Growth hormone in adults

Physiological and clinical aspects

Second edition

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Physiological regulators of growth hormone secretion

Mark L. Hartman

Growth hormone (GH) has diverse metabolic actions that regulate body composition, fluid homeostasis, glucose and lipid metabolism, bone metabolism, exercise performance and cardiac function. These actions improve the quality of life of adults, and confer beneficial effects when adults with GH deficiency are treated with recombinant human GH (rhGH). These important findings and the regulation of the GH-insulin-like growth factor-I (GH-IGF-I) axis are reviewed in detail in this volume. Shortly after the development of radioimmunoassays for GH in the early 1960s, multiple factors such as age, gender, pubertal status, nutrition, sleep, body composition, stress, exercise and several hormones were found to regulate GH secretion (for early review see Reichlin, 1974). Subsequent research has demonstrated that GH is secreted in discrete pulses, separated by periods of secretory quiescence. The pattern of GH release may modulate its metabolic actions. The amplitude and frequency of GH secretory pulses are regulated by physiological factors via effects on the hypothalamus and by direct actions of various hormones and metabolites on the GH-secreting pituitary cells, the somatotrophs. The pituitary integrates these signals and releases GH in a precisely regulated manner to ensure that the correct amount of GH reaches its target tissues. Although GH deficiency is usually diagnosed in the setting of hypothalamic and pituitary disease, alterations in the central and peripheral signals that normally regulate GH may account for the relative GH deficiency observed with ageing and obesity. Understanding the mechanisms by which GH secretion is normally regulated may suggest strategies for enhancing endogenous GH secretion in states of relative GH deficiency. These strategies may include pharmacological approaches such as the use of GH secretagogues or modification of nutrition, sleep and exercise habits. For patients with intact pituitary glands, such interventions may offer advantages over administration of rhGH since a normal pattern of GH secretion may ensue.

Neural regulation of pulsatile GH secretion

GH-releasing hormone and somatostatin

The pulsatile release of GH by the anterior pituitary gland is controlled by two hypothalamic peptides that are secreted at the median eminence into the hypophyseal-portal circulation. GH-releasing hormone (GHRH), synthesized in the arcuate nucleus and the ventromedial nucleus, stimulates both GH synthesis and secretion (Wehrenberg et al., 1982; Barinaga et al., 1983; Lechan et al., 1984; Fukata, Diamond & Martin, 1985). The human GHRH receptor, expressed in pituitary cells, mediates these effects (Gaylinn et al., 1993). Somatostatin, arising from the periventricular and paraventricular nuclei, inhibits GH release without affecting GH synthesis (Lechan et al., 1983; Fukata et al., 1985). Five somatostatin receptor subtypes have been cloned and characterized to date (for review see Viollet et al., 1995). In human pituitary cells (both normal and tumoral), the type II and V somatostatin receptors predominate. The type V receptor appears to mediate most of the suppression of GH secretion by somatostatin (Shimon et al., 1997a, b).

Several lines of evidence suggest that GHRH initiates GH pulses and somatostatin modulates the amplitude of GH pulses. Blocking the action of GHRH, either by passive immunization in rats or with a GHRH antagonist in rats or humans, abolishes pulsatile GH release (Wehrenberg et al., 1982; Lumpkin, Mulroney & Haramati, 1989; Jaffe, DeMott-Friberg & Barkan, 1993; Ocampo-Lim et al., 1996). The GH response to exogenously administered GHRH is highly variable in both rats and humans (Thorner et al., 1983; Tannenbaum & Ling, 1984). Passive immunization with somatostatin antiserum eliminates the variability in GH responses to GHRH in rats (Tannenbaum & Ling, 1984). Based on this observation, it was proposed that GH pulses result from the coincidence of peaks of GHRH and troughs of somatostatin secretion (Tannenbaum & Ling, 1984). Direct sampling of hypophyseal-portal blood in anaesthetized male rats reveals that GHRH concentrations in portal blood are maximal when somatostatin concentrations are at their nadir (Plotsky & Vale, 1985). Similar studies in unanaesthetized ovariectomized ewes demonstrate that both hypothalamic peptides are secreted in pulses with GHRH and somatostatin peaks of 25–40 and 65–160 ng/l, respectively. A significant association exists between GHRH and GH pulses but a clear relationship between troughs of somatostatin secretion and GH pulses is not always present in sheep (Frohman et al., 1990). In humans, acute termination of a somatostatin infusion triggers an acute rebound of GH release (Hindmarsh et al., 1991; Jaffe, DeMott-Friberg & Barkan, 1996). This effect of somatostatin withdrawal cannot be blocked by administration of a GHRH antagonist (Jaffe et al., 1996). In addition, GH secretion remains pulsatile in the presence of continuously high GHRH concentrations either as a result of infusions or ectopic GHRH secretion (Vance et al.,

1985). These data support the concept that intermittent somatostatin secretion can produce detectable GH pulses in humans. Modulation of GH pulse amplitude by somatostatin is also supported by increased GH response to GHRH after administration of pharmacological agents that decrease the release and/or action of somatostatin such as pyridostigmine, a cholinesterase inhibitor (Massara et al., 1986), and GH-releasing peptide (GHRP), a hexapeptide that acts through non-GHRH receptors (Bowers et al., 1990).

GH exerts a negative feedback effect on its own secretion. Daily subcutaneous administration of exogenous GH for two to five days decreases the endogenous GH response to GHRH (Nakamoto et al., 1986; Rosenthal et al., 1986; Ross et al., 1987). This effect may be mediated by an increase in serum IGF-I concentrations (see below). However, this inhibitory effect has also been observed as early as three hours after an intravenous injection of GH, before any rise in serum IGF-I was detected, suggesting that GH may increase hypothalamic somatostatin secretion (Ross et al., 1987). In rats, the negative feedback effect of an exogenous GH injection on endogenous GH pulses is eliminated by passive immunization with somatostatin antiserum (Lanzi & Tannenbaum, 1992). In vitro, GH stimulates somatostatin release from rat hypothalami (Sheppard et al., 1978).

Recent evidence suggests that GHRH- and somatostatin-secreting neurons may interact within the hypothalamus. Somatostatin receptors have been demonstrated on GHRH-synthesizing neurons in the rat arcuate nucleus (McCarthy, Beaudet & Tannenbaum, 1992; Bertherat et al., 1992). GHRH secretion and messenger ribonucleic acid (mRNA) levels are increased in rat hypothalami that have been depleted of somatostatin by either surgical or electrolytic lesions (Katakami, Downs & Frohman, 1988) or by cysteamine treatment (Bertherat et al., 1991). These observations suggest that somatostatin may inhibit GHRH secretion. In vitro, somatostatin inhibits GHRH release (Yamauchi et al., 1991) and GHRH stimulates somatostatin release from perfused rat hypothalami (Aguila & McCann, 1985). Such intrahypothalamic interactions between these two neuropeptides may contribute to the regulation of pulsatile GH release.

Other peptides

A synthetic hexapeptide, His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2, with potent GH-releasing properties was developed in the laboratories of Bowers and Momany in the early 1980s (Momany et al., 1981; Bowers et al., 1984). This was the first of a family of peptides, termed GH-releasing peptides (GHRPs). Co-administration of GHRP and GHRH stimulates GH secretion in a synergistic fashion, suggesting that GHRPs act via a non-GHRH receptor (Bowers et al., 1990). Oral administration of GHRP to humans stimulates GH release, although the oral bioavailability is poor (Hartman et al., 1992b). Continuous infusion of GHRP for 24-hours in

humans enhances pulsatile GH secretion (Huhn, et al., 1993). These and other observations suggested that development of a long-acting GHRP-like secretagogue would be efficacious in enhancing pulsatile GH secretion if a compound with greater oral bioavailability could be developed.

Subsequently, orally active non-peptide analogs of GHRP were developed at Merck Research Laboratories (for review, see Smith et al., 1997). These compounds stimulate GH secretion via activation of a novel G-protein coupled receptor that activates phospholipase C, resulting in activation of Ca^{2+} channels and inhibition of K^+ channels. This signal transduction pathway is distinct from that of GHRH (Smith et al., 1997). This receptor, now termed the GH secretagogue receptor, was cloned by Smith and co-workers in 1996. It is expressed in somatotroph cells in the anterior pituitary and in the hypothalamus (Smith et al., 1997). These observations suggest that an endogenous GHRP-like neuropeptide may activate this receptor in vivo and be involved in the regulation of GH secretion. However, the endogenous ligand for this receptor has not been identified to date. The several possible sites of action for GHRP-like compounds in the regulation of GH secretion include: (1) a direct stimulatory action on the pituitary; (2) stimulation of hypothalamic GHRH release; (3) functional antagonism of somatostatin action on the pituitary; (4) attenuation of GH autofeedback; (5) opposition of the inhibitory effect of somatostatin on GHRH neurons; and (6) stimulate release of an unknown ('U') hypothalamic factor that may synergize with GHRH (Bowers et al., 1991; Smith et al., 1997; Guistina & Veldhuis, 1998). The hypothalamic actions of GHRP-like compounds may be the most important for stimulation of GH secretion. In humans, 80% of the acute GH-releasing effect of GHRP is abolished by prior administration of a GHRH antagonist (Pandya et al., 1998). In sheep, systemic injection of hexarelin, a GHRP-6 analogue, increases the release of GHRH into the portal blood of sheep without any change in somatostatin concentrations (Guillaume et al., 1994). Administration of the spiropiperidine MK-677 to healthy older adults increases pulsatile GH secretion, with an increase in the amplitude but not in the number of GH pulses (Chapman et al., 1996). The potential therapeutic applications of these GHRP-like compounds are reviewed in a later chapter.

Other peptides that have been proposed to regulate GH secretion include galanin, pituitary adenylate cyclase-activating protein (PACAP), opioid peptides, thyrotropin-releasing hormone (TRH), neuropeptide Y (NPY), substance P bombesin, melatonin and leptin. Few studies of these peptides have been performed in humans, although limited data suggest that galanin, substance P and melatonin may enhance the GH response to GHRH. TRH has a stimulatory effect on GH secretion in pathophysiological states such as acromegaly and diabetes but has no effect in normal subjects (for review, see Guistina & Veldhuis, 1998).

Extrahypothalamic regulation of GH secretion

Pulsatile GH secretion persists in rats that have undergone complete hypothalamic deafferentation, suggesting that the neural mechanisms for episodic release of GHRH and somatostatin reside in the hypothalamus (Willoughby et al., 1977). Nevertheless, experimental studies, including electrical stimulation techniques, focal destructive brain lesions and pharmacological studies, have provided strong evidence for regulation of GH secretion by extrahypothalamic regions of the brain. Neural inputs from extrahypothalamic sites are relayed to the mediobasal hypothalamus where they synapse on the hypothalamic neurons that secrete GHRH and somatostatin, resulting in integrated control of GH secretion by the central nervous system (Martin, 1984).

Modulation of GH secretion by neurotransmitters

A number of central neurotransmitters modulate GH secretion. This topic has been comprehensively reviewed elsewhere (Müller, 1987; Guistina & Veldhuis, 1998) and so only a brief summary will be provided here. Pharmacological studies in humans reveal that activation of α_2-adrenergic receptors and muscarinic cholinergic receptors stimulate GH secretion; antagonists of these receptors suppress GH release (Müller, 1987; Guistina & Veldhuis, 1998). The influence of α_2-adrenergic neurons appears to be dominant since co-administration of clonidine (an α_2-adrenergic agonist) and atropine (a muscarinic cholinergic antagonist) stimulates GH release. Furthermore, treatment with yohimbine (an α_2-adrenergic antagonist) can completely block the stimulatory effects on GH secretion of enhancing cholinergic tone with pyridostigmine, a cholinesterase inhibitor (Devesa et al., 1991). In contrast, β-adrenergic receptors appear to mediate significant inhibitory effects on GH release. Blockade of β-adrenergic receptors enhances the GH response to GHRH and other provocative stimuli but appears to have no effect on spontaneous GH secretion in boys with constitutional delay of growth (Müller, 1987; Guistina & Veldhuis, 1998; Martha, Blizzard & Rogol, 1988). Administration of salbutamol, a β_2-adrenergic agonist, inhibits GH secretion and is able to block the stimulation of GH release by L-arginine or pyridostigmine (Ghigo et al., 1994). Nicotinic cholinergic and α_1-adrenergic receptors appear to have lesser effects on GH secretion (Müller, 1987; Guistina & Veldhuis, 1998).

Although α-adrenergic and cholinergic neurotransmission are likely to have important roles in regulating GH secretion in humans, it is still unknown whether the stimulatory effects on GH secretion of these pathways are mediated by suppression of somatostatin release or stimulation of GHRH secretion or both. In rats, passive immunization with antiserum to GHRH but not to somatostatin suppresses the stimulatory effects of clonidine, suggesting that clonidine stimulates GHRH release (Miki, Ono & Shizume, 1984). In sheep, clonidine increases the

hypophyseal-portal blood concentrations of GHRH (Magnan et al., 1994). In humans, administration of a GHRH antagonist significantly suppresses the stimulatory effect of clonidine on GH release (Jaffe et al., 1996). However, the fact that clonidine potentiates the GH response to GHRH in both rats and humans suggests that clonidine may decrease somatostatin secretion (Devesa et al., 1991; Lima et al., 1993). In rabbits, yohimbine suppresses spontaneous and GHRH-stimulated GH secretion in anti-somatostatin immunized animals, suggesting that α_2-adrenergic receptors may affect both GHRH and somatostatin secretion (Minamitani et al., 1989). Most experimental evidence supports the hypothesis that activation of β-adrenergic receptors increases hypothalamic somatostatin secretion (Guistina & Veldhuis, 1998).

Evidence that cholinergic pathways suppress hypothalamic somatostatin release include: (1) GHRH-stimulated GH release is potentiated by cholinergic agonists and blocked by cholinergic antagonists in rats and humans (Locatelli et al., 1986; Massara et al., 1986; Kelijman & Frohman, 1991); (2) pyridostigmine reverses the inhibitory effect of intravenous GH infusions on the GH responses to GHRH or insulin-induced hypoglycaemia (Kelijman & Frohman, 1991); and (3) depletion of hypothalamic somatostatin content by anterolateral deafferentation of the mediobasal hypothalamus or treatment with cysteamine eliminates the effect of cholinergic agonists and antagonists on GH secretion in rats (Locatelli et al., 1986). In contrast, administration of neostigmine to sheep increases hypophyseal-portal blood concentrations of GHRH (Magnan et al., 1993). In humans, administration of a GHRH antagonist significantly suppresses the stimulatory effect of pyridostigmine on GH release (Jaffe et al., 1996). These results suggest that pyridostigmine stimulates GH secretion by suppressing somatostatin release, which triggers a rebound increase in GHRH release via hypothalamic neuronal interactions (Guistina & Veldhuis, 1998).

Dopaminergic agonists stimulate spontaneous GH release and enhance the GH response to GHRH in normal subjects (Müller, 1987; Vance et al., 1987). Although some experimental evidence suggests that dopaminergic agonists stimulate GH release via suppression of somatostatin (Guistina & Veldhuis, 1998), administration of a GHRH antagonist significantly suppresses the stimulatory effect of L-dopa on GH release (Jaffe et al., 1996). In contrast, bromocriptine and other dopaminergic agonists inhibit GH release in patients with GH-secreting pituitary tumours (Jaffe & Barkan, 1992). In normal subjects, prior infusion of dopamine inhibits the GH response to L-dopa, L-arginine and insulin-induced hypoglycaemia (Woolf, Lantigua & Lee, 1979; Bansal, Lee & Woolf, 1981a). Administration of bromocriptine to normal subjects also inhibits the GH response to insulin-induced hypoglycaemia (Bansal, Lee & Wolf, 1981b). These observations suggest that results of GH stimulation tests being performed for the purpose of establishing the diagnosis of

GH deficiency may be affected by concomitant therapy with dopaminergic agonists for pituitary tumours.

Other neurotransmitters that may stimulate GH secretion include serotonin, gamma-hydroxybutyrate (GHB), and excitatory amino acids, such as N-methyl-D, L-aspartate (NMDA) (Müller, 1987; Guistina & Veldhuis, 1998). The effects of histamine on GH secretion appear to be inhibitory in rats (Müller, 1987; Guistina & Veldhuis, 1998). However, in humans blockade of histamine type 1 receptors reduces the GH response to other pharmacological stimuli (Guistina & Veldhuis, 1998).

Adrenergic, cholinergic and serotoninergic pathways may mediate the effects of a number of physiological factors that regulate GH secretion. Alpha-adrenergic pathways may mediate the GH response to insulin-induced hypoglycaemia, exercise and certain stresses since these responses can be blocked by administration of phentolamine (Martin, 1973). The GH response to stress may involve α-adrenergic pathways in the limbic system because blockade of catecholamine synthesis in the rat inhibits GH release induced by electrical stimulation of the hippocampus and the basolateral amygdala (Martin, 1973). Serotoninergic and cholinergic pathways have been implicated in the increase in GH secretion associated with sleep (Martin, 1984; Müller, 1987).

Evaluation of pulsatile GH secretion in humans

Since hypophyseal-portal blood cannot be sampled in vivo in humans, analyses of pulsatile pituitary hormone release have been undertaken to infer the patterns of secretion of hypothalamic releasing hormones. This approach has been particularly useful to study pituitary hormones that are regulated primarily by one releasing hormone. Thus, studies of pulsatile gonadotropin secretion led to novel therapies with pulsatile administration of gonadotropin-releasing hormone (Santoro, Filicori & Crowley, 1986). The interactions between GHRH and somatostatin make analysis of the hypothalamic regulation of GH secretion more complex than is the case with luteinizing hormone secretion.

Three general categories of analytical methods have been developed for analysis of pulsatile hormone release: (1) peak detection methods for objective identification of pulses in hormone concentrations; (2) deconvolution techniques for estimation of hormone secretion rates; and (3) methods to evaluate the orderliness of pulsatile hormone release.

Commonly used peak detection methods include Ultra (Van Cauter et al., 1981), Pulsar (Merriam & Wachter, 1982), Detect (Oerter, Guardabasso & Rodbard, 1986) and Cluster (Veldhuis & Johnson, 1986). These computer-assisted algorithms employ different mathematical assumptions and approaches to identify pulses in time series of hormone concentrations. Therefore, results obtained with different

algorithms may not be directly compared. The statistical parameters used with these algorithms must be adjusted for each hormone and different sampling frequencies. The performance of these algorithms and methods for optimizing the sensitivity and positive accuracy of peak detection have been previously reviewed (Urban et al., 1988; Urban, Johnson & Veldhuis, 1989).

Deconvolution techniques resolve underlying hormone secretory events by mathematically removing the effects of metabolic clearance on series of plasma hormone concentrations obtained at frequent intervals. Two general categories of deconvolution methods have been devised: (1) methods assuming a known hormone half-life; and (2) methods to calculate both hormone half-life and secretion rates simultaneously assuming a specific shape of the underlying secretory event (for review, see Veldhuis & Johnson, 1992). With the latter method, termed multiple-parameter deconvolution, each burst of GH secretion is typically assumed to comprise a Gaussian distribution of secretory rates (Veldhuis, Carlson & Johnson, 1987). Estimates of 24-hour GH production rates and the half-life of endogenous GH obtained with this method agree well with those obtained by other methods (Hartman et al., 1991). Until recently, GH secretion has typically been modelled as entirely pulsatile with negligible basal (non-pulsatile) secretion. With the development of new chemiluminescence assays for GH with enhanced sensitivity, low levels of basal secretion have been detected, accounting for 6% or less of the daily GH production rate in young, healthy men (Iranmanesh, Grisso & Veldhuis, 1994). Conditions with altered GH half-lives have also been encountered. Recent studies have demonstrated that the metabolic clearance of GH is a function of the plasma GH concentration and the glomerular filtration rate (Haffner et al., 1994). An advantage of the multiple-parameter deconvolution method is that subject-specific half-lives of endogenous GH may be estimated. In addition, the use of deconvolution analysis has made it possible to estimate the frequency, amplitude, mass and duration of GH secretory bursts that give rise to GH concentrations in circulating blood in a variety of physiological and pathological states. Finally, the time course of the effect of physiological variables on GH secretion may be determined more precisely by calculating GH secretion rates.

Figure 1.1 depicts the pulsatile patterns of serum GH concentrations and deconvolution-resolved GH secretion rates over 24 hours in two normal young men sampled at five-minute intervals. Removal of the effects of metabolic clearance reveals that pulses of circulating GH concentrations arise from multiple bursts of GH secretion. In this study of 12 normal men, 96% of GH was secreted in volleys composed of multiple (4.0 ± 0.4) discrete secretory bursts. Such volleys of GH secretion were separated by 171 ± 19 min, whereas their constituent individual secretory events occurred every 36 ± 1.7 min ($p = 0.0001$). Between secretory volleys, calculated GH secretory rates fell asymptotically to zero (Hartman et al.,

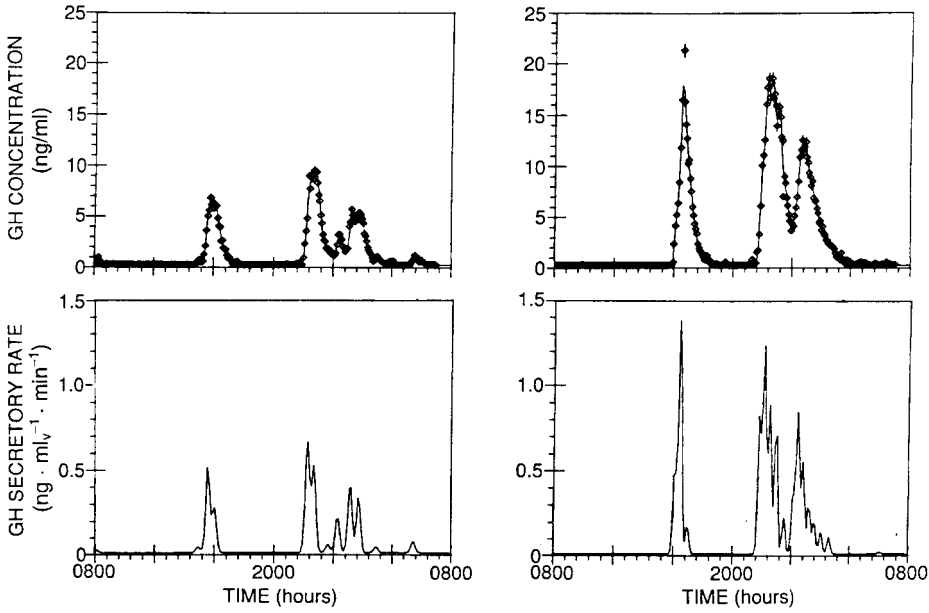

Figure 1.1. Representative 24-hour profiles of pulsatile serum growth hormone (GH) concentrations and deconvolution-resolved GH secretory rates in two normal men. For each individual, the *upper* panels depict serial serum GH concentrations measured in blood collected at 5-minute intervals over 24 hours. The continuous line through the data represents the curve fit by the multiple-parameter deconvolution model. In the *lower* panels, the calculated GH secretory rate (ng per ml of distribution volume [ml_v] per min) is plotted vs. time. The secretory rate is derived by removing the influence of subject-specific endogenous GH clearance on the GH concentration profile. Note that the resolved detectable GH secretory pattern consists of clusters or volleys of multiple secretory bursts with intervening periods of apparent secretory quiescence. These complex volleys of GH secretory bursts are acted upon by metabolic clearance to give rise to the multiform peripheral GH concentration pulses shown in the *upper* panels. (Reproduced from Hartman et al., 1991, with copyright permission of the American Physiological Society.)

1991). These distinct distributions of interpulse intervals suggest that this pattern of 'pulses within pulses' results from the interaction of multiple bursts of hypothalamic GHRH secretion stimulating the pituitary gland during a period of diminished somatostatin secretion (Figure 1.2) (Hartman et al., 1991). This inferential model offers a basis for investigating neuroendocrine mechanisms subserving alterations in GH secretion in humans. Other investigators have also observed a multiphasic pattern of GH secretion using a different deconvolution algorithm (Van Cauter et al., 1992a).

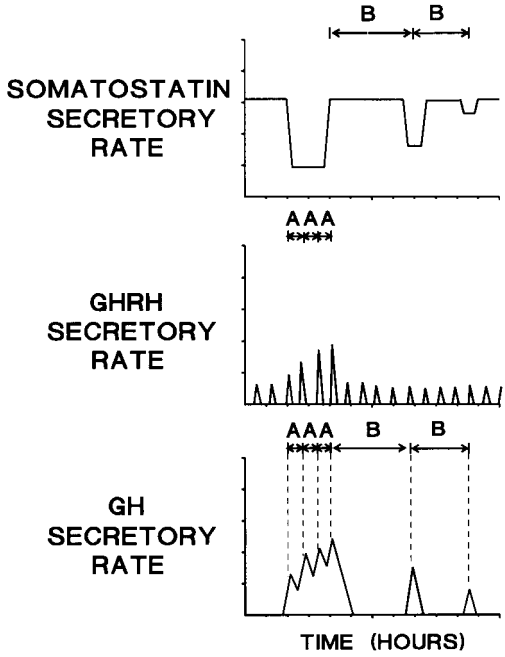

Figure 1.2. Hypothetical model for the physiological basis of a volleyed burst-like mode of growth hormone (GH) secretion in humans. Intra-volley interburst intervals (A) are considered to reflect the frequency of bursts of GH releasing hormone (GHRH) secretion, while inter-volley interburst intervals (B) represent periods of relatively or absolutely increased somatostatin secretion. Thus, multiple GHRH bursts during an interval of decreased somatostatin secretion may give rise to volleys of GH secretion. During periods of increased somatostatin secretion, the GH response to GHRH is inhibited. The frequency of GHRH release is illustrated here as constant, although some physiological variability occurs based on a mean intra-volley interval coefficient of variation of 25 ± 1.6%. (Reproduced from Hartman et al., 1991, with copyright permission of the American Physiological Society.)

A third approach to the analysis of pulsatile hormone release is evaluation of the regularity or pattern orderliness of hormone release over time, using the approximate entropy statistic (Pincus, 1991). This statistic assumes no particular model of hormone secretion and is not affected by differences in mean hormone concentrations (Pincus & Keefe, 1992). This method is complementary to pulse detection and deconvolution methods in that it conveys different information. For example, although GH is secreted in pulses in both normal subjects and in patients with acromegaly, GH secretion is significantly more disorderly in the latter group. This

observation suggests that the mechanisms responsible for generation of GH pulses in acromegaly differ from those in normal subjects (Hartman, et al., 1994). The orderliness of GH release, as well as the mean mass of GH secreted per pulse, are highly conserved in individual healthy men across a wide range of ages (Friend, Iranmanesh & Veldhuis, 1996). However, gender and age affect the orderliness of GH secretion in normal subjects as reviewed below.

Influence of insulin-like growth factor-I (IGF-I) and GH binding proteins

IGF-I

A role for IGF-I in the negative feedback regulation of GH secretion was first suggested by the observation that intracerebroventricular injections of plasma-derived IGF preparations markedly diminished GH pulse amplitudes in rats (Abe et al., 1983; Tannenbaum, Guyda & Posner, 1983). These early IGF preparations may have contained both IGF-I and IGF-II since a combination of both recombinant human IGF-I (rhIGF-I) and IGF-II (rhIGF-II) was required to reproduce these observations in subsequent experiments (Harel & Tannenbaum, 1992). In cultured rat pituitary cells, IGF-I decreases GH secretion and mRNA levels (Berelowitz et al., 1981; Yamashita & Melmed, 1986). The effects of IGF-I on the hypothalamus are less certain. In studies with incubated rat hypothalami, IGF-I has been reported to increase somatostatin secretion and mRNA levels (Berelowitz, et al., 1981; Aguila, Boggaram & McCann, 1993); GHRH release was increased in one study (Aguila, et al., 1993) and decreased in another (Shibasaki et al., 1986). Intracerebroventricular, but not systemic, infusions of IGF-I decreased GHRH and increased somatostatin hypothalamic mRNA levels in GH-deficient dwarf rats; effects of IGF-I on GHRH and somatostatin release were not evaluated (Sato & Frohman, 1993).

IGF-I circulates bound to several binding proteins that prolong the plasma half-life of IGF-I and modulate its bioavailability and action. The IGF-binding proteins (IGFBP) are differentially regulated. IGFBP-3, the predominant plasma binding protein, is regulated slowly and in parallel with serum GH concentrations. Plasma IGFBP-1 concentrations are decreased rapidly by increases in insulin levels (Clemmons, 1991).

The effects of systemic rhIGF-I infusions on pulsatile GH release in humans have been reported. Figure 1.3 illustrates the effects of a six-hour intravenous infusion of rhIGF-I at 10 μg/kg per h in 10 normal men who were fasted for 32 hours to enhance GH secretion; plasma glucose concentrations were maintained at basal levels by a variable glucose infusion. Mean serum GH concentrations fell from 6.3 $\pm$ 1.6 to 0.59 $\pm$ 0.07 mg/l after 120 min. GH secretion rates, calculated by deconvolution analysis, were rapidly suppressed within 60 min and remained suppressed thereafter. Infusion of rhIGF-I decreased the mass of GH secreted per pulse by 84%

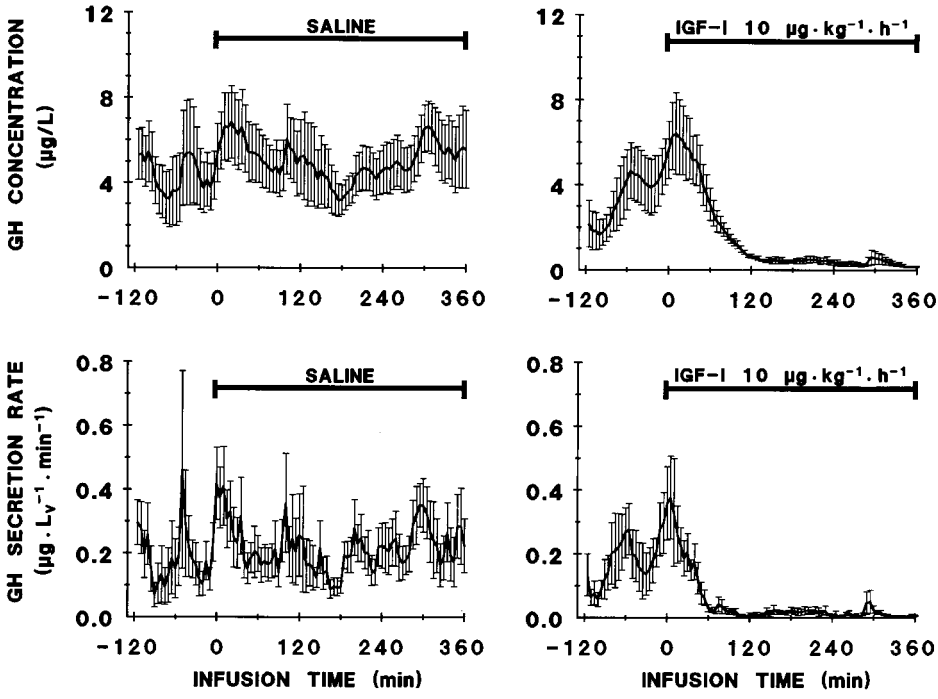

Figure 1.3. Mean (±SE) serum growth hormone (GH) concentrations (*upper panels*) and GH secretion rates (μg per L of distribution volume [L_v] per min) (*lower panels*), calculated by a waveform-independent deconvolution method, for 2 hours prior to and during 6-hour infusions of saline (*left panels*) and 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ recombinant human insulin-like growth factor-I (rhIGF-I) (*right panels*) in 10 normal men on the second day of a fast (32–40 hours of fasting); plasma glucose concentrations were maintained at basal levels by a euglycaemic clamp. Note that whereas pulsatile GH secretion remained elevated during saline (as expected for fasted subjects), rhIGF-I rapidly suppressed GH secretion rates during the first hour of rhIGF-I infusion. (Reproduced from Hartman et al., 1993, with copyright permission of the American Society for Clinical Investigation.)

($p < 0.01$) and the number of detectable GH secretory pulses by 32% ($p < 0.05$). These data demonstrate that IGF-I has a rapid negative feedback effect on pulsatile GH secretion in man (Hartman et al., 1993).

Subsequent studies have reported the dose-response relationship between rhIGF-I infusions and suppression of GH release in humans, and have provided insight into the mechanisms responsible for this effect. Intravenous infusion of rhIGF-I at 3 μg/kg per h was able to suppress GH release in both young and older subjects although the older subjects appeared to be less sensitive to the suppres-

sive effects of rhIGF-I. Infusion of rhIGF-I at a dose of 1 μg/kg per h was not able to suppress GH release in either age group (Chapman, et al., 1997). After discontinuing a four-hour rhIGF-I infusion (3 μg/kg per h), a rebound increase in GH concentrations occurred five to seven hours later. At this time point, free IGF-I levels had returned to baseline levels but total IGF-I levels were still elevated. The close temporal association between the resolution of GH suppression and the fall in free (but not total) IGF-I concentrations suggest that unbound (free) IGF-I is the major IGF-I component responsible for GH suppression during an rhIGF-I infusion (Chapman et al., 1998). Prolonged (48 hours) intravenous infusions of rhIGF-I suppress 24-hour mean GH concentrations by 85% and attenuate both the GH response to GHRH and the thyrotropin response to thyrotropin-releasing hormone (Bermann et al., 1994). This latter observation supports the hypothesis that IGF-I stimulates somatostatin release in humans although a direct effect on the pituitary is also likely. A single subcutaneous dose of 40 mg/kg body weight rhIGF-I has been reported to decrease GH secretion rates by 40% during a 22-hour study period in patients with insulin-dependent diabetes mellitus (Cheetham et al., 1994). This observation supports the hypothesis that reductions in serum IGF-I in patients with poorly controlled diabetes mellitus result in increased GH secretion.

GH-binding proteins

Two distinct GH-binding proteins (GHBP) have been identified in plasma of humans: (1) a 60 kD protein that is identical with the extracellular portion of the GH receptor and has high affinity for the 22 kD (predominant) form of GH; and (2) a low-affinity 100–170 kD GHBP that may not be a single protein. The regulation and possible physiological significance of these proteins have been reviewed (Mercado & Baumann, 1993). Plasma concentrations of the high-affinity GHBP are quite stable throughout the day in a given individual. Current evidence suggests that GH does not regulate plasma GHBP levels to a significant degree. However, plasma GHBP concentrations are decreased in patients with malnutrition, insulin-dependent diabetes mellitus, hepatic cirrhosis, renal failure, hypothyroidism and critical illness. Oral oestrogen therapy and obesity are associated with increased GHBP levels. In normal subjects, GHBP concentrations are positively correlated with percentage body fat and measures of subcutaneous abdominal fat and intra-abdominal visceral fat (Fisker et al., 1997). Decreased or undetectable plasma levels of GHBP have been described in patients with certain types of congenital short stature (Mercado & Baumann, 1993). In normal children, plasma GHBP levels are inversely related to the 24-hour GH secretion rate (Martha et al., 1991). In GH-deficient children, the increase in IGF-I concentrations and growth velocity induced by treatment with GH is correlated with serum

GHBP levels (Martha et al., 1992b). Plasma GHBP concentrations have been proposed to reflect the number of tissue GH receptors and to provide an index of tissue responsivity to GH. The number of GH receptors may influence GH secretion via IGF-I feedback. Thus, a lower number of GH receptors may result in relative GH resistance, decreased IGF-I synthesis and enhancement of GH secretion (Martha et al., 1992b; Mercado & Baumann, 1993). In adults, serum GHBP levels are inversely related to the ratio of 24-hour mean GH over IGF-I concentrations. Therefore, GHBP levels may reflect tissue sensitivity to GH in the adult as well (Fisker et al., 1997).

The high-affinity GHBP may enhance the actions of GH by prolonging its half-life in plasma. Mathematical modelling reveals that for an individual with a typical monoexponential GH half-life of 18 minutes, the half-lives of free and bound GH are approximately 9 and 29 minutes, respectively. Since GH is secreted in a pulsatile manner, a highly dynamic (nonequilibrium) system ensues in which the half-life of free GH, its instantaneous secretion rate, and the GHBP affinity and capacity all contribute to defining momentary levels of free, bound and total GH, the percentage of GH bound to protein and the percentage occupancy of GHBP. Over a 24-hour period, the percentage of GH bound to GHBP varies from 10–80% under conditions of pulsatile GH secretion. The percentage of GH bound to GHBP rises following a burst of GH secretion, as free GH is removed more rapidly than bound. Dissociation of GH from GHBP during periods of low or absent GH secretion maintains some free GH in plasma (Veldhuis et al., 1993). The potential importance of these effects of GHBP on GH pharmacokinetics is supported by preliminary data indicating that co-administration of GHBP and GH enhances the growth-promoting effects of GH in rats (Clark et al., 1991).

Physiological regulators of GH secretion

Many physiological factors influence GH secretion, most likely by effects on GHRH and somatostatin secretion, and/or by altering IGF-I levels or target tissue sensitivity to IGF-I. The precise mechanisms that mediate the effect of a physiological factor on GH secretion in humans may be difficult to determine. Studies with experimental animals often are not helpful since GH secretion in response to sleep, nutrition, and stress may differ from those observed in humans. For this reason, this review will focus primarily on human studies and will be limited to normal physiology, with the exception of obesity. The pathophysiology of GH secretion in states of excessive or deficient serum levels of glucocorticoids, thyroid hormone, and glucose, as well as other disease states will not be covered (for review, see Guistina & Veldhuis, 1998). The effect of ageing will be discussed briefly, as this is the topic of a later chapter.

Gender, menstrual cycle, puberty and gonadal steroids

Gender

Twenty-four hour integrated serum GH concentrations were approximately 50% higher in young menstruating women than young men in two early studies (Ho et al., 1987; Hartman et al., 1990). With frequent blood sampling (e.g., every 5 minutes), an increased number of detectable GH pulses was observed in women compared to men in one of these studies (Hartman et al., 1990). However, this gender difference in GH pulse frequency may have been an artifact of inadequate assay sensitivity since GH concentrations were more frequently undetectable in men ($46 \pm 7.6\%$ of samples) than in women ($17 \pm 6.8\%$ of samples) using the immunoradiometric assay (IRMA; sensitivity 0.25 μg/l) (Pincus et al., 1996). Three recent studies have examined gender differences in GH secretion using new GH assays with enhanced sensitivity and deconvolution analysis. Two of these studies used an immunofluorometric assay (IFMA; sensitivity 0.01 μg/l) and included men and women with a broad age range (27–59 years; mean ~ 40 years) (Van den Berg et al., 1996; Vahl et al., 1997). Both studies reported no gender difference in the number of GH secretory pulses. Compared to men, women had 2 to 3-fold higher 24-h mean GH concentrations and production rates. This was the result of a greater (1.5 to 2.4-fold) mass of GH secreted per pulse in women compared to men (Van den Berg et al., 1996; Vahl et al., 1997). GH secretory pulse amplitudes were greater in women but there was no gender difference in the duration of GH secretory pulses or in basal secretion rates (Van den Berg et al., 1996). In one study, the GH half-life was slightly longer in women than men (Vahl et al., 1997), but this was not the case in the other study (Van den Berg et al., 1996). The third study used a sensitive (0.01 (g/l) chemiluminescence assay but differed from the other two studies in that the mean age of the subjects was ~ 25 years and a different deconvolution algorithm was employed (Jaffe et al., 1998). In this study there was no significant difference in the mean 24-hour GH secretion rate or GH secretory pulse amplitude between the young women and men. However, women had nearly twice as many GH secretory pulses per 24 hours compared to the men (Jaffe et al., 1998). These disparate findings are somewhat difficult to reconcile. Perhaps, the differences in pulsatile GH secretion between men and women change with ageing.

A gender difference in the GH response to GHRH may also exist, although the data have not been entirely consistent. A study of the dose-response relationship revealed a lower ED_{50} for women in the mid-follicular phase of the menstrual cycle (0.2 μg/kg) than for men (0.4 μg/kg). However, maximal responses to 1 and 10 μg/kg GHRH did not differ between men and women (Gelato et al., 1984). In contrast, a larger study investigating only a single dose of GHRH (1 μg/kg) found significantly greater maximal responses in premenopausal women than in age-matched men.

There was no gender difference in the GH response in postmenopausal women compared to age-matched men (Lang, et al., 1987).

Other gender differences in the pattern of GH secretion have been reported. Men have large nocturnal GH pulses with very small pulses during the day. In contrast, women have a more continuous mode of GH secretion with more uniform pulse amplitudes. Jaffe and coworkers quantified these visual impressions by reporting a significantly greater standard deviation of GH secretory pulse amplitudes in men compared to women. In addition, the proportion of serum GH concentrations above 0.5 μg/l was significantly greater in women (53 ± 6%) than men (35 ± 4%) (Jaffe et al., 1998). These observations are complemented by the report that 24-hour pulsatile GH release in women is significantly more disorderly than in men, as measured by the approximate entropy statistic. This finding has been reported in studies employing both a GH IRMA and a GH IFMA, suggesting that assay sensitivity does not affect this finding (Pincus et al., 1996; Vahl et al., 1997). This greater process irregularity in women probably reflects a more complex hypothalamic control of GH secretion (Pincus et al., 1996). In support of this hypothesis, spontaneous GH secretion in young women was significantly less responsive to the negative feedback effects of a rhIGF-I infusion (10 μg/kg per h) than that of men. The GH response to exogenous GHRH was significantly attenuated by rhIGF-I infusion in men but not in women, despite the fact that total IGF-I concentrations were higher in the women than men (Jaffe et al., 1998). The neuroendocrine mechanisms responsible for the sexually dimorphic patterns of GH secretion have not been completely established in humans.

Such gender differences in GH secretion have diagnostic implications for disorders of GH secretion, particularly when newer enhanced sensitivity GH assays are employed. For example, Figure 1.4 illustrates that after an oral glucose load normal suppression of GH in plasma is less than 0.057 mg/l for young men and 0.71 μg/l for young women during the early follicular phase, a 12-fold difference (Chapman et al., 1994). Thus, earlier criteria for 'normal' glucose suppression of serum GH concentrations overlooked a significant gender difference.

Menstrual cycle

During the late follicular phase of the menstrual cycle, GH pulse amplitudes and integrated GH concentrations are increased (approximately doubled) compared to the early follicular and mid-luteal phases. GH pulse amplitudes are positively correlated with serum oestradiol and negatively correlated with progesterone concentrations, suggesting that changes in gonadal steroid concentrations during the menstrual cycle possibly regulate GH secretion to a significant degree (Faria et al., 1992). A recent study employing a GH IFMA (sensitivity 0.01 μg/l) and deconvolution analysis reported that 24-hour mean GH concentrations and production

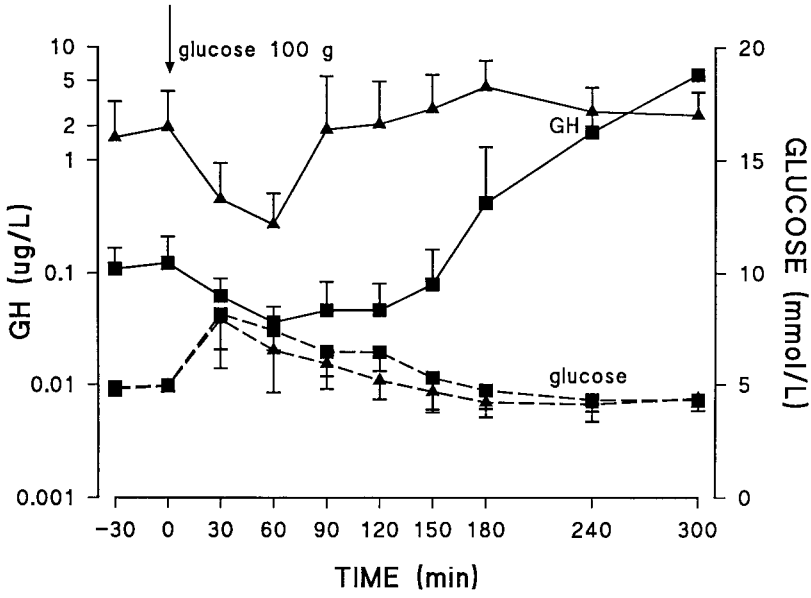

Figure 1.4. Serum growth hormone (GH) (—) and plasma glucose (– – –) concentrations (mean ± SEM) in normal young adult males (■); $n = 9$) and females (▲; $n = 6$; early follicular phase) during a 100-g oral glucose tolerance test. Serum GH concentrations were measured with an enhanced sensitivity chemiluminescence assay. Note the logarithmic scale for GH. (Reproduced from Chapman et al., 1994 with copyright permission of The Endocrine Society.)

rates were 1.6-fold higher during the periovulatory phase than the early follicular phase. The number of GH secretory pulses per 24 hours was significantly higher (1.3-fold) in the periovulatory period but the trends for increased mass of GH secreted per pulse and GH pulse amplitude did not reach statistical significance. Serum oestradiol concentrations during the periovulatory period were significantly correlated with GH secretory pulse amplitude, frequency, and 24-hour GH production rate (Ovesen et al., 1998). These studies demonstrate that changes in gonadal steroid concentrations possibly mediate the changes in spontaneous GH secretion during the menstrual cycle. In contrast, GH responses to either a GHRH or arginine stimulation test did not differ by menstrual cycle phase (Evans et al., 1984; Gelato et al., 1984; Ovesen et al., 1998).

Puberty

A longitudinal study of late pre-pubertal boys demonstrated a fourfold variation in mean 24-hour GH concentrations (1.6–7.0 μg/l) across the group. However, within

individual subjects, the 24-hour mean GH concentration, as well as properties of pulsatile GH secretion, varied much less during a follow-up period of 9–19 months, suggesting that GH secretion is relatively constant during late prepuberty (Martha et al., 1996). Twenty-four hour GH production rates increase threefold during puberty and are maximal during late puberty when linear growth velocities are greatest (Martha et al., 1989; Martha et al., 1992a). In a cross-sectional study, increased GH concentrations during puberty in boys was found to result from an increase in the mass of GH secreted per burst without changes in detectable GH pulse frequency or estimated half-life compared with prepubertal boys. An increase in GH secretory burst amplitude without a change in the duration of secretory bursts accounts for the increased mass of GH secreted per pulse (Martha et al., 1992a). In addition, during mid- to late-puberty pulsatile GH secretion in boys is significantly more disorderly than that of young men, as assessed by the approximate entropy statistic (Veldhuis et al., 1997).

Gonadal steroids

An increased concentration of gonadal steroids is one plausible proximate stimulus to enhanced GH secretion during pubertal development (Kerrigan & Rogol, 1992). Administration of ethinyl oestradiol (100 ng/kg daily for 1 or 5 weeks) to girls with Turner's syndrome or testosterone enanthate (100 mg monthly for 3 months) to prepubertal boys with constitutional delay of adolescence doubles 24-hour endogenous GH production rates (Mauras, Rogol & Veldhuis, 1990; Ulloa-Aguirre et al., 1990). Testosterone increases the mass of GH secreted per pulse (Ulloa-Aguirre et al., 1990); oestradiol increases the number of detectable GH secretory pulses per 24 hours and may also increase GH secretory pulse amplitudes (Mauras et al., 1990). A dose-response study of testosterone enanthate treatment of boys with idiopathic hypogonadotrophic hypogonadism demonstrated that an intramuscular dose as low as 25 mg every two weeks for six weeks was able to increase pulsatile GH secretion (Guistina et al., 1997). Administration of oestrogen to girls and testosterone to boys also induced a greater irregularity of GH secretion, as assessed by the approximate entropy statistic (Veldhuis et al., 1997). These observations suggest that the increased disorderliness of GH secretion observed during puberty may also be attributed to rising concentrations of gonadal steroids. Since approximate entropy values did not change when 5-α-dihydrotestosterone (a non-aromatizable androgen) was administered to boys, aromatization of testosterone to oestrogen is likely to be responsible for the increase in irregularity of GH secretion when testosterone is administered to boys (Veldhuis et al., 1997).

The decline in serum GH concentrations with age in men and women correlates with changes in gonadal steroid levels. When the entire age range of men and women was compared, serum oestradiol (but not testosterone) levels largely

accounted for the differences in 24-hour integrated GH concentrations (Ho et al., 1987). However, when men aged 21–71 years were studied, serum testosterone (not oestradiol) was the best correlate of 24-hour GH secretion (Iranmanesh, Lizarralde & Veldhuis, 1991). A recent study evaluated 24-hour GH secretion in men with a wide range of ages (18–63 years) and body mass indices (BMI; 18–39 kg/m^2), using an ultrasensitive (0.002 μg/l) chemiluminescence GH assay and deconvolution analysis. The 24-hour GH production rate and the GH secretory pulse mass were positively correlated with serum testosterone but not oestradiol concentrations. However, serum oestradiol was positively correlated with the GH half-life and inversely related to basal GH secretion rates. Higher testosterone levels were associated with greater regularity of GH secretion, as assessed by approximate entropy. These data suggest that pulsatile and basal GH secretion may be differentially regulated by testosterone and oestradiol in men (Veldhuis et al., 1995). The increment in GH secretion in response to a three-day intravenous pulsatile GHRH infusion in men was also positively correlated with serum testosterone but not with oestradiol levels (Iranmanesh et al., 1998).

Oral oestrogen replacement therapy increases 24-hour spontaneous and GHRH-stimulated GH release in menopausal women (Dawson-Hughes et al., 1986; Weissberger, Ho & Lazarus, 1991). Animal studies suggest that oestrogen likely stimulates GH release via effects on hypothalamic somatostatin and GHRH secretion (Wehrenberg & Guistina, 1992). However, reduced negative feedback by IGF-I is also possible since oral oestrogen administration in women decreases serum IGF-I levels (Dawson-Hughes et al., 1986; Weissberger et al., 1991), probably by inhibiting the stimulation by GH of hepatic IGF-I synthesis (Murphy & Friesen, 1988). The route of oestrogen administration may alter its effect on GH secretion. In one study, oral ethinyl oestradiol decreased serum IGF-I and increased 24-hour mean GH concentrations whereas transdermal 17β-oestradiol increased IGF-I but had no effect on 24-hour GH concentrations (Weissberger, et al., 1991). In another study, both oral and transdermal 17β-oestradiol increased GH release in postmenopausal women when higher doses of the transdermal preparation were administered (Friend et al., 1996).

The stimulatory effect of testosterone on GH secretion may be mediated directly by the androgen receptor or through aromatization to oestradiol. Administration of a nonaromatizable androgen, oxandrolone, to prepubertal boys increased GH secretion to a similar degree as testosterone (Ulloa-Aguirre et al., 1990). In contrast, androgen receptor blockade with flutamide increases and oestrogen receptor blockade with tamoxifen decreases GH release, suggesting that aromatization of testosterone to oestradiol is important in the stimulation of GH (Metzger & Kerrigan, 1993; Weissberger & Ho, 1993). In another study, induction of hypogonadism in normal men with leuprolide administration did not significantly alter

spontaneous GH secretion or serum IGF-I concentrations. The lack of effect on GH secretion may have been due to the short period (2 weeks) of hypogonadism. In contrast, administration of supraphysiological doses of testosterone enanthate (3 mg/kg weekly for 3 weeks) increased 24-hour GH secretion and serum IGF-I by 22% and 21%, respectively, above that observed when the subjects were eugonadal. In contrast, administration of a nonaromatizable androgen, stanozolol, did not increase GH secretion or serum IGF-I levels (Fryburg et al., 1997).

In summary, current evidence favours the hypothesis that both androgens and oestrogens regulate GH secretion and contribute to gender differences in GH secretion, increases in GH release during puberty and declining serum GH concentrations with ageing and after menopause.

Nutrition

Plasma IGF-I concentrations are reduced by fasting and are restored with refeeding; both adequate protein and energy intake are necessary to return IGF-I levels to normal (Clemmons & Underwood, 1991). Five days of fasting in healthy men increases pulsatile GH release, presumably because of a reduction in IGF-I negative feedback (Ho et al., 1988). After two days of fasting, 24-hour GH secretion rates, estimated by deconvolution analysis, are increased four- to fivefold without significant decreases in serum total IGF-I concentrations. As shown in Figure 1.5, increases in detectable GH secretory pulse frequency and amplitude account for the enhanced GH secretion during short-term fasting; no change in calculated GH half-life occurs (Hartman et al., 1992a). Analysis of the interpulse intervals in this study revealed that the frequency of GH secretory pulses within volleys of GH secretion was increased; in addition, the intervals between volleys of GH secretion were decreased. This suggests that both increased GHRH pulse frequency and decreased somatostatin secretion occur in response to nutrient withdrawal (Hartman et al., 1992a). When subjects who have fasted for 34 hours are refed balanced eucaloric meals, fasting-enhanced GH secretion rates are rapidly suppressed (within 60 minutes) and thereafter remained indistinguishable from control (fed) levels (Hartman & Thorner, 1990). Similarly, the GH response to exogenous GHRH administration is enhanced by fasting and attenuated by prior ingestion of a mixed meal (Kelijman & Frohman, 1988; DeMarinis et al., 1988). The GH response to L-692, 429 (a nonpeptide mimetic of GHRP) in non-obese subjects is also attenuated by prior meal ingestion to a similar degree as observed in fasted obese subjects (Kirk et al., 1997).

The mechanisms responsible for the effects of nutrition on GH secretion are poorly understood. Acute increases in plasma glucose or free fatty acid concentrations are known to decrease the GH response to GHRH (Masuda et al., 1985; Imaki et al., 1985). However, several amino acids are known to stimulate GH