adults subsequently participated in the GH treatment study. This sample was augmented with an additional 11 patients (6 males and 5 females; 3 IGHD and 8 MPHD) who were GHD both as children and adults, had not been treated with GH in the past year, and had not participated in the GH discontinuation study. Upon reintroduction of GH to only those patients meeting adult criteria for GHD, IGF-I levels increased between 0 and 6 months in both IGHD and MPHD, but without further change by 12 months. Accompanying this increase, scores on the insecure and depression scales (of the SCL-90) decreased across the entire 12 months for both IGHD and MPHD groups, whereas anxiety (assessed by the State-Trait Anxiety Scale) decreased significantly only from baseline to 6 months. QoL scores showed a significant improvement from 0 to 6 months of GH treatment. IGF-I levels were negatively correlated with negative mood states, but positively correlated with vigor, QoL, and short-term memory. The investigators concluded that GH-modulation of IGF-I concentrations is responsible both for deteriorating mood states during GH discontinuation and improved psychological status during the return to treatment.

Stouthart PJ, et al. Quality of Life of Growth Hormone (GH) Deficient Young Adults During Discontinuation and Restart of GH Therapy. *Psychoneuroendocrinology* 2003;28:612-626.

Editor's Comment: As recognition has grown that the actions of GH extend beyond linear growth, the practice of treating GHD in adulthood has become more widely accepted. Unlike most studies assessing the benefits of adult GH replacement, these outcome variables were psychological rather than metabolic. In this study, both the IGHD (73% of whom retested GH-sufficient by adult criteria) and MPHD subgroups exhibited similar deterioration in emotional state upon discontinuation of GH with improvement after reinstating GH therapy. The investigators related these psychological changes to lower and subsequently improved IGF-I concentrations.

Several methodological features of this study should be taken into account before factoring them into clinical management algorithms. For instance, the investigators provide no indication of how representative study participants were of those in this clinic in meeting diagnostic and age criteria. Were those who agreed to participate more emotionally symptomatic? Research suggests considerable variability among patients in responsiveness to the QoL benefits of adult GH replacement.^{1,2} The potential contribution of a placebo effect to mental health indices also needs to be considered. A meta-analysis suggests that placebo effects are stronger in small trials with continuous subjective outcomes.³ The investigators may be attributing some psychological benefits to GH that are potentially due to response bias or placebo effect. Nonetheless this study is of great interest and provides important information.

David E. Sandberg, PhD

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Non-Hormonal Genetic Influence on Brain Development

Current dogma holds that differences in brain development and behavior between males and females depend primarily on gonadal steroid hormones, especially testosterone and its metabolites that induce the masculine pattern and inhibit the female pattern of brain development. However, there is also evidence that genetic factors may act directly on the developing brain contributing to these differences. Until recently, this alternative view has been difficult to document, but Dewing et al provide new and convincing evidence for non-hormonal genetic effects.

Their work was done in a mouse embryo 10.5 days

after conception. This is just before the first sign of sexual differentiation of the genital ridges occurs, thus the influence of gonadal hormones could be excluded. Their strategy was to harvest whole heads from the embryos, isolate RNA into separate pools for males and females and then analyze for differential gene expression in the male and female brains. For screening analysis, they used gene (microarray) chip (Affymetrix) technology which allowed the relative expression of nearly 10,000 characterized mouse genes and over 3,000 less well defined expressed sequences (Expressed Sequence Tags – ESTs) to be determined. The normalized gene

chip results reported as fold change or difference between male and female brain RNA revealed 36 genes or ESTs with enhanced expression in females and 18 genes or ESTs with enhanced expression in males. These genes exhibited a significant fold difference of greater than 1.1 and 7 genes or ESTs for each sex displayed a fold difference of 2.0 or more. The gene showing highest differential expression in females was *Xist*, which was 18.5 fold higher in females, while genes showing the highest differential expression in males included DEAD box peptide (*Dby*) and eukaryotic translation initiation factor 2,Y (*Eif2s3Y*) with fold differences of 10.0 and 8.8, respectively. *Xist* maps to the X chromosome, while the latter two genes reside on the Y chromosome.

Real-time quantitative analysis (RT-PCR) of littermate-matched male and female embryonic brain RNA confirmed and validated the results of the gene chip screening for a small number of genes based on their potential roles in brain development. The authors concluded that developmental differences in male and female brains in mice are due in part to the differential expression of genes before gonadal secretion starts.

Dewing P, et al. Sexually dimorphic gene expression in mouse brain precedes gonadal differentiation. *Mol Brain Res* 2003;118:82-90.

First Editor's Comment: *This is an important paper that documents the differential expression of genes in the male and female brain prior to any influence from gonadal hormones. If confirmed, it will have a substantial impact on understanding how genetic factors influence brain development. The design of the study allows for the identification of non-hormonal factors that act before the gonads are formed. However, there is no reason to think that genes act through mechanisms that do not involve gonadal hormones after gonadal hormone secretion begins, although other investigational approaches will be needed to demonstrate this. Dewing and colleagues provide no insight into the nature of the non-hormonal mechanisms through which genes may act before the appearance of gonadal hormones, although they could presumably be multiple and diverse.*

*One should note that the most dramatic differences were found for genes whose expression is expected to be limited to one sex or the other. For example, one would expect genes located on the Y chromosome to be expressed only in the male brain and *Xist* mRNA, which is expressed only by the inactive X chromosome in XX females, to be detected only in the female brain. That they were detected at all, seemingly reflects how the assays distinguish negative results from background signals. When these results are excluded the differences were diminished. Microarray gene chip and related approaches for studying gene expression are relatively new and evolving rapidly as is bioinformatics, the discipline that deals with analysis of the vast amounts of data this technology generates. Its novelty combined*

with the complexity of its data has led to a certain amount of caution in the biomedical field with regard to the biological significance of microarray results. Initially, a 2-fold difference in expression was considered an informal threshold for biological significance. Many of the results in this study fall below this level and therefore would not be considered significant by this criteria even though they are statistically significant. However, as the analytical methods advance, the threshold is being progressively lowered such that a cut-off, such as the 1.1-fold difference used in this paper, is becoming acceptable. It is still probably wise, however, to view small differences in gene expression with caution until they are confirmed by others and placed in a biological context.

William A. Horton, MD

Second Editor's Comment: *The findings of this study are important and exciting, and will likely contribute to a transformation of the dominant conceptual model regarding sexual differentiation of somatic phenotype, brain, and behavior. There is a risk that the findings may be misinterpreted in a manner potentially harmful to the clinical decision-making process in cases involving intersexuality. The findings force us to rethink the classic view of brain sexual differentiation and behavior which posits that the role of genes in the development of sex differences is restricted to the process of sex determination, i.e., the development of a bipotential and undifferentiated gonad into either an ovary or a testis. Evidence of a direct role of genes (not mediated by sex hormones) may lead clinicians to question the flexibility in decision-making they may currently exercise when sex assignment is in question. But should they?*

The basic finding of the study is that over 50 candidate genes are differentially expressed in the brains of male and female mice, ostensibly prior to gonadal production of sex hormones. Although a remarkable observation, these findings are not necessarily relevant for one psychological outcome variable of great importance in intersex cases, that is the stability of gender identity across the lifespan. (Gender identity refers to the individual's self identification as either girl/woman or boy/man.) Readers of media reports of this article will likely draw different conclusions. The headline of one well-publicized report of this study states "Sexual Identity Hard-Wired by Genetics."¹ Quotes within the article imply that gender identity springs directly from our genome. If so, then how do we account for the consistent finding in the literature that 46,XY individuals with complete androgen insensitivity syndrome develop an unambiguous gender identity as girls, and later women?²

The conflict between research findings and their interpretation is likely more apparent than real and is promoted by an oversimplification of the process of psychosexual differentiation in humans. An individual's

gender identity need not be congruent with their gender-role (which refers to behaviors that differ in frequency or level between males and females in this culture and time such as toy play or maternal interest), and sexual orientation (the pattern of sexual arousal). At the present time, the clinical research literature suggests that gender identity generally conforms with the gender of rearing, even when gender assignment is discordant with genetic sex. The picture is quite different, however, with respect to the variables of gender-role behavior and sexual orientation. It is clear that many new findings will stem

from the line of research described in this report. However, it would be unfortunate if these data were to be interpreted as suggesting that gender assignment must conform with genotype to foster a stable gender identity.

David E. Sandberg, PhD

IGF, Learning & Memory

Lupien et al tested the following hypotheses: (1) IGF treatment can prevent brain disturbances that contribute to impaired spatial learning/memory; (2) IGF-I can support cognitive function across the blood-brain barrier; (3) IGF can preserve brain function in diabetes independently of hyperglycemia; and (4) brain IGF contributes to hippocampal-based cognitive functions.

The first three hypotheses were tested by comparing normal rats versus streptozocin (STZ) diabetic rats. Four weeks after STZ, minipumps were implanted to deliver continuous infusions of 20 µg/day IGF-I or vehicle (10 mM acetic acid, pH 6.0) for 7.5 weeks. (For reference, daily IGF-I production by the adult rat liver is about 31 µg/day.) The hidden platform or "place" test was performed to assess spatial learning and memory; the "probe" test to examine memory; and the "cued" test to detect sensorimotor deficits. Following these tests, the mean blood glucose levels were 125.0±11 mg/dl in the non-diabetic rats versus 515±73 in the STZ + vehicle and 495±99 in the STZ + IGF rats. Body weights of both STZ groups were about half that of the non-diabetic rats.

All 3 groups decreased their latency times to escape the hidden platform, but there was a 3-day lag before latencies began to decline in the STZ + vehicle group. STZ+IGF performed similarly to the non-diabetic rats, and both groups decreased their latencies by shortening their search paths. The STZ + vehicle group decreased their latencies by increasing their swim velocity; their paths did not shorten. The average latency was more prolonged in the STZ + vehicle, than in the STZ + IGF rats. The STZ + vehicle rats also swam the furthest distance; STZ + IGF were again like the non-diabetics. Swim velocities were not significantly different, thus motor or proprioceptive disturbances were not the cause of the poorer performance of the STZ + vehicle rats. IGF infusion improved learning/memory performance without ameliorating the hyperglycemia or the catabolism of the STZ rats. Total brain weight and hippocampal weight were significantly reduced in the STZ rats, and these were not attenuated by IGF infusion. The second experimental design tested IGF's contribution to normal learning/memory by passive avoidance of electric shocks after two-weeks of continuous infusion into the lateral ventricle of either 40% anti-IGF-II

antisera or 40% preimmune serum. Whereas the latencies of the preimmune serum rats increased, those of the IGF-II antisera rats were significantly diminished. The authors concluded that IGF treatment can prevent brain disturbances that contribute to impaired spatial learning/memory in experimental diabetes in rats.

Lupien SB, et al. Systematic insulin-like growth factor-I administration prevents cognitive impairment in diabetic rats, and brain IGF regulates learning/memory in normal adult rats. *J Neurosci Res* 2003;74:512-523.

Editor's Comment: *The authors integrated their results into a review of prior studies of the effects of diabetes and IGF on neurologic function. Experimentation in rats allowed controlled manipulations that cannot be made in humans, like the examination of brain tissues and the continuous intraventricular infusion of IGF antisera. These data add to the evidence supporting IGF benefits for neurologic function. Aleman and colleagues demonstrate significant associations between circulating IGF-I concentrations and performance on perceptual-motor performance and mental processing speed in healthy men aged 65-76 years.¹ Although it is tempting to attribute the better performance to the higher IGF-I levels, associations are NEVER sufficient to prove causation and require corroborative evidence.*

While the associations between high circulating IGF-I concentrations and increased cancer risk have garnered a lot of attention, the neurologic effects of IGF should be considered, particularly pertaining to diabetes-induced learning/memory impairments and increased risk of dementia. Gasparini and Xu recently reviewed IGF-I and insulin as it related to the pathophysiology of Alzheimer's disease.² It appears that there may also be risks to having low IGF-I levels; IGF-I does more than promote somatic growth.

Adda Grimberg, MD

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Beta Cell Capacity and Insulin Sensitivity in Prepubertal Children Born Small for Gestational Age

The association between intrauterine growth retardation (IUGR) and the development of type 2 diabetes mellitus (T2DM) in adulthood has been demonstrated in several studies. Veening et al studied beta cell capacity and insulin sensitivity in 28 children born small for gestational age (SGA) and 22 children born appropriate for gestational age (AGA). All were Caucasian, born at term, and pre-pubertal (mean age 9.1 and 9.0 years, respectively). Insulin sensitivity was determined using a hyperinsulinemic-euglycemic clamp, while beta cell capacity was determined using a hyperglycemic clamp combined with arginine infusion. Anthropometric studies were obtained and relationships between catch-up growth, change in BMI, and clamp findings were determined.

Family history of T2DM and hypertension was not different between the two groups and at the time of the studies, mean actual length and BMI were similar in both groups. Insulin sensitivity was significantly lower in the SGA group. However, arginine-stimulated insulin secretion, a measure of beta cell capacity, was similar in both groups. Changes in BMI values between 0 and 1 year, 0 and 2 years, and 2 to 9 years, were categorized into tertiles. In SGA children, insulin sensitivity was significantly lower in those with the highest BMI change between years 2 to 9, compared to those with the smallest BMI change. Insulin secretion was significantly higher in SGA children with the highest BMI change in years 2 to 9, compared to those with the lowest BMI change during those years. No similar changes were seen among the responses in the AGA children.

The authors conclude that insulin sensitivity, but not beta cell capacity, is reduced in children born SGA. Thus, insulin sensitivity is the primary effect promoting the

development of T2DM in later life. But studies have shown that insulin resistance is not by itself sufficient to cause T2DM. SGA children whose BMI was greater during childhood had more insulin resistance. Thus, being overweight is clearly an important factor in the insulin resistance of SGA children and adults. The authors suggest that SGA children with excessive gain in BMI after the second year of life should be screened for the development of T2DM and associated cardiovascular risk factors.

Veening MA, et al. *Diabetes* 2003;52:1756-1760.

Editor's Comment: *This is an important paper. These investigators have performed complex studies in a large group of SGA and AGA children and showed that insulin sensitivity rather than beta cell capacity is abnormal in the SGA children. Since we know that the risk for T2DM is increased among adults who were born SGA, and we know that T2DM requires both insulin resistance and reduced beta cell capacity, this paper implies that reduced beta cell capacity must occur later than 9 years of age. Whether reduced capacity occurs at a later age or is related in some way to increasing BMI remains to be demonstrated. The findings with regard to BMI tertiles support the need for weight control among these individuals. What role exogenous GH administration will pay in this complex metabolic process also remains to be seen. It is clearly very important that careful metabolic studies be performed in children born SGA before and during treatment with exogenous GH. Such studies should be an important part of every database that records the effects of such treatment with these children.*

William L. Clarke, MD

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