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Regulation and Clinical Assessment of Growth Hormone Secretion

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Regulation of Growth Hormone Secretion

Introduction

Pituitary growth hormone (GH) is secreted in a pulsatile pattern reflecting the interplay upon the somatotroph of hypothalamic GH-releasing hormone (GHRH), GH-release inhibiting hormone or somatostatin (SRIH), and the recently characterized endogenous GH secretagogue (s) (GHS). The release of these neurohormones is in part self-regulated, but their secretion is primarily controlled by input from catecholaminergic, dopaminergic, serotonergic, cholinergic, and peptidergic neurotransmitters and neuromodulators, and by feedback from GH and insulin-like growth factor-I (IGF-I), the peripheral effector of the anabolic effects of GH, as well as by the metabolic status of the subject (**Fig. 1**) *(1)*. In this article, the authors present an overview of this regulatory system in man and discuss the diagnostic difficulties encountered in establishing the clinical status of endogenous GH secretion.

The Hypothalamic-Pituitary Growth Hormone Axis

Both 40 and 44 amino acid isoforms of GHRH are synthesized by neurons of the hypothalamic arcuate, dorsomedial, and ventromedial nuclei (**Table 1**). GHRH interacts with a G_s-coupled receptor to activate adenylyl cyclase, cyclic AMP, and protein kinase A to increase transcription of *GH1* and of cytosolic Ca^{2+} levels and thereby stimulate synthesis and secretion of GH (**Fig. 2**). There are 14 and 28 amino acid isoforms of SRIH synthesized in hypothalamic periventricular and arcuate nuclei that act through a family of five G_i-coupled receptors to inhibit secretion but not synthesis of GH. SRIH-R subtypes II and V are predominantly expressed in the human adenohypophysis, and SRIH–28 preferentially binds to SRIH-R subtype V. The interaction of SRIH with its receptor inhibits adenylyl cyclase activity and "opens" K^+ channels, thus increasing cytosolic levels of K^+, polarizing and inhibiting inflow of Ca^{2+} into the somatotroph, consequently decreasing the release of GH.

While investigating the GH-releasing effects of enkephalin, Bowers and colleagues *(2)* synthesized peptidyl GH releasing agents distinct from GHRH and suggested the likelihood of a third (possibly hypothalamic) factor that regulates endogenous GH secretion *(3–5)* (*see* Note Added in Proof). Subsequent pharmacologic modeling led to development of 6 and 7 amino acid peptides and then to nonpeptidyl structures that are orally active with potent and relatively specific GH-releasing activity termed GH secretagogues (GHS) (**Fig. 3**). These compounds bind to a G_q ($G_{\alpha 11}$) -coupled cell membrane receptor that activates phospholipase C_β and processing of membrane phosphoinositide. GHS facilitates pituitary GH secretion by raising cytosolic Ca^{2+} concentrations within the somatotroph by (1) increasing cytoplasmic levels of inositol trisphosphate, thus mobilizing Ca^{2+} from the endoplasmic reticulum and (2) inhibiting K^+ influx leading to cellular depolarization, thereby permitting entry of Ca^{2+} into the cell (Fig. 2) *(6)*. The GHS receptor is expressed in the adeno- and neurohypophyses and supraoptic nuclei and by GHRH containing neurons in the hypothalamic arcuate and ventromedial nuclei *(7)*. Thus, GHS may also stimulate GH release indirectly by increasing GHRH release and possibly by inhibiting SRIH secretion. In vivo in normal subjects, GHS acts synergistically with GHRH to stimulate GH secretion. It is inactive in subjects with GHRH or GHRH-R deficiency suggesting that GHS acts principally through release of GHRH and that the direct pituitary action of GHS on GH release is secondary *(8)*. However, there are also data supporting a GHRH-independent mechanism for GHS action; for example, large doses of GHRH block the GH releasing effect of GHRH, but not that of GHS and acromegalic subjects unresponsive to GHRH respond to GHS with further GH release *(6)*. These and other inferential data suggest that GHS may act through yet another unidentified GH-releasing agent termed the "U" (unknown)-factor! Thus, GHS may have multiple sites and mechanisms of action on GH secretion.

Both GHRH and SRIH inhibit their own secretion; GHRH stimulates release of SRIH, while SRIH depresses

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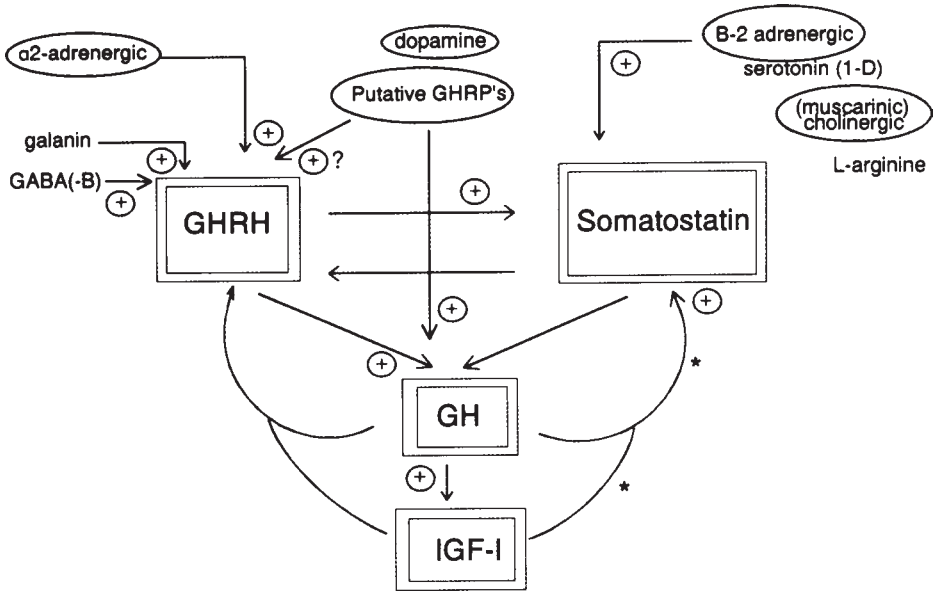

Fig. 1. Regulation of growth hormone secretion in humans. Depicted are some of the factors that increase the secretion of pituitary growth hormone (GH) by enhancing release of GH-releasing hormone GHRH), others that do so by suppressing secretion of somatostatin. The GH secretagogues (GH-releasing peptides or GHRP) act on the pituitary somatotroph and within the hypothalamus. GH and insulin-like growth factor-I (IGF-I) provide inhibitory and stimulatory feed back at the hypothalamic and pituitary levels. See Table 2 for additional details. Reproduced with permission from Giustina, A. and Veldhuis, J. D. (1998). *Endocrine Rev.* **19,** 717–797.

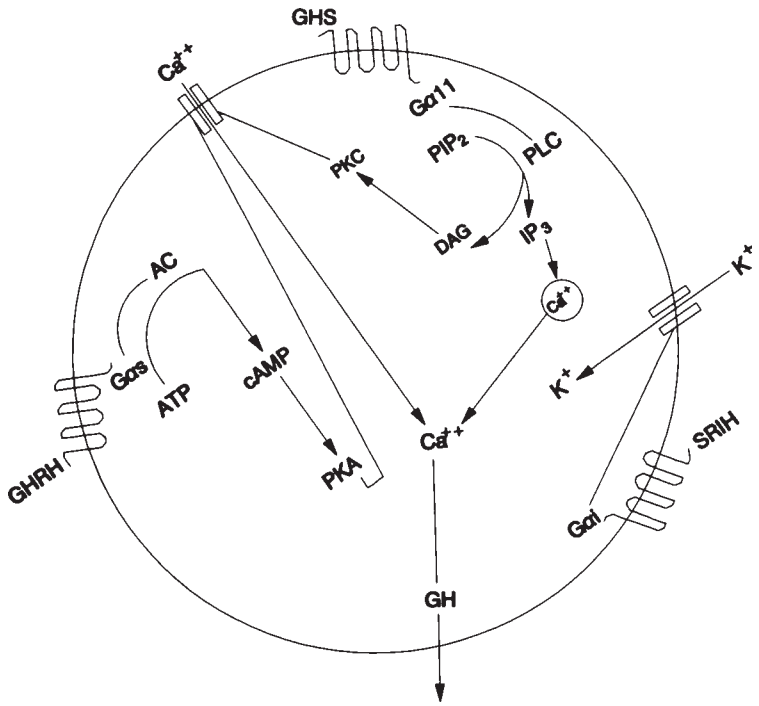

Fig. 2. Mechanisms of actions of GHRH, SRIH, GHS on GH synthesis and secretion at the somatotroph. Cellular action of growth hormone releasing and inhibiting agents. Acting through cyclic AMP, GHRH increases PKA activity leading to phosphorylation of L-type Ca^{2+} channels and increased transport and intracellular concentrations of Ca^{2+}. SRIH increases transport of K^+, thus raising intracellular levels of K^+ and inhibiting Ca transport. GHS increases intracellular concentrations of Ca^{2+} by activating PLC that 1) inhibits K+ transport, 2) mobilizes Ca from calciosomes (endoplasmic reticulum) through $1P_3$, and 3) enhances Ca transport through DAG activation of PKC. (GHRH — GH releasing hormone; GHS — GH secretagogue; SRIH — somatostatin; ATP — adenosine triphosphate; cAMP — cyclic adenosine monophosphate; PKA — protein kinase A; PLC — phospholipase C; PIP_2 — phosphatidylinositol; $1P_3$ -inositol trisphosphate; DAG — diacylglycerol; PKC — protein kinase C) (Solid line — stimulatory effect). Reproduced with permission from Root, A. W., Bercu, B. B., Diamond, F. B., Jr. (1997). *Growth Genet. Horm.* **13,** 33–38.

Table 1
Components of the Hypothalamic-Pituitary Growth Hormone-Insulin-Like Growth Factor-I Axis

Component (*GENE*)	Chromosome site	Chemistry	Function
GHRH (*GHRH*)	20q11.2	40 and 44 amino acid isoforms derived from 108 amino acid preprohormone	Stimulates synthesis and secretion of GH through the GHRH-receptor
GHRH-Receptor (*GHRHR*)	7p15-p14	423 amino acid G_s coupled 7-transmembrane domain receptor for GHRH	Stimulates synthesis and secretion of GH through activation of adenylyl cyclase and cyclic AMP
SRIH (*SST*)	3q28	14 and 28 amino acid isoforms derived from 116 amino acid precursor	Inhibits secretion of GH
SRIH-Receptor		Five (I-V) subtypes of G_s coupled 7-transmembrane domain receptors for SRIH	Inhibits secretion of GH by depressing activation of adenylyl cyclase
(*SSTR1*)	14q13		
(*SSTR2*)	17q24		
(*SSTR3*)	22q13.1		
(*SSTR4*)	20p11.2		
(*SSTR5*)	l6p13.3		
GHS (Ghnelin)	?	28 amino acid peptide	Stimulates secretion of GH at the somatotroph and hypothalamic levels
GHS-Receptor (*GHSR*)	3q26.2	366 amino acid G coupled 7-transmembrane domain receptor for GHS	Stimulates synthesis and secretion of GH through activation of phospholipase C and hydrolysis of membrane phosphoinositide
GH (*GH1*)	17q22-q24	191 (22 kDa) and 176 (20 kDa) isoforms	Stimulates growth through IGF-I and affects metabolism of proteins, carbohydrates, and fats
GH-Receptor (*GHR*)	5p13-p12	Straight chain 620 amino acid protein with extracellular, transmembrane, and intracellular domains	After dimerization, signal transduction proceeds through JAK2 tyrosine kinase and STAT activation
GHBP			Extracellular domain of GH receptor; modulates activity of GH
IGF-I (*IGF1*)	12q22-q24.1	70 amino acid peptide homologous to insulin	Anabolic effector of GH — stimulates expansion of chondrocytes
IGF-I-Receptor (*IGF1R*)	15q25-q26	1337 amino acid protein processed to α and β subunits; two α/β chains linked by S-S bonds	IGF-I receptor with intrinsic tyrosine kinase activity in intracellular domain, propagates IGF-I signal
IGFBP-3 (*IGFBP3*)	7p14-p12	264 amino acid peptide	Carrier protein for IGF-I in serum in association with ALS
ALS	6?	578 amino acid protein	Part of the 150 kDa tripartite complex in which IGF-I is transported in serum

[a]ALS, Acid-labile subunit; GH, Growth hormone; GHBP, GH binding protein; GHRH, GH releasing hormone; GHS, GH secretagogue; IGF, Insulin-like growth factor; JAK, Janus kinase; SRIH, Somatostatin; STAT, Signal transducers and activators of transcription.

that of GHRH. GH and IGF-I inhibit secretion of GHRH and enhance SRIH release in the hypothalamus and suppress GH discharge at the pituitary level. The principal biologically active species of human GH in the pituitary and serum are the 191 and 176 amino acid isoforms that account for 85% and 15% of circulating GH, respectively. The DNA base sequence encoding amino acids 32–46 is alternatively spliced from the 191 amino acid isoform during transcription of *GH1* to produce the 176 amino acid molecule. Both GH species are biologically active but may be differentiated immunologically. Human GH interacts with its cell membrane receptor (GHR) through two sites (numbered 1 and 2) within the GH structure, leading to dimerization of the single, long-chain GHR and initiation of signal transduction within the target cell *(9)*. The extracellular domain of the GHR may be proteolytically removed and circulate as GH binding protein (GHBP). Approximately 50% of circulating GH is bound to GHBP; this peptide may sequester and thus dampen fluctuations in biologically active (free) GH values in serum and/or deliver GH to the GHR. GH stimulates synthesis of IGF-I in liver, cartilage, fibroblasts, and other tissues; in serum, the

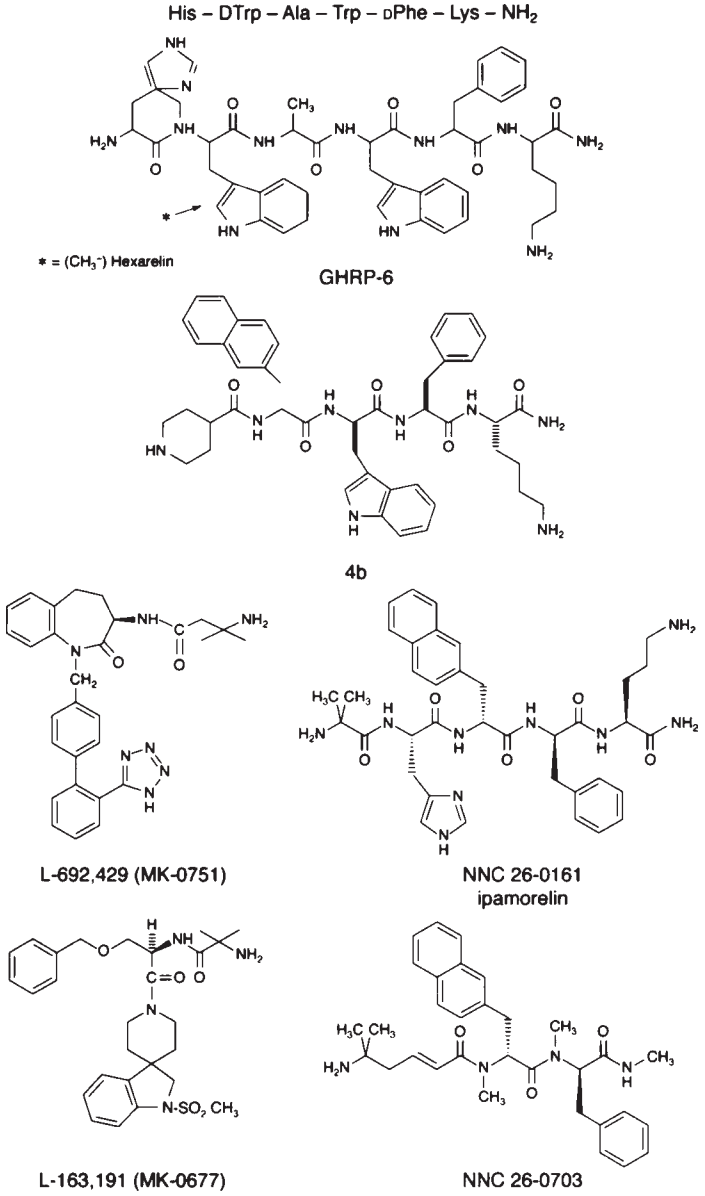

Fig. 3. Synthetic GH secretagogues. Chemical structures of several growth hormone releasing peptides (GHRP) and nonpeptidyl growth hormone secretagogues (GHS) that interact with the GHS receptor to stimulate GH secretion. 4b is a cyclic analogue of GHRP-2. L-692,429 (MK-0751) is a benzolactam; L-163,191 (MK-0677) is a spiropiperidine. NNC 26-0161 and NNC 26-0703 are newly designed GHSs. Reproduced with permission from Casanueva, F. F. and Dieguez, C. (1999). *Trends Endocrinol. Metab.* **10,** 30–38.

bulk of IGF-I is present in a triplex composed of IGF-binding protein-3 (IGFBP-3) and acid labile subunit (ALS); the synthesis of IGF-I, IGFBP-3, and ALS is directly regulated by GH.

Measurement of Growth Hormone

Serum concentrations of GH may be measured by a number of immunological and biological assays of varying specificity and sensitivity. Polyclonal radioimmunoassays (RIAs) usually recognize both forms of GH as well as circulating GH fragments, dimers, and oligomers and usually read quantitatively higher GH concentrations than do more specific methods of GH measurement. RIAs for GH employ various polyclonal antibodies that recognize different epitopes (antibody recognition sites) of the GH molecule resulting in inconsistent quantitative measurement of GH in the same specimen *(10)*. Other causes of GH assay variability include the use of differing GH standards (pituitary derived or biosynthetic), assay design (competitive vs two-site), and test matrices (buffers, proteins). Because of the limited sensitivity of most RIAs, GH concentrations are frequently undetectable in normal subjects. Relatively arbitrarily, investigators have selected minimal "cut-off" peak-challenged (stimulated) GH values of 3, 5, 7, or 10 ng/mL (RIA) to differentiate between GH sufficient and insufficient subjects often without data to substantiate these levels. Marin et al. *(11)* demonstrated that GH concentrations are often low in normally growing, healthy children; after insulin hypoglycemia, arginine infusion, or standard exercise, peak GH (RIA) values may not exceed 1.9 ng/mL in some prepubertal children and 7 ng/mL in pubertal subjects; after pretreatment with estrogen or androgen normal prepubertal children achieve peak GH levels >7 ng/mL.

The dual (polyclonal or monoclonal) antibody immunoradiometric (IRMA) and enzyme-linked immunosorbent (ELISA) assays are several-fold more sensitive than the RIA and usually specific for the 22 kDa form of GH because two GH epitopes are required for ligand recognition, but in many sera from normal subjects GH values still remain undetectable (<0.05 ng/mL) even with these methods *(12)*. The dual monoclonal antibody immunochemiluminometric (ICMA) and immunofluorometric (IFMA) GH assays have increased the sensitivity of serum GH measurement 10 fold (0.002–0.005 ng/mL). Although these highly sensitive assays have made possible the determination of very low levels of GH in serum and have been useful in the study of endogenous GH secretion in normal subjects and in patients with pituitary dysfunction, they have not improved the accuracy of the diagnosis of GH deficiency nor have they identified the short child who will have a sustained linear growth response to the administration of GH *(12–14)*.

The radioreceptor assay for GH employs hepatic cell or lymphocyte membrane GH-receptors to measure serum GH concentrations; it has yielded physiologically interesting information but is relatively insensitive. The immunofunctional assay (IMFA) for GH measures circulating forms of GH that are able to bind to and permit GHR dimerization and signal transduction *(9)*. The IMFA employs both a monoclonal antibody directed against GHR binding site 2 and recombinant GHBP (that can bind to either GHR binding site 1 or 2 of the GH molecule). The IMFA GH

Table 2
Factors that Regulate
Secretion of Growth Hormone in Humans

Increase	Decrease
Adrenergic α2	Adrenergic β2
Amino acids	Aging
Dopamine	IGF-I
Muscarinic	Nicotinic
Glucocorticoids	Glucocorticoids
Estrogen	ilyperglycemia
Exercise	Fatty acids
GABA (B)	Obesity
Galanin	ilypothyroidism
GHRH	ilyperthyroidism
GHS	
Histamine	
Hypoglycemia	
Opiates	
Serotonin	
Sleep	
Starvation	
Stress	
Testosterone	

Adapted with permission from Giustina, A. and Veldhuis, J. D. (1998). *Endocr Rev.* **19,** 717–797.

recognizes the 22 kDa and 20 kDa isoforms of GH and GH fragment 1–134. The IMFA-GH is 10-fold less sensitive (0.05 ng/mL) than the IFMA, and recognizes approx 52–93% of the GH in a specimen measured by a polyclonal RIA, substantiating the fact that the RIA recognizes forms of GH without both receptor binding sites. The IMFA-GH correlates well with the Nb2 cell bioassay for human GH *(v.i.)*. Whether it will be superior to other assays in distinguishing between GH-deficient and GH-sufficient subjects is not yet known.

In the "22 kDa GH exclusion assay," after determination of total GH in a serum sample by polyclonal RIA, serum samples are cleared of 22 kDa GH by binding to a monoclonal antibody targeted to amino acids 32–46 of 22 kDa GH; residual GH is then remeasured in the polyclonal RIA; while quantitating the amount of non-22 kDa GH in serum, the clinical utility of this assay remains untested *(15)*. This assay has been adapted to distinguish between 22 kDa recombinant human GH (rhGH) and circulating levels of the isomers of pituitary-derived GH and can thus help to identify the subject receiving rhGH *(16)*.

The bioactivity of human GH in serum may be estimated by its prolactin-like effect on the growth and metabolism of Nb2 cells in tissue culture after neutralization of endogenous prolactin. Measurement of Nb2 cell division in response to GH is a slow procedure with limited capacity. The activated Nb2 cell is able to reduce tetrazolium (and thus change its color from yellow to blue); this property led to development of the eluted stain assay (ESTA) for serum GH, a method that permits rapid assay of larger numbers of samples *(15)*. The ESTA measures higher quantities of GH than does an IRMA highly specific for 22 kDa GH, reflecting the fact that ultra specific immunoassays may not measure all of the bioactive GH in serum.

Endogenous Secretion of Growth Hormone

Growth hormone is secreted in pulses at approx 120-min intervals; in general, a burst of GH secretion is the response to a coincident decline in SRIH release and abrupt increase in GHRH secretion (and possibly GHS), modified by the input of neurotransmitters and the metabolic status upon the hypothalamus and somatotroph (**Table 2**). The secretion of GH is relatively low during waking hours and increases substantially with the onset of slow wave sleep, primarily due to increase in the amplitude of pulsatile GH release and to higher nadir (interpulse) GH values, consistent with decline in SRIH "tone" during sleep. Giustina and Veldhuis *(1)* introduced the concept of approximate entropy, an expression of the patterned (dis) orderliness of pulsatile GH secretion. In human females, the secretion of GH is distinctly less orderly (i.e., more irregular and random) than in males, attributable in part to the effect of estrogen. Among the neurotransmitters that increase GH secretion in man are those that enhance GHRH release (α_2 adrenergic agonists such as clonidine; γ-amino butyric $acid_B$ receptor agonists such as baclofen, and galanin) and those that inhibit (dopamine, serotonin; muscarinic cholinergic agonists such as pyridostigmine; β_2 adrenergic antagonists such as propranolol or atenolol; and L-arginine) SRIH release (Fig. 1).

In a study of 24-h GH secretory profiles in young adult male monozygotic and dizygotic twin pairs, Mendlewicz et al. *(17)* found a significant genetic influence on the daytime secretion of GH, reporting a heritability estimate of 74% for GH secretion during wakefulness. Serum levels of IGF-I are also genetically determined to a large extent *(18)*. Serum GH levels increase in response to exercise and physical and emotional stress, fall after ingestion of carbohydrates, rise as glucose concentrations fall, and increase in response to protein ingestion.

The secretion of GH is quite high in the neonate (when serum IGF-I values are relatively low despite rapid linear growth), declines during later infancy, and is relatively stable during childhood *(1)*. The nocturnal GH secretory pulse amplitude and nadir GH values are greater in prepubertal girls than boys suggesting a gender-related effect on GH secretion that may reflect the low levels of bioactive estrogen measurable in the serum of prepubertal females *(19,20)*. With the onset of clinical signs of puberty and rising sex hormone concentrations characteristic of sexual maturation, GH secretion increases as do serum levels of IGF-I; peak GH secretion and IGF-I values are reached in mid- to late adolescence. The pubertal rise in GH secretion

also reflects increases in amplitude of the GH secretory pulse and higher nadir GH values. In pubertal and adult subjects, GH secretion is greater in females than males, the result of augmented GH mass per secretory burst. In the young adult, GH secretion falls to values that are 25–50% of those recorded in the adolescent and declines steadily thereafter to extremely low levels in the sixth decade of life in many older subjects, the result of decreased release of GHRH, increased secretion of SRIH, and possibly declining production of endogenous GHS *(1)*.

Body composition and fat distribution influence GH secretion. In normal prepubertal boys and girls, body mass index (BMI = weight [kg]/height $[m]^2$) is inversely related to the 24-h, daytime and nocturnal integrated concentrations of GH as well as the peak amplitude of the GH pulse, area under the curve of the 24-h GH profile, and GH pulse frequency with no gender differences and without relationship to chronologic age, height, weight, or skeletal maturation *(21)*. In female children and adolescents, the waist/hip ratio (a measure of abdominal fat) is inversely related to nocturnal GH secretion *(20)*. In adults the secretion of GH is inversely related to the amount of visceral fat *(22)*. The secretion of GH is decreased in obese children and adults and increased by fasting and in malnourished subjects. Although fatty acids suppress stimulated GH secretion directly at the pituitary somatotroph, obesity does so by augmenting SRIH release as well as by increasing the metabolic clearance of GH and decreasing the GH secretory burst mass *(1)*. The (muscarinic) cholinergic agonist, pyridostigmine, enhances the GH secretory response to GHRH in obese children and adults by suppressing SRIH release.

That GH secretion is related inversely to the BMI and the amount of intra-abdominal fat, is possibly due to the influence of the adipocyte cytokine-like product leptin on this process. Administration of leptin to fed rats increases GH secretion, perhaps by suppression of SRIH release *(23)*. Also in rats, the single chain leptin receptor is expressed in arcuate and paraventricular nuclear GHRH producing cells *(24,25)*. In man, GH increases serum concentrations of leptin initially; leptin values fall during long-term administration of GH as body fat stores decline *(26,27)*. The effect of leptin on GH secretion in man has yet to be reported. Hyperglycemia lowers GH concentrations (to a greater extent in men than women) and suppresses stimulated GH secretion probably by increasing SRIH release *(1)*. L-arginine and other amino acids also stimulate GH secretion, in part by suppressing release of SRIH.

Thyroid hormones, gonadal sex steroids, and adrenal glucocorticoids influence the secretion of GH. In hypothyroidism there is decreased pituitary expression of GH and of the GHRH and SRIH receptors and decreased spontaneous and provoked GH secretion, although the hypothalamic release of GHRH is exaggerated *(1)*. Sex hormones (particularly estrogens and testosterone, an aromatizable androgen) amplify GH release during puberty and in response to stimuli as previously related. They act at hypothalamic and pituitary levels to increase transcription rates for both GHRH and GH. Glucocorticoids exert opposing effects on GH secretion. In low dose, acute administration of dexamethasone increases the secretion of GH in children and adults, possibly by enhancing pituitary transcription of GH and the GHRH receptor; however, increased endogenous secretion and/or long-term administration of supraphysiologic amounts of glucocorticoids reduce GH release by decreasing GHRH synthesis and release and increasing those of SRIH *(1)*. In subjects with either hypo- or hyperadrenocorticism, GH secretion is low due to decreased pituitary synthesis of GH or increased SRIH tone, respectively.

Approximately 0.01% of circulating GH is excreted in the urine; the majority of filtered GH is reabsorbed in the proximal renal tubule. Urinary GH excretion has been quantitated by sensitive immunoassays, either directly or after dialysis and concentration, and has been reported to be low in subjects with GH deficiency and increased in those with hypersomatotropism. Interpretive problems have been encountered in expression of urinary GH values as ng/24 h, ng/night, pg/h, ng/mg creatinine, or ng/L. Overnight urinary GH concentration and excretion are dependent on urine volume but independent of length of the collection interval. In healthy children, overnight urinary GH excretion is unrelated to age, sex, or height, and inversely related to body weight and BMI. Urinary GH excretion peaks in mid- to late puberty and then declines to prepubertal values in adulthood *(28,29)*. There are wide ranges and much overlap between sexes and stages of sexual maturation as well as marked infradian variability in urinary GH excretion *(30)*. The clinical usefulness of urinary GH measurements is limited in children and adults *(31)*.

Clinical Evaluation of Growth Hormone Secretion

The approaches to the diagnosis of GH deficiency (GHD) in the child and in the adult differ. In children the possibility of GHD is considered when the child is inappropriately small for his apparent genetic (i.e., familial) potential, when the rate of growth has declined, or in a neonate with hypoglycemia or micropenis (male) *(32,33)*. After appropriate evaluation has excluded familial, genetic, intrauterine, nutritional, psychosocial, or other definable systemic or other endocrinologic causes of poor growth, the diagnosis of GHD is then investigated *(34)*. In the presence of known insults to the hypothalamic-pituitary axis (**Table 3**), clearly abnormal growth, delayed skeletal maturation, and subnormal secretion of GH, the diagnosis of GHD in childhood is rather straight forward. However, despite sophisticated immunochemical methods for measuring GH in serum, mathematical analyses for assessing

Table 3
Causes of Growth Hormone Deficiency[a]

I. Genetic mutations
  A. Multiple pituitary hormone deficiencies
    1. Type I — Prophet of Pit1 (*PROP1*) — AR — deficiencies of growth hormone, thyrotropin, prolactin, gonadotropins
    2. Pou Domain, Class 1, Transcription factor (*POU1F1=PIT1*) — AR — deficiencies of growth hormone, thyrotropin, prolactin
  B. Growth hormone releasing hormone (*GHRH*)[b]
  C. GHRI-I-receptor (*GHRHR*)
  D. Isolated GHD
    1. Type IA — (*GH1*) — AR — absent GH
    2. Type lB — AR — reduced GH
    3. Type II — AD — reduced GH
    4. Type III — X-linked recessive - reduced GH
    5. Error in posttranslational processing of GH
  E. GH-Receptor (*GHR*)
    1. GHBP absent or low
    2. GHBP normal or elevated
II. Congenital malformations of brain, hypothalamus, adenohypophysis
  A. Pituitary aplasia/hypoplasia
  B. Septo-optic dysplasia
  C. Pituitary stalk interruption syndrome
  D. Empty sella syndrome
  E. Hydrocephalus
  F. Anencephaly
  G. Holoprosencephaly
III. Acquired insults to hypothalamus and/or adenohypophysis
  A. in utero insults: Infectious, toxic
  B. Perinatal insults: Asphyxia, trauma
  C. Head trauma: Shaken baby syndrome, surgical invasion
  D. Tumor: Craniopharyngioma, germinoma, glioma
  E. Infiltrative disease: Histiocytosis, sarcoidosis
  F. Meningitis/Encephalitis
  G. Autoimmune hypophysitis
  H. Radiation therapy
  I. Psychosocial insults
IV. Abnormalities of IGF-I synthesis or action (*see* Table 4)

[a]AR, autosomal recessive; AD, autosomal dominant.
[b]Isolated deficiency of GHRH due to loss-of-function mutation not described to date.

Table 4
Causes of Insulin-Like Growth Factor-I Deficiency

I. Growth hormone dependent/related
  A. Deficiency of growth hormone (see Table 3)
  B. Loss-of-function mutations of growth hormone receptor or within the signal transduction system
  C. Loss-of-function mutations of IGF1
  D. Abnormalities in synthesis or function of IGFBP-3
  E. Loss-of-function mutations in IGF1R
  F. Errors within the IGF-I signal transduction system
II. Secondary
  A. Normal variation (infant, young child)
  B. Malnutrition
  C. Hypothyroidism
  D. Chronic liver or other disease
  E. Growth hormone/growth hormone receptor-inhibiting antibodies

Adapted from Rosenfeld, R. G. (1997). *J. Clin. Endocrinol. Metab.* **82,** 349–351.

its daily secretion, and assays for determination of GH-dependent factors (IGF-I, IGFBP-3), the diagnosis of subtle states of GHD remains difficult. Rosenfeld *(32)* suggests that in the absence of other identifiable cause GHD be considered in the extremely short child (height <3 SD below mean for age), the child with an abrupt deceleration in growth velocity to <5th percentile for chronologic age, the subject with modest short stature (height >2 but <3 SD below mean for age) whose growth rate is at or below the 25th percentile for age, or in the patient with other pituitary hormonal defects (e.g., thyrotropin deficiency detected in neonatal screening programs for congenital hypothyroidism).

The historical review offers clues to the diagnosis of GHD when a history of perinatal asphyxia, neonatal hypoglycemia, or prolonged postpartum jaundice is elicited. Extreme short stature (height >3 SD below mean for age and gender) and/or deceleration of linear growth rate not associated with other definable cause of growth attenuation are the hallmarks of GHD. (An exception is the child with craniopharyngioma who, prior to and even after neurosurgical removal of the tumor, may have sustained normal growth despite GHD.) Classically, physical examination reveals the infantile features and chubby body habitus of the hyposomatotropic child and often a small phallus in the affected male. Careful neurologic and visual assessment are critical in the evaluation of the GHD child when possible as septo-optic dysplasia is commonly associated with GHD and craniopharyngioma can occasionally present with growth failure rather then visual loss or signs of increased intracranial pressure. Bone age is usually substantially delayed behind the chronologic age in the GHD child. However, many times classical findings are not present. A reasonable and rapid screen for GHD is measurement of the serum IGF-I concentration. Indeed, Rosenfeld *(32)* argues that the diagnosis of GHD should be superseded by the diagnosis of IGF-I deficiency as a primary entity (**Table 4**). A clearly normal IGF-I level (in relationship to skeletal maturation and stage of sexual maturity if the patient is pubertal rather than to chronologic age) usually excludes GHD in the majority of short subjects. (The authors do not find the measurement of serum IGFBP-3 concentrations particularly helpful.) When confronted with a low IGF-I level it is necessary to exclude

normal variation (IGF-I values may be quite low in infants and young children), nutritional deficiency, psychosocial dwarfism, hypothyroidism, and chronic disease.

If the IGF-I concentration is clearly low without apparent cause, assessment of GH secretion in the **euthyroid**, **fasting** state is the next step. Depending on the index of suspicion for GHD, one may examine GH secretion with an exercise test if the child is old enough to cooperate. If **vigorous** exercise fails to evoke significant GH secretion or if the clinical likelihood of GHD is high, these authors select carefully monitored insulin-hypoglycemia and arginine infusion as the GH stimulatory tests of choice. In the infant or child who may already be hypoglycemic, glucagon and L-dopa are safer GH secretagogues. Depending on the clinical situation, priming with propranolol or sex hormones may be considered if the distinction between GHD and normal GH secretion is equivocal. Because of the heterogeneity of the GH immunoassays, it is essential that the GH assay be stable; if a GH assay must be altered, the new assay has to be characterized in relation to the prior assay. Each clinician should be fully aware of the type and characteristics of the GH assay utilized. These writers find that the polyclonal RIA for GH remains a quite useful method for measurement of serum GH values, the more specific and sensitive analyses offering few if any advantages. There are many other agents (clonidine, dexamethasone, GHRH, and GHS) that have been advocated as GH stimulating substances, but the clinical superiority of any one stimulus has not been proven. Repetitive measurements of spontaneous 6-, 12-, or 24-h GH concentrations are of little practical assistance in the diagnosis of GHD in childhood except in some children who have been exposed to cranial radiation leading to GH neurosecretory dysfunction with normal provoked but subnormal spontaneous GH release.

Once the diagnosis of GHD has been established, imaging of the hypothalamic-pituitary region is important; in many subjects an interrupted pituitary stalk will be found with an ectopic posterior pituitary lobe beneath the hypothalamus; occasionally, one will identify a craniopharyngioma that has been neurologically and ophthalmologically silent.

It is essential to confirm the diagnosis of GHD in adulthood in patients in whom the diagnosis of isolated, idiopathic GHD was made in childhood, particularly when no structural abnormality of the hypothalamic-pituitary unit is present. As many as 70% of such patients will **not** be GHD as adults *(35–37)*. Low serum IGF-I concentrations are present in the majority of adults with persistent childhood-onset GHD, but less informative in patients with adult-onset GHD, especially after age 60 yr *(37)*. To this point, subnormal GH secretion in response to insulin hypoglycemia has been the test of choice for the identification of adult GHD *(37)*. Infusion of arginine with/without a preceding intravenous bolus of GHRH has been reported to be as sensitive as insulin hypoglycemia in identifying adult GHD, particularly in the adult >60 years of age *(31,38)*. Urinary levels of GH, IGF-I, and IGFBP-3 are not useful in the diagnosis of GHD in the aged adult *(31)*.

Although the GH secretory responses to GHS are reproducible with few nonresponders in a normal population, current data do not support the superiority of these agents in establishing the diagnosis of GHD relative to other GH releasing agents in either children or adults *(6)*. However, in adults the GH responses to combined administration of GHRH/GHS are only slightly affected by age, gender, and body composition; thus, the GHRH/GHS test may become useful in the diagnosis of GH deficiency in the adult *(6)*.

In the child with exaggerated linear growth not due to familial or nutritional factors or an overgrowth syndrome such as cerebral gigantism, and in the adult with symptoms and physical signs of acromegaly, the presence of a persistently elevated serum IGF-I concentration (relative to chronologic and bone ages, gender, and pubertal status) is highly suggestive of GH excess. Failure to suppress GH concentrations to low levels with oral glucose is consistent with hypersomatotropism; however, adolescents may have a paradoxical increase in GH release in response to glucose (as well as to other atypical GH secretagogues such as thyrotropin and gonadotropin releasing hormones). Occasionally, in order to establish the diagnosis of excessive GH secretion, serial measurements of serum GH concentrations may be necessary to demonstrate that low but invariant GH levels are associated with loss of pulsatile GH secretion.

Conclusions

The pulsatile secretion of GH is under the control of interactive hypothalamic neurotransmitters, neuromodulators, and neuropeptides, pituitary, and peripheral regulatory factors as well as nutritional and hormonal status. Although serum concentrations of GH can be measured with great sensitivity and precision, its measurement is confounded by the variety of assays and reagents utilized that "read" different GH levels in the same specimen. Hence, stability of the GH assay employed is essential. Except in those patients with classical clinical, hormonal, and radiographic findings, the diagnosis of GH deficiency or of GH-related IGF-I deficiency remains problematic and requires an integrated auxological and hormonal investigative approach *(39)*.

Note Added in Proof

Since preparation of this manuscript, Kojima et al. *(40)* have isolated and identified the human and rat growth hormone secretagogue (GHS). It is a 28 amino acid peptide termed ghrelin present mainly in the stomach but also detected in the arcuate nucleus of the hypothalamus. It stimulates secretion of GH in vitro and in vivo by activating the GHS receptor. Its structure is unique in that for

bioactivity its third amino acid—serine—must be acylated (octanoylated = $C_7H_{15}CO$). Ghrelin is derived from a 117 amino acid precursor protein. Human ghrelin differs from rat ghrelin at two loci (amino acids 1 and 12). In normal human subjects the mean plasma concentration of ghrelin is 117.2 fmol/mL.

References

1. Giustina, A. and Veldhuis, J. D. (1998). *Endocr. Rev.* **19,** 717–797.
2. Bowers, C. Y. (1996). In: *Growth Hormone Secretagogues*. Bercu, B. B. and Walker, R. F. (eds.). Springer-Verlag: New York, pp. 9–28.
3. Bercu, B. B. and Walker, R. F. (eds.). (1996). *Growth Hormone Secretagogues*. Springer-Verlag: New York pp. 1–460.
4. Root, A. W., Bercu, B. B., and Diamond, F. B., Jr. (1997). *Growth Genet. Horm.* **13,** 33–38.
5. Nargund, R. P., Patchett, A. A., Bach, M. A., Nurphy, M. G., and Smith, R. G. (1998). *J. Med. Chem.* **41,** 3103–3127.
6. Casanueva, F. F. and Dieguez, C. (1999). *Trends Endocrinol. Metab.* **10,** 30–38.
7. Tannenbaum, G. S., Lapointe, M., Beaudet, A., and Howard, A. D. (1998). *Endocrinology* **139,** 4420–4423.
8. Maheshwari, H. G., Rahim, A., Shalet, S. M., and Baumann, G. (1999). *J. Clin. Endocrinol. Metab.* **84,** 956–959.
9. Strassburger, C. J., Wu, Z., Pflaum, C.-D., and Dressendorfer, A. (1996). *J. Clin. Endocrinol. Metab.* **81,** 2613–2620.
10. Celniker, A. C., Chen, A. B., Wert, R. M., Jr., and Sherman, B. M. (1989). *J. Clin. Endocrinol. Metab.* **68,** 469–476.
11. Marin, G., Domene, H. M., Barnes, K. M., Blackwell, B. J., Cassorla, F. G., and Cutler, G. B., Jr. (1994). *J. Clin. Endocrinol. Metab.* **79,** 537–541.
12. Iranmanesh, A., Grisso, B., and Veldhuis, J. D. (1994). *J. Clin. Endocrinol. Metab.* **78,** 526–535.
13. Albertsson-Wikland, K., Jansson, C., Rosberg, S., and Novamo, A. (1993). *Clin. Chem.* **39,** 1620–1625.
14. Root, A. W., Duckett, G. E., Geiszler, J. E., Hu, C. H., and Bercu, B. B. (1997). *J. Pediatr. Endocrinol. Metab.* **10,** 2–10.
15. Strassburger, C. J. and Dattani, M. T. (1997). *Acta Pediatr.* suppl. **423,** 5–11.
16. Wu, Z., Bidlingmaier, M., Dall, R., and Strassburger, C. J. (1999). *Lancet* **353,** 895.
17. Mendlewicz, J., Linkowski, P., Kerkhofs, M., Leproult, R., Copinschi, G., and van Cauter, E. (1999). *J. Clin. Endocrinol. Metab.* **84,** 856–862.
18. Hong, Y., Pedersen, N., Brismar, K., Hall, K., and de Faire, U. (1996). *J. Clin. Endocrinol. Metab.* **81,** 1791–1797.
19. Klein, K. O., Baron, J., Colli, M. J., McDonald, D. P., and Cutler, G. B., Jr. (1994). *J. Clin. Invest.* **94,** 2475–2480.
20. Roemmich, J. N., Clark, P. A., Mai, V., Berr, S. S., Weltman, A., Veldhuis, J. D., and Rogol, A. D. (1998). *J. Clin. Endocrinol. Metab.* **83,** 1440–1447.
21. Martin-Hernandez, T., Galvez, M. D., Cuadro, A. T., and Herrera-Justiniano, E. (1996). *Clin. Endocrinol.* **44,** 327–334.
22. Vahl, N., Jorgensen, J. O. L., Skjaerbaek, C., Veldhuis, J. D., Orskov, H., and Christiansen, J. S. (1997). *Am. J. Physiol.* **272,** E1108–E1116.
23. Carro, E., Senaris, R., Considine, R. V., Casanueva, F. F., and Dieguez, C. (1997). *Endocrinology* **138,** 2203–2206.
24. Hakansson, M. L., Brown, H., Ghilardi, N., Skoda, R. C., and Meister, B. (1998). *J. Neurosci.* **18,** 559–572.
25. Tannenbaum, G. S., Gurd, W., and Lapointe, M. (1998). *Endocrinology* **139,** 3871–3875.
26. Kristrom, B., Carlsson, B., Rosberg, S., Carlsson, L. M. S., and Albertsson-Wiklund, K. (1998). *J. Clin. Endocrinol. Metab.* **83,** 2735–2541.
27. Gill, M. S., Toogood, A. A., Jones, J., Clayton, P. E., and Shalet, S. M. (1999). *J. Clin. Endocrinol. Metab.* **84,** 1288–1295.
28. Gelander, L., Karlberg, J. P. E., Larsson, L. A., Rosberg, S., and Albertsson-Wikland, K. (1998). *Horm. Res.* **49,** 8–16.
29. Main, K. M., Jarden, M., Angelo, L., Dinesen, B., Hertel, N. T., Juul, A., Muller, J., and Skakkebaek, N. E. (1994). *J. Clin. Endocrinol. Metab.* **79,** 865–871.
30. Thalange, N. K. S., Gill, M. S., Gill, L., Whatmore, A. J., Addison, G. M., Price, D. A., and Clayton, P. E. (1996). *J. Clin. Endocrinol. Metab.* **81,** 100–106.
31. Gill, M. S., Toogood, A. A., O'Neill, P. A., Thorner, M. O., Shalet, S. M., and Clayton, P. M. (1998). *J. Clin. Endocrinol. Metab.* **83,** 2562–2565.
32. Rosenfeld, R. G. (1997). *J. Clin. Endocrinol. Metab.* **82,** 349–351.
33. Rosenfeld, R. G. (1997). *Acta Pediatr.* **suppl. 423,** 17–19.
34. Rosenfield, R. L. (1996). *Endocrinol. Metab. Clin. NA* **25,** 743–758.
35. Maghnie, M., Stigazzi, C., Tinelli, C., Autelli, M., Cisternino, M., Loche, S., and Severi, F. (1999). *J. Clin. Endocrinol. Metab.* **84,** 1324–1328.
36. Tauber, M., Moulin, P., Pienkowski, C., Jouret, B., and Rochiccioli, P. (1997). *J. Clin. Endocrinol. Metab.* **82,** 352–356.
37. Toogood, A. A. and Shalet, S. M. (1997). *Acta Pediatr.* suppl. **423,** 117–120.
38. Aimaretti, G., Corneli, G., Razzore, P., Bellone, S., Baffoni, C., Arvat, E., Cammani, F., and Ghigo, E. (1998). *J. Clin. Endocrinol. Metab.* **83,** 1615–1618.
39. Preece, M. A. (1997). *J. Pediatr.* **131,** S61–64.
40. Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., and Kangawa, K. (2000). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* **402,** 656–660.