Growth Hormone and Gender

Studies in Healthy Adults and in Patients with Growth Hormone Disorders

BY

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ABSTRACT


The use of a new, more sensitive immunoassay for growth hormone (GH) revealed that the serum levels in men were lower than expected in sera drawn ambulatory in the morning after an overnight fast and that the gender difference was more than 10 times greater than reported. These observations led to a more thorough study on the impact of gender and sex steroids on the levels of GH and other hormones in ambulatory morning samples and over a 24-hour period. Furthermore, the impact of gender was studied in GH deficient (GHD) patients and healthy young adults treated with GH, and in patients with acromegaly treated with octreotide.

An 80-fold gender difference in the morning GH levels was observed in young individuals as a reaction to ambulation, with decreased levels in men and increased in women. Oral contraceptives (OCs) given to women further increased the morning GH levels. During the day, higher outputs of epinephrine and lower levels of GH were seen in the men, while no gender differences were seen at night. The gender difference in morning GH levels decreased with age due to opposite changes in men and women. Administration of 17β-estradiol (E2) via subcutaneous implants in postmenopausal women, which increased the E2-concentrations to luteal phase levels, had no effect on the morning GH levels, indicating that the different reactions to ambulation do not appear to result from a direct sex steroid effect alone.

Short-term administration of GH to young, healthy adults resulted in larger effects on insulin-like growth factor I (IGF-I) and other key metabolic parameters in men than in women. The smallest response was noted in women taking OCs. The clinical studies involving long-term GH treatment of patients with GHD demonstrate a gender difference in GH responsiveness, with women being less sensitive than men, a fact which should have a therapeutic impact in patients with GH disorders. A further gender difference of therapeutic importance was observed in men and women with acromegaly. Long-term treatment with a slow-release formulation of octreotide resulted in higher IGF-I levels in the men, despite equal doses of the drug and similar levels of GH.

Key words: Growth hormone, gender, ambulation, oral contraceptives, epinephrine, GH treatment, age, E2-implant, IGF-I, octreotide.

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To my family
The thesis is based on the following papers, which will be referred to in the text by their Roman numerals


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<th>Abbreviation</th>
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<tr>
<td>BALP</td>
<td>Bone-specific alkaline phosphatase</td>
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<tr>
<td>BMC</td>
<td>Bone mineral content</td>
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<td>BMD</td>
<td>Bone mineral density</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>E₂</td>
<td>17β-estradiol</td>
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<td>EE</td>
<td>Ethinylestradiol</td>
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<td>FIA</td>
<td>Fluoroimmunoassay</td>
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<td>FSH</td>
<td>Follicle-stimulating hormone</td>
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<td>GH</td>
<td>Growth hormone</td>
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<td>GHBP</td>
<td>Growth hormone binding protein</td>
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<td>GHD</td>
<td>Growth hormone deficiency</td>
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<td>GHRH</td>
<td>Growth hormone releasing hormone</td>
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<td>GHRP</td>
<td>Growth hormone releasing peptide</td>
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<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
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<tr>
<td>ICTP</td>
<td>Carboxy-terminal crosslinked telopeptide of type I collagen</td>
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<td>IGF</td>
<td>Insulin-like growth factor</td>
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<td>IGFBP</td>
<td>Insulin-like growth factor binding protein</td>
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<tr>
<td>kDa</td>
<td>Kilodalton</td>
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<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
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<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
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<tr>
<td>OCs</td>
<td>Oral contraceptives</td>
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<tr>
<td>PICP</td>
<td>Carboxy-terminal propeptide of type I procollagen</td>
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<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
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<td>SHBG</td>
<td>Sex hormone binding globulin</td>
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<tr>
<td>U-Dpyr/cr</td>
<td>Deoxypyridinoline in urine/creatinine</td>
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INTRODUCTION

Growth hormone
Growth hormone (GH) was isolated from the bovine pituitary gland in 1944, and from the human pituitary in 1956/57. There are several GH isohormones secreted. The most abundant isoform is a polypeptide single-chain of 191 amino acids, the 22 kilodalton (kDa) (22K) GH. Under basal conditions, more than half of 22K is bound to high affinity growth hormone binding protein (GHBP) in the circulation, while a small proportion is bound to a separate, low affinity GHBP. The high-affinity GHBP corresponds to the extracellular domain of the GH receptor (1, 2). Plasma levels of the GHBPs rise during childhood and remain relatively constant in adulthood, but declining levels after the age of 60 have been reported (3).

Regulation of GH secretion

Neuroregulatory mechanisms
Multiple neurotransmitter pathways, as well as peripheral feedback signals, regulate GH secretion either by acting directly on the anterior pituitary gland and/or by modulating GH releasing hormone (GHRH) or somatostatin release, or both, from the hypothalamus (Fig 1). GHRH stimulates the synthesis and release of GH, and somatostatin inhibits the secretion of GH but not its biosynthesis (4). Studies in humans have suggested a role of somatostatin withdrawal in the generation of GH pulsatile release (5, 6). It has been reported that the release of GH must also be associated with a concomitant GHRH pulse (7). With advancing age there is a reduction in GHRH release paralleled by an increase in the somatostatinergic hypothalamic tone (8). GH itself participates in an autonegative feedback system, probably by stimulating somatostatin release from the hypothalamus. In addition, a concomitant withdrawal of GHRH in response to a GH stimulus cannot be excluded. There are also studies indicating that GH feeds back to suppress the hypothalamic expression of the GH receptor itself (4).

Synthetic GH secretagogues was first described in 1977, and in 1984 the existence of a natural GH releasing peptide (GHRP) hypothalamic hormone was hypothesized. In 1996 the GHRP-GH secretagogue receptor was cloned (9). This discovery
led to engineering of GHRPs such as GHRP-1, GHRP-2, GHRP-6, and hexarelin as well as nonpeptidyl GH secretagogues, which have been shown to be effective releasers of GH in both animals and humans. The GH-releasing effect of GHRPs is the same in both sexes, but undergoes age-related variations (8). The recent finding of ghrelin, an endogenous ligand specific for the GH secretagogue receptor, which releases GH both \textit{in vivo} and \textit{in vitro}, indicates that ghrelin is involved in a third system for regulating GH secretion. Human ghrelin is homologous to rat ghrelin apart from two amino acids. The peptide was first purified from the gastric mucosa. It has also been found in the hypothalamus. Further, PCR analysis indicates that the GH secretagogue receptor is expressed also in heart, lung, pancreas, intestine and adipose tissue (10). Ghrelin possibly functions not only in the control of GH secretion, but also in the regulation of diverse processes of the digestive system (11). In a study in 6 healthy men, intravenous ghrelin administration (0.2, 1.0, and 5.0 µg/kg, respectively) strongly released GH in a clearly dose-dependent manner, and was found to be more potent for GH release in humans than GHRH (12).

\begin{center}
\textbf{Fig 1.} GH, IGF-I, GHRH, ghrelin, somatostatin (SRIH) feedback loop in the regulation of GH secretion. An inhibitory effect of β-adrenergic stimulation on GH release has also been documented, (not shown in the figure). (Modified from Shlomo Melmed, M.D., The Pituitary, 1995. Reprinted by permission of Blackwell Science, Inc).
\end{center}
Several neurotransmitters are also involved in the regulation of GH secretion. In normal subjects, acute administration of dopamine and dopamine agonists causes GH release. Phentolamine, a non-specific $\alpha_1$- and $\alpha_2$-receptor blocking agent, reduces the GH response to many stimuli in humans whereas agents acting at the $\alpha_1$-receptor alone does not seem to influence GH secretion. Stimulation of $\alpha_2$-receptors with agonists induces GH release in man. Experiments performed in humans using $\beta$-adrenergic receptor-blocking agents, support the hypothesis that $\beta$-adrenergic receptors mediate inhibitory effects on GH release. The endogenous neurotransmitter primarily involved in the $\beta$-receptor-mediated inhibition of GH release has been reported to be L-epinephrine. Experimental studies suggest that $\beta$-receptors modulate the hypothalamic somatostatin tone (4).

Factors influencing the regulation of GH secretion

The secretion of GH is augmented after onset of sleep and also under catabolic conditions of fasting and stress, and by certain amino acids. Conversely, food intake and exposure to glucose, high levels of free fatty acids (FFA) and obesity inhibit GH release (13-16). Hypoglycemia has been reported to raise the GH concentration more in men than in women (17, 18), suggesting a sex difference in the glucose threshold for hormone release (17), while similar glucose thresholds have been observed by Fanelli et al. (18). In a recent study, reduced central nervous system efferent input was found to be responsible for the lowered neuroendocrine responses to hypoglycemia in women (19). In response to exercise, raised GH levels were found in men only (20), while others have reported similar increases in GH concentrations in men and women (21, 22).

Studies of the 24-hour GH serum concentration have shown a reduction with advancing age in men (23-25), and in both men and women (26-29). With each advancing decade, the GH production rate in men has been reported to decrease by 14% and the GH half-life to fall by 6% (24). Obese subjects are characterized by reduced serum GH concentrations (30). Since fat mass tends to increase with age the negative association between GH secretion and age may reflect a concomitant change in body composition (31). An inverse, age-independent relationship between the rela
tive adiposity and the mass of GH secreted per burst has been described (32) and in nonobese adults, intra-abdominal fat was found to be the dominant determinant of estimates of GH secretion (33).

The GH/IGF-I axis
GH has both direct and indirect actions on peripheral tissues. The indirect actions of GH are mediated mainly by IGF-I. The IGFs (IGF-I, IGF-II) and insulin are structural homologues. IGF-I and insulin act through similar cell surface receptors and share many biological properties, although the affinity for the IGF receptor is about 100-fold lower for insulin than for IGF-I. IGF-I is generated in response to GH at the sites of GH action. Most of the circulating IGF-I is of hepatic origin, although GH receptors have been identified in most tissues. IGF-I exerts negative feedback on the hypothalamus and pituitary to inhibit GH release (34). In extracellular tissues IGF-I is bound to IGF binding proteins (IGFBPs). So far, six IGFBPs are known. More than 75% of circulating IGF-I is carried in a trimeric complex composed of IGFBP-3 and a liver derived glycoprotein known as the acid-labile subunit (ALS). All three components of this complex are induced by GH. The remaining plasma IGF-I is mainly bound to IGFBP-1 or IGFBP-2. Ninety-nine percent of IGF-I is bound to IGFBPs in serum (35).

Higher IGF-I levels have been shown in girls than in boys during late puberty (36, 37) while the concentrations have been reported to be similar in adult men and women (36, 38). Further, a linear inverse correlation has been found between IGF-I and age without gender difference (39).

Effects of GH and IGF-I on growth
According to the original somatomedin (now known as IGFs) hypothesis, GH stimulates body growth by stimulating liver production of somatomedin, which in turn stimulate longitudinal bone growth in an endocrine manner (40). The dual-effector theory proposes that GH has both a direct action and an indirect action mediated by IGF-I, and that locally produced IGF-I contributes to the stimulatory effects of GH, particularly stimulation of longitudinal growth (41, 42). Both endocrine and para
crine/autocrine IGF-I have been reported to be necessary for normal growth, especially in tissues with high levels of IGF-I expression (for example ovary, kidney, lung) (40). In a recent important study in mice, IGF-I production was abolished in the liver by using the Cre/loxP recombination system resulting in complete inactivation of the IGF-I gene in the hepatocytes (43). The concentration of IGF-I in the serum was reduced by 75%, confirming that the liver is the principal source of IGF-I in the blood. The reduction in IGF-I had no effect on postnatal growth, indicating that autocrine/paracrine-produced IGF-I is more important than liver-derived IGF-I for body growth.

**Metabolic effects of GH**

GH has a wide range of anabolic and metabolic effects. The most rapid effect *in vivo* is an acute decrease in forearm glucose uptake within 10 minutes after an intravenous GH pulse. An acute insulin antagonistic effect with maximal effect on lipolysis is seen after 2 hours. These effects are reversed after 4 hours underlining the potential role of GH as a principal physiological regulator of diurnal substrate levels and fuel utilization in humans (44, 45). A single pulse of GH resulted in increased lipid intermediates after 5 hours with a higher response in men (46). In another study, increased IGF-I mRNA expression was found in human liver tissue after 5 hours (47), while no increase was seen in serum levels of IGF-I or insulin. Two weeks administration of GH to healthy young women increased circulating lipid fuel substrates, energy expenditure and lipid oxidation, together with elevated insulin levels, indicating that after more prolonged GH exposure, metabolic actions of GH prevail, despite normal daytime levels of GH (48). Furthermore, GH has been shown both to increase the mobilization of triglycerides from fat depots and to inhibit lipoprotein lipase activity in human adipose tissue, suggesting that GH also may have an impact on adipose tissue accumulation (49, 50).

A lack of nocturnal GH release depresses the rate of lipolysis (51), while surges of nocturnal GH correlate with ketone concentrations (52), demonstrating that GH is a potent regulator of lipolysis at night.
Catecholamines are the major lipolysis-stimulating hormones in humans. They influence lipolysis in adipocytes by binding to lipolytic β-adrenoceptors and antilipolytic α2-adrenoceptors resulting in the break-down of triglycerides to the end-products glycerol and non-esterified fatty acids (53). Experimental studies have demonstrated that GH acts in synergy with epinephrine to increase lipolysis (54-56). Treatment with GH for 6 months in GH deficient adults increases the lipolytic response to β-adrenergic agonists in abdominal fat cells (57).

**Gender differences in GH secretion pattern**

*Studies in rats*

Gender differences in GH was first described in rats. Early studies in male rats have shown striking regularity of the GH pulses, occurring at 3- to 4-hour intervals, while values are low or undetectable between peaks (58). In contrast, more continuous secretion of GH has been found in female rats (59, 60). A more recent study has demonstrated that the secretion is significantly more irregular in female than in male rats with nearly complete separation between female and male GH profiles over time (61).

Both *in vitro* and *in vivo* studies in rats have shown that the episodic pulses of GH secretion are due in part to episodic removal of the inhibitory activity of somatostatin, while other studies indicate that the GH pulses are due to stimulation by GHRH rather than to periodic removal of somatostatin (34). Gonadal steroids probably interfere with both of these hypothalamic control systems. Prepubertal gonadectomy of male rats results in elevated basal GH levels, an effect that is reversed by testosterone replacement. In contrast, estrogens seem to elevate basal GH levels in adult male rats. Gonadectomy of female rats may result in a decrease in the GH trough levels but they do not reach the low levels observed in intact male rats, indicating that testosterone is important for these low trough levels (34, 62).
Studies in humans

As in animals, humans secrete GH in a pulsatile fashion. Earlier studies over 24 hours have shown either no gender difference in GH levels (26) or higher concentration in women than in men (27). The divergent results were probably due to the use of less sensitive GH assays. When an ultrasensitive assay for GH was used, and samples were taken every 20 min over 24 hours, pulsatile GH secretion was shown to be oscillatory rather than episodic (63). The women had larger mean GH concentrations (1.5-fold), more frequent values above 1 µg/L, higher mean peak amplitudes overall, and higher mean nadirs and trough levels than the men. The number of peaks per 24 hour was about 13 in both sexes, with a dominant, but not strictly periodic, 2-hour rhythmicity.

In a more recent study in men and premenopausal women, GH was measured every 10 min over 24 hours (64). Women had 2.9-fold higher mean and integrated 24-hour serum GH concentrations than men, accounted for by higher maximal and incremental peak amplitudes. The GH-interpulse interval and GH pulse frequency (10-11/24 h) were similar in men and women. Total daily GH production rate and total GH secretion were higher in women than in men. The half-life and basal secretion rate of endogenous GH were similar in men and women and in both sexes, more than 90% of GH production was secreted in a pulsatile mode. Larger GH mass secreted per burst was seen during the night compared to daytime.

In a study by Pincus et al. (61) samples drawn at 10-min intervals showed two-fold higher serum GH concentrations in women over a 24-hour period. Female GH release was consistently more irregular than male release both during dark and light periods, with an almost complete gender separation.

Relevance of male vs. female pattern of GH pulsatility to target tissue

Results from both animal and human studies suggest that high peaks and low troughs of plasma GH (i.e. a male secretory pattern) induce a higher rate of somatic growth than a low peak - high trough plasma pattern of GH (i.e. a female secretory pattern). Experimental data in rats indicate that the gender specific pattern of GH delivery to the individual target tissue influences growth, and specific muscle and hepatic enzyme
and receptor expression, differently. The mechanism underlying this phenomenon is unknown, although changes in GH responsiveness dependent on previous GH exposure have been documented (34, 65).

**GH secretion and sex steroids**

**Puberty**

In prepubertal boys and girls, the mean 24 hour GH secretion rates are comparable. The rate increases during puberty, earlier in girls (Tanner stage 2) than in boys (Tanner stage 4), but decreases at stage 5 in both sexes. The number of high amplitude peaks increases during puberty in both boys and girls. The calculated GH baseline is consistently higher in pubertal girls than in boys and an increase is seen in girls at Tanner stage 3 and 4, and a decrease at stage 5. Such an increase of baseline levels is not found in boys. Before puberty, a marked day-night rhythm is observed, which disappears in midpuberty in boys owing to a greater increase in GH secretion during the day than at night (66).

In growing boys, total daily GH secretion varied directly with the serum testosterone level. The strongest relationship to serum testosterone concentration existed for the mass of GH secreted per burst (67). During female puberty, physiological alterations of endogenous estrogen levels and/or the accelerated growth rate related positively to spontaneous GH concentrations, and to plasma IGF-I (68). The GH axis seems very sensitive to the stimulatory effect of estrogens since a rise in GH levels has been reported in girls before any signs of sexual development (69). Moreover, estrogen-associated increases in GH production are accompanied by rising plasma IGF-I concentrations, *i.e.* additional estrogen-dependent hypothalamo-pituitary mechanisms must operate to sustain amplified GH secretion with concurrently elevated plasma IGF-I levels in normal female puberty (65).

**Influence of estrogens on GH secretion**

There is ample evidence in the literature to suggest that estrogens play a major role in increased GH secretion in women compared with men. Faria *et al.* (70) found that, during the normal menstrual cycle, serum GH concentrations rose two-fold in the late
follicular phase and correlated positively with estradiol, whereas some other studies have shown no influence of the phase of the menstrual cycle on the 24-hour GH concentration (26, 71). Two early studies reported no effect on basal GH values whereas there was a rise in GH in the midcycle period in response to ambulation or exercise (72, 73). Similar results have been reported when arginine and insulin have been given near the time of ovulation (74, 75). In one study, no variation in the magnitude of GH response was detected throughout the normal menstrual cycle after GHRH injections (76), while in another greater stimulated GH release was seen in the late follicular phase (77). A recent investigation within the same menstrual cycle showed an increase in GH in the preovulatory phase parallel with a rise in estradiol levels. A concomitant increase in IGF-I values suggests a central stimulation of the GH/IGF-I axis (78). Superovulation treatment of infertile women stimulates serum GH concentrations up to 4-fold, while down-regulation of the gonadotropic axis reduces basal and GHRH-stimulated GH release (4). In the study by Ho et al. (27) in young and elderly men and women, serum concentrations of free estradiol correlated to mean GH concentration and pulse amplitude in both men and women.

Oral estrogens suppress the GH-dependent production of IGF-I in the liver, resulting in reduced IGF-I concentrations and negative feedback action on the GH axis. Administration with transdermal estrogens to serum 17β-estradiol (E2) levels within the premenopausal physiological range had no effect on GH or IGF-I levels (79, 80, 81). The effect on the liver seems to be dose-dependent, since higher doses of transdermal estrogens cause serum GH levels to rise with a concomitant lowering of IGF-I (82). Furthermore, in a study of postmenopausal women, oral estrogens decreased IGF-I levels, reduced lipid oxidation, increased fat mass, and reduced lean body mass compared with the transdermal route suggesting important clinical implications for postmenopausal health (83).

**Influence of androgens on GH secretion**

Studies in pubertal boys and girls demonstrated that aromatization of testosterone to estrogen in boys, or estrogen itself in girls, is the likely stimulus amplifying secretory activity of the GH axis in puberty (84, 85). On the other hand, treatment with testo
sterone or a nonaromatizable androgen to constitutionally delayed boys resulted in similar increases in GH production suggesting that androgens exert stimulatory effects on GH secretion via the androgen receptor (86). In contrast to the situation in pubertal boys, no correlation was found between mean 24-hour GH and testosterone concentrations in adult men (27), while in another study of aging men, declining levels of testosterone were suggested to contribute to the fall in GH secretion (28). Testosterone alone administered to GH deficient prepubertal boys failed to alter IGF-I concentrations while GH alone or in combination with testosterone increased the IGF-I levels. This indicates that a normal hypothalamic-somatotrope function is required for an increased production of IGF-I in response to androgens (87). Furthermore, there are both clinical and experimental investigations suggesting that androgens may potentiate the effect of GH (88-91).

**GH deficiency**

GH deficiency (GHD) in adulthood is accompanied by an increased abdominal fat mass, reduced lean body mass and bone mineral content, reduced exercise capacity, deranged lipoprotein and carbohydrate metabolism, reduced cardiac function, and decreased extracellular water content (92, 93). In addition, patients with hypopituitarism have increased mortality, in particular due to cardio- and cerebrovascular diseases, to which GHD may contribute (94-96). Furthermore, a three-fold increase in fracture frequency (97) and impaired quality of life (98) have been described in patients with GHD. Several studies in GH treated GHD patients have shown beneficial effects on body composition, metabolic abnormalities, general well-being, quality of life, bone turnover, and after long-term treatment also on bone mineral density and muscle strength (99-107).

**Acromegaly**

The delay from onset of symptoms to diagnosis in acromegaly often ranges between 5 and 15 years. In addition to the considerable morbidity associated with acromegaly, the mortality is two to four times that of the general population (108, 109). The major cause of death is usually cardiovascular disease, although increased mortality from
respiratory and malignant diseases also have been reported. GH excess results in typical acral and soft tissue changes, and the tumor mass causes local effects such as headache, visual impairment, and deficiency of other pituitary hormones. The cardiovascular disease includes hypertension, cardiac hypertrophy, cardiomyopathy, ischemic heart disease, congestion heart failure, and arrhythmias. Major metabolic effects are peripheral insulin resistance and impaired glucose tolerance. In addition, there are symptoms such as sweatings, fatigue, and joint pain (110). Successful treatment and normalization of GH levels in patients with acromegaly decrease the mortality rate to that of age- and sex-matched controls (111-113).

**Morning GH**

In 1965, Frantz & Rabkin (72) reported that women had six-fold higher GH concentrations than men in samples taken ambulatory in the morning. In contrast, almost no gender difference was noted when sera were drawn in the morning before any significant activity. When 25 mg diethylstilbestrol was given twice a day for four weeks to men, a marked rise in plasma growth hormone was seen in all the ambulatory specimens, while GH levels in samples taken at rest in the morning showed little if any elevation with estrogen.

As part of the training in clinical chemistry for medical students at Uppsala University Hospital, blood samples were obtained from ~120 students each year. The samples were taken on an ambulatory basis in the morning, after an overnight fast. The concentrations of various hormones were determined with methods used in routine at the Hormone laboratory, Department of Clinical Chemistry. When a more sensitive time-resolved sandwich fluoroimmunoassay (FIA), specific for the pituitary GH 22 kDa isoform, was introduced at the laboratory we found that the female students had up to 100-fold higher median GH concentrations in the morning than the male students. The highest values were found in women taking oral contraceptives (OCs). Such a large difference had previously not been documented for any hormone. In the study by Frantz & Rabkin (72), a less sensitive polyclonal competitive radioimmunoassay (RIA) was used. The detection limit of their assay was ~0.3 µg/L, and most of the values for men were below or close to that limit. With the noncompetitive FIA, all
men had measurable GH concentrations above the detection limit of 0.009 mIU/L (Fig 2). This made the large gender difference apparent. Except for the report of Frantz & Rabkin (72) no other reports were found in the literature where samples had been taken under similar conditions. The observations led to the present more thorough study on the impact of gender and sex steroids on ambulatory morning GH values.

**Fig 2.** GH analyzed with both the competitive radioimmunoassay (RIA; µg/L) and the non-competitive fluoroimmunoassay (FIA; mIU/L). Sera drawn from medical students in the ambulatory state in the morning after an overnight fast. The results in µg/L were converted to mIU/L using a factor of 2.

- men; O women; ▲ women taking OCs.
AIMS OF THE PRESENT INVESTIGATION

The aims of the present investigations were:

• to examine the impact of gender and age, on the serum levels of GH and other hormones, in samples taken in the ambulatory state in the morning after an overnight fast in healthy adults
• to investigate the effect of oral contraceptives (OCs) in healthy young women, and the effect of subcutaneous implants of 17β-estradiol in postmenopausal women, on serum GH concentrations in the ambulatory state in the morning after an overnight fast
• to examine the influence of ambulation vs. rest, on serum GH concentrations in the morning after overnight fasting in healthy young men, women, and women taking OCs
• to study the influence of gender and OCs on GH and epinephrine secretion during a 24-hour period in healthy young adults
• to investigate an influence of gender on the effects of short-term administration of GH on serum IGF-I and other key metabolic parameters in healthy young men, women, and women taking OCs
• to assess an influence of gender on the effects of long-term treatment with GH on bone metabolism and bone mineral density in men and women with GH deficiency
• to investigate an influence of gender on the effects of treatment with octreotide on serum GH and IGF-I concentrations in men and women with acromegaly
MATERIALS AND METHODS

Study subjects and study design

Paper I
This prospective study included 291 medical students divided into six groups according to gender, age, and the use of hormonal contraceptives. In the younger age interval, samples were obtained from 125 male students and 75 female students, 21-26 years old, and in the higher age interval from 25 male students and 25 age-matched female students, 27-43 years old. Female students using two different kinds of OCs were also investigated; 19 women taking OCs with ethinylestradiol (EE) and levonorgestrel (21-26 years), and 22 women taking OCs with EE and desogestrel (21-24 years). Sera were taken in the morning when the subjects came to the hospital, in the ambulatory state and after an overnight fast, and were analyzed for 12 different hormones and SHBG. In the women, the samples were taken at random in the menstrual cycle.

Paper II
Healthy medical student volunteers (20-29 years old) were included in the study - 7 men, 7 women with normal menstrual cycles, and 7 women taking OCs. All were non-smokers. Serum samples were drawn and analyzed for GH, in the ambulatory state in the morning after an overnight fast, when the subjects came to the hospital. They returned at 18:00 h for the beginning of a 24-hour GH profile. Samples were then taken every second hour. The students remained in bed during the night until sera were taken at 08:00 h the next morning in the fasting state. During the day, they were free to walk around. Urine was collected in 4-hour periods for the assay of epinephrine and norepinephrine. In the women, samples were taken at random in the menstrual cycle.
Paper III
This study included healthy medical students (59 men aged 21-34 and 25 women aged 21-39), male volunteers from the Uppsala county (99 middle-aged men aged 41-59, and 96 elderly men aged 60-75), and postmenopausal women (19 women, aged 51-79) treated with subcutaneous implants of 20 mg E₂ every 6 months after a preceding hysterectomy, and 15 apparently healthy women without estrogen replacement therapy. Serum samples for analysis of GH, SHBG, E₂, and, in the men, also testosterone, were obtained in the morning when the subjects came to the outpatient clinic after an overnight fast. In the younger women, the samples were taken at random in the menstrual cycle, and in the E₂-treated women, at random between 1 and 5 months after the implantation.

Paper IV
Three groups of healthy medical students (20-30 years old) were included in an open, prospective 2-week study; 6 men, 6 women with normal menstrual cycles, and 6 women taking OCs. The subjects came to the hospital in the ambulatory state in the morning after an overnight fast. The first day (day 0), serum and second-void morning urine were sampled for assay of hormones, lipids, and biochemical bone markers. The blood and urine collections were repeated on days 3, 7, 10, and 14. rhGH (Norditropin®, Novo-Nordisk Pharma, Copenhagen, Denmark) was administered subcutaneously by the students themselves every evening before bedtime. The daily dose during the first week (days 0-6) was 1 IU m⁻² body surface and during the second week (days 7-13) 3 IU m⁻². The mean doses were 2.0 ± 0 and 6.2 ± 0.3 IU for the men, 1.7 ± 0.3 and 5.4 ± 0.5 IU for the women, and 1.6 ± 0.2 and 5.1 ± 0.2 IU in the women taking OCs, respectively. In the women, samples were taken at random in the menstrual cycle.

Paper V
Twenty-one men (mean age 45 ± 7 years) and 15 women (mean age 47 ± 7 years) with GHD participated in a placebo-controlled trial, randomized to either rhGH (Norditropin®, Novo Nordisk A/S, Copenhagen, Denmark) or placebo for 9 months, and after
3 months of wash-out the other treatment was given for an additional 9 months. In all but two patients, hypopituitarism was acquired in adulthood. Eight women were on estrogen replacement therapy (n = 4 oral, n = 4 transdermal) and all men were receiving testosterone. Thirty-three of the patients continued in an open study with GH treatment for up to a total of 45 months. At the end of the placebo-controlled part of the study the dose was adjusted according to side-effects and to maintain the serum concentrations of IGF-I within the normal age-related reference interval. Measurements of bone mineral density (BMD) and bone mineral content (BMC) of the total body were performed at baseline, after the first 9 months of treatment with GH or placebo, after 3 months of wash-out, and after the next 9 months with GH or placebo. Thereafter, bone mineral measurements of the total body, lumbar spine, and hip were carried out every 6 months up to a total of 33 months, and thereafter again after 45 months of GH therapy. At the same time-points, serum and urine samples were collected after an overnight fast.

**Paper VI**

In the study of short-acting octreotide (Sandostatin®, Novartis Pharma AG, Basel, Switzerland), 100 µg injections were given twice daily in 21 men and 15 women (mean age 53.5 years; 26-78 and 54.3 years; 22-74, respectively). Two men had undergone surgery alone, and 4 men and 1 woman had been treated with surgery and radiation therapy. Three of the men and none of the women had sex hormone replacement therapy. Mean levels of serum GH 1, 3, 5, and 7 hours after the injection on the third day of treatment were compared to the GH values prior to the injection in the morning. The mean suppression (%) was then calculated. Median GH levels did not differ between men and women prior to treatment.

Thirteen men and 12 women (mean age 56.2 years; 34-78 and 63.8 years; 28-81) were included in a follow-up study with slow-release injections of octreotide (Sandostatin LAR®, Novartis Pharma AG, Basel, Switzerland). Among the males, 2 had been treated with surgery alone, 4 with surgery and radiation therapy, and 5 with radiation therapy alone. Among the females, 1 had undergone surgery, and 1 surgery and radiation therapy. Seven men were on testosterone replacement therapy and 4 women had
oral estrogen replacement therapy. The patients were switched from ongoing treatment with short-acting octreotide to intramuscular injections every fourth week. Doses were adjusted to age-matched reference intervals for IGF-I. A 4-hour GH profile and morning IGF-I were taken at the onset and after 3, 9, 15, and 21 months of treatment. Samples for IGF-I were also taken prior the monthly injections of octreotide.

Methods

Growth hormone in 50 µL serum (S-GH 22 kDa) was measured with a non-competitive sandwich time-resolved FIA (AutoDELFIA™ hGH kit, Wallac Oy, Finland) specific for the pituitary 22 kDa GH isoform. The results were expressed in mIU/L, using the 1st international reference preparation of GH (80/505) as a reference standard. The minimum detection limit was 0.009 mIU/L. The within- and between-assay CVs were 1.1% and 2.3%, respectively (Paper I-IV, VI).

Serum GH was assayed by a RIA, using polyclonal antibodies. The lowest level of detection was 0.3 µg/L and intra- and interassay variations were below 8% (Paper V, VI). The results were converted to mIU/L using a factor of 2 (Paper VI).

Serum IGF-I was measured with a commercial RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA) after extraction of binding proteins with acid ethanol (Paper I, IV, V).

Serum IGF-I was measured by a non-extraction IGF-I immunoradiometric assay (IRMA), (Nichols Institute Diagnostics, San Juan Capistrano, CA) using two region-restricted affinity purified polyclonal antibodies (Paper VI).

The serum concentrations of insulin, cortisol, E2, free thyroxine, triiodothyronine, and testosterone were measured with competitive immunoassays (Pharmacia Insulin RIA, Pharmacia Diagnostika, Uppsala, Sweden; AutoDELFIA Cortisol kit, AutoDELFIA Estradiol kit, AutoDELFIA FreeThyroxin [FT4] kit, and AutoDELFIA Triiodothyronine [T3] kit from Wallac Oy; and Coat-A-Count® Total Testosterone kit from Diagnostic Products Corporation).
The serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone, prolactin, SHBG, and parathyroid hormone (PTH) were measured with noncompetitive sandwich immunoassays (AutoDELFIA hFSH kit, AutoDELFIA hLH Spec kit, AutoDELFIA hTSH Ultra kit, AutoDELFIA Prolactin kit, and AutoDELFIA SHBG kit from Wallac Oy; INTACT PTH kit from Nichols Institute Diagnostics).

Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were measured in serum using routine methods at the Department of Clinical Chemistry, University Hospital, Uppsala. Low-density lipoprotein (LDL) cholesterol concentrations were calculated according to the formula suggested by Friedewald et al. (114).

Osteocalcin in serum was determined by RIA (CIS Biointernational, Oris Industries, Gif-Sur-Yvette, France). Serum concentrations of carboxy-terminal crosslinked telopeptide of type I collagen (ICTP) and carboxy-terminal propeptide of type I procollagen (PICP) were measured by commercially available RIAs (Orion Diagnostica, Espoo, Finland). Bone-specific alkaline phosphatase (bALP) activity in serum was calculated from measurements of the enzyme activity in untreated serum and after extraction with wheat germ lectin (Boehringer Mannheim, Mannheim, Germany).

Deoxypyridinoline (Dpyr) was measured in urine with a competitive ELISA (Pyrilinks-D™, Metra Biosystems, Mountain View, CA, USA). Creatinine was measured in urine by a routine method at the Department of Clinical Chemistry, Uppsala, Sweden.

The urinary content of epinephrine and norepinephrine were determined by HPLC (115, 116).

BMD and BMC were determined by dual energy x-ray absorptiometry (DXA; DPX-L equipment from Lunar Corp., Madison, WI) of the total body, the lumbar spine, and the femoral neck. The area of the femoral neck within a box of 12 x 50 mm and the area of the L2-L4 segment were also determined.
Statistics
The median and the 2.5th and 97.5th percentiles of the hormone concentrations were calculated for each group of individuals. The Mann-Whitney nonparametric test was used to calculate the significance of differences between groups (Paper I, III, VI).

Correction of P-values for comparisons between the groups was done according to the Bonferroni method (Paper III).

Correlations between age and serum concentrations of analytes and, after adjustments for age, between GH and other analytes were calculated using Spearman rank correlation test. The adjustment for age was made in a linear regression model after log transformation of the values (Paper III).

All serum values for hormones and hormone-related proteins were transformed into logarithms before analysis and were presented as geometric means (± SD) (Paper II, IV).

The urine values for epinephrine and norepinephrine (Paper II), the serum values for lipids and serum and urine values for bone markers (Paper IV), the serum values for IGF-I and bone markers, and changes in bone mass (Paper V) were presented as the means (± SD), and the suppression of GH and serum values for GH and IGF-I were presented as the means (± SEM) (Paper VI).

When the ANOVA factorial overall test was found to be significant (P<0.05) for differences amongst groups, unpaired two-tailed Student’s t-test was used as a post-hoc test (Paper II, IV).

Statistical comparisons within the same group were made on paired observations, using the two-tailed Student’s t-test (Paper II, IV, V).

Unpaired two-tailed Student’s t-test was used to calculate the significance of differences between groups (Paper V, VI).
RESULTS AND COMMENTS

Influence of gender and oral contraceptives (OCs) on ambulatory morning GH levels, and on GH and epinephrine secretion during a 24-hour period

Influence of gender on ambulatory morning GH levels in healthy young adults (Paper I and II)

In the young medical students (Paper I), aged 21-26, the median morning GH value was 80 times higher in the women than in the men (14.4 mIU/L and 0.18 mIU/L, respectively) when samples were taken in the ambulatory state after an overnight fast (Fig 3). Corresponding median IGF-I values were 322 µg/L and 290 µg/L ($P<0.001$). In the group of older medical students, aged 27-43, the gender difference was 68-fold (Fig 3) (Table 2 in Paper I). The median IGF-I values did not differ and were lower than in the younger men and women ($P<0.001$).

In the 24-hour study among 21 medical students (Paper II) the gender difference was 28-fold in the ambulatory state in the morning and 4.6-fold in the supine state. In the men, the values were threefold lower in the ambulatory state compared with the resting state, whereas in both groups of women they were twofold higher, i.e. the levels in men and women changed in opposite directions (Table 1 in Paper II).

Comments

The most striking finding in these two studies in young adults is the influence of ambulation on morning GH values. The 80-fold gender difference in GH values (Paper I) in the morning was larger than those of the classical sex steroids testosterone (male/female ratio 14.4) and $E_2$ (female/male ratio 2.2). Few previous studies have specifically studied morning GH levels. In the study by Frantz & Rabkin (72) women had six-fold higher GH concentrations than men (ages 20-80) in the ambulatory state in the morning when a competitive RIA was used. In a more recent study by Chapman et al. (117) the subjects (ages 18-34) came to the hospital in the morning, and the blood sampling started after 1 hour of rest. A sensitive chemiluminescence assay was used. The gender ratio of the baseline mean GH values was 20. After a glucose toler
ance test lower nadir GH concentrations were noted in the men. Since the fractional decline in mean GH levels was equivalent in men and women it was suggested that the lower GH levels in men after glucose ingestion were due to lower baseline values and not to a greater suppressive effect of glucose.

Fig 3. Distribution of GH concentrations (mIU/L) in sera, drawn in the ambulatory state in the morning after an overnight fast. The subjects were 291 medical students; 125 men and 75 women, aged 21-26 years; 19 women taking EE and levonorgestrel OCs (EELEV); 22 women taking EE and desogestrel OCs (EEDES); and 25 men and 25 women, aged 27-43 years.
Admission to hospital on the morning of an insulin tolerance test, has recently been described to reduce the GH response in healthy adults compared to an overnight hospital stay (118). The results were not separated by gender, and more men than women were included (13 out of 19) which might explain that lower responses were observed.

In the 24-hour study (Paper II) the larger gender difference in GH values in the ambulatory state compared to the resting state, was due to an increase in the women and a decrease in the men. The decrease in GH levels in the men was surprising since physical activity is generally considered to increase GH secretion in men (13, 20-22, 119). However, in the investigation of men, by Sotsky et al. (119), GH increased only at the most intense exercise level. Taken together, young men and women seem to differ in their GH response to physical activity.

**Influence of gender and (OCs) on GH levels during a 24-hour period in healthy young adults (Paper II)**

During the day the women had 7-fold higher GH concentrations than the men while the difference was 4.6-fold during the 24-hour study period. At night there was no significant difference between the sexes. The women taking OCs had slightly, but not significantly higher GH levels than the women not taking OCs (Fig 4). The mean maximum values differed by two-fold and the mean nadir values differed by three-fold between men and women (Table 1 in Paper II). Figure 5 shows the diurnal pattern in men, women and women taking OCs.

**Comments**

In the 24-hour study (Paper II), we found a larger gender difference in GH values during the day (7-fold), and over 24 hours (4.6-fold), than earlier reported. In studies from other laboratories in which samples were taken every 10 to 20 min over a 24-hour period, the gender ratio was 1.5, 2.9 and 2.2, respectively (61, 63, 64). In those studies the subjects were generally older, and they came to the hospital the day before the study. In our study most of the students were walking or bicycling directly to the hospital for the first sampling, and in daytime samples were taken during rou
tine daily activity. The large gender difference that we observed during the day has recently been confirmed in a study in 15 young men and women, who entered the hospital on the day of the test (16). Samples for analysis of GH were taken every 30 min. The gender difference was 10-fold in the morning hours (8 to 12 am) accounting for most of the differences between sexes.

![Graph showing GH concentrations in men, women, and women taking OCs](image)

**Fig 4.** GH concentrations (mIU/L, geometric mean ± SE) in sera drawn at 2-hour intervals from seven men, seven women, and seven women taking OCs during a 24-hour period.

- □ men; ○ women; ● women taking OCs.

At night, the men had nearly 8 times higher GH concentrations than during the day, while the values in the women were only about two-fold higher. As a result, no significant gender difference was found (Paper II). This is in accordance with the findings of van den Berg *et al.* (64) of an increased nocturnal GH-secretory release that was larger in men than in women.
Fig 5. GH concentrations (mIU/L) in sera drawn at 2-hour intervals from seven men, seven women, and seven women taking OCs during a 24-hour period.
The young age of the students might contribute to the large gender difference in morning GH values since the difference was larger in the younger (80-fold) than in the older (68-fold) students (Paper I). The gender difference in IGF-I values in the youngest students (Paper I) was probably also due to the young age of the subjects since higher IGF-I values have been shown in adolescent females than in males (36, 37) whereas in adult men and women IGF-I concentrations are similar (36, 38).

**Influence of oral contraceptives on morning GH and IGF-I levels (Paper I and II)**

The median morning GH level was 117 times higher in women taking OCs with EE and levonorgestrel and 125 times higher in women taking OCs with EE and desogestrel, than in young men of similar age (Paper I) (Fig 3). The median GH values were significantly higher than in the group of women not taking OCs (21.0 mIU/L; \(P<0.05\), and 22.5 mIU/L; \(P<0.01\), respectively, vs 14.4 mIU/L). Corresponding IGF-I values were 305 \(\mu g/L\) and 280 \(\mu g/L\), significantly lower \((P<0.01)\) in the desogestrel-group compared to the women not taking OCs (322 \(\mu g/L\)).

**Comments**

The influence of OCs on GH levels has previously not been explored. In the study by Frantz & Rabkin (72), diethylstilbestrol was given in pharmacological doses to men which resulted in GH concentrations similar to those in women. Several more recent reports have described that estradiol given orally to postmenopausal women increases GH levels. This has been explained by decreased IGF-I production in the liver which through negative feed back will increase the GH secretion from the pituitary (79, 80).

In the present study in Paper I, the drug containing desogestrel had a larger impact on GH and IGF-I values (as well as on concentrations of SHBG, E₂, FSH, LH; Table 1 in Paper I) than the drug containing levonorgestrel, which has been reported to have a weaker androgen effect (120). These findings suggest that intake of ethinylestradiol together with progestogens contributes to the higher GH concentrations in these women.
**Urinary epinephrine and norepinephrine during 24 hours (Paper II)**

The men had a higher output of epinephrine than the women during the day whereas there was no gender difference at night. Over 24 hours there was a tendency toward higher outputs among the males (Table 2 in Paper II). The women taking OCs had significantly lower ($P<0.05$) epinephrine outputs than the women not taking OCs both in the daytime and over 24 hours while the amounts were similar at night (Table 2 in Paper II). Regarding the output of urinary norepinephrine, there was no difference between the three groups.

**Comments**

Higher urinary epinephrine outputs during the day were found in the men than in the women (Paper II). Higher concentrations of plasma epinephrine in men than in women have been reported in response to physical activity (20, 22, 121) and to hypoglycemia (17, 122, 123). In accordance with our findings, larger amounts of epinephrine have previously been found in men, both in urine (124, 125) and in plasma (126) over a 24-hour period. $\beta$-adrenergic agonists have been reported to inhibit the GH response to exercise in adult patients with asthmatic bronchitis (127), and to abolish the GH response to GHRH in normal women (128). Similarly, administration of a $\beta$-blocker has been found to enhance the GH response to different stimuli in both men and women (129-131). Somatostatin release has been suggested to be inhibited by $\beta$-adrenergic blockade (130, 131) and increased by $\beta$-adrenergic receptor activation in the brain (132). Therefore, increased epinephrine output during the day might cause an inhibition of GH release via somatostatin release. GH and epinephrine act in synergy to increase lipolysis. The finding of a reciprocal relationship between GH and epinephrine during the day (higher epinephrine output and lower GH secretion in the men than in the women, Paper II) suggests a gender difference in the utilization of substrates for energy production. This is supported by recent studies of young adults during exercise, in which women derived more energy from fat oxidation and less from carbohydrate oxidation than did men (22, 133). The differences in fuel oxidation were associated with differences in the catecholamine response to exercise in that men.
had a greater elevation than women in both epinephrine and norepinephrine levels. It was speculated that women have a greater priority for carbohydrate conservation than men under conditions of increased energy demand.

The even lower output of epinephrine in the women taking OCs in our study was a new finding. Recent studies have shown unchanged epinephrine levels in OC users (134, 135). In postmenopausal women treated with transdermal estrogen unchanged levels were reported in one study (136), while attenuated epinephrine responses to mental stress were observed in another (137). Larger increases in plasma epinephrine in response to stress have been shown in postmenopausal women compared to premenopausal women (138), and in patients who had undergone bilateral salpingo-oophorectomy higher levels of epinephrine were seen than in women who had undergone hysterectomy only (139). Together with our own results, the data point to an attenuating effect of female sex steroids on epinephrine secretion in women.

Influence of age and gender on ambulatory morning GH levels in healthy adults, and the effect of estradiol on morning GH levels in postmenopausal women (Paper III)

Effects of age and sex on ambulatory morning GH values
The median morning GH level was 102 times higher ($P<0.0001$) in the young women than in the young men (16.4 mIU/L vs. 0.16 mIU/L). In the postmenopausal women, the median GH level was 12-fold higher ($P<0.0001$) than in the elderly men (4.30 mIU/L vs. 0.36 mIU/L) (Fig 1 in Paper III). This was due to an increase in GH values among the elderly men compared to the young and middle-aged men ($P<0.001$), and a decrease among the postmenopausal women compared to the younger ones ($P<0.01$) (Table I in Paper III).

The median concentrations of testosterone were identical in the three groups of men. The SHBG concentration increased and the values for free androgen index decreased with age (Table I in Paper III).
The morning GH values significantly ($r_s = 0.35; P<0.0001$) increased with age in men, aged 41-75 (Fig 6). After adjustment for age, an inverse correlation was found between the levels of GH and free androgen index ($P<0.05$) and a direct correlation between GH and SHBG ($P<0.05$) while no significant correlation was observed between the levels of GH and $E_2$ or total testosterone (Table II in Paper III).

Fig 6. Individual levels of GH in sera drawn in the morning after an overnight fast plotted in relation to age of 195 men aged 41-75 years. The linear regression line is indicated. The increase with age was highly significant (Spearman $r = 0.35; P<0.0001$).

Comments
The finding in this study of an increase in morning GH levels with age in men, contrasts to reports of decreased GH secretion over 24 hours (23-29). In the women, the morning GH values decreased with age. Thus, the marked gender difference was reduced with age from over 100-fold in young adults to about 12-fold in the elderly. Gender differences in 24 hour GH concentration have been reported to be five-fold in
young individuals (140), three-fold around the age of 40 (64), and two-fold in elderly men and women (141). Obviously, the large gender difference in morning GH values is not representative of 24 hour GH secretions.

The inverse correlation between free androgen index and morning GH in the men, values adjusted for age, is in contrast with earlier reports over 24-hour GH secretion in men, where close relations have been found between decreased GH secretion and declining levels of testosterone with aging (28, 32). A positive correlation has also been shown between serum testosterone and both the mass and amplitude of the GH secretory burst over 24 hours (24). The higher total estrogen values observed in the elderly men could be due to increased SHBG levels and to increased peripheral conversion of testosterone to estrogens (142, 143). It was recently reported in a study including more than 500 men (aged 20-80) that total serum levels of testosterone, E2, and IGF-I declined with age from the third decade, whereas SHBG levels increased from the sixth decade, resulting in an accentuated decline in bioavailable amounts of testosterone and E2 (144). The authors observed an age-independent and positive effect of BMI on serum levels of E2. Higher urinary outputs of epinephrine were reported in men than in women (Paper II), and lower epinephrine secretion has been shown in elderly individuals than in younger ones (125). One could speculate that both the change in the androgen/estrogen ratio and a reduced secretion of epinephrine and thereby less somatostatin release, may contribute to the elevated morning GH values in elderly men.

Changes in body composition occur with aging, such as a decrease in lean body mass and increases in total body fat, abdominal fat, and waist/hip ratios (145). A recent study by Vahl et al. (33) reported visceral adiposity to be the major determinant of GH secretion in healthy nonobese adults. Others have described that age and BMI each correlate negatively with secretion GH (24), and that an age-related increase in obesity could be a cause as well as a consequence of diminished GH secretion (28). It is likely, that changes in body composition as well as other, age-dependent factors, aside from sex-hormones, will influence morning GH levels in elderly men.
Effect of $E_2$ treatment on morning GH levels in postmenopausal women

The median GH values were slightly lower in the $E_2$-treated than in the control group of women (median values 2.80 mIU/L and 4.30 mIU/L, respectively) (Fig 3 in Paper III).

The treatment with $E_2$ implants raised the $E_2$ level 13-fold compared to that in the control group (median levels 464 and 35.5 pmol/L, respectively). The SHBG concentrations in the $E_2$-treated postmenopausal women were similar to those in the untreated control group and in the group of young women (Table 1 in Paper III).

Comments

Despite $E_2$ levels similar to those in the mid-luteal phase of the cycle, we did not find any difference in GH concentrations in the $E_2$ treated women compared to the control group of women. This treatment has been shown to counteract effectively both the qualitative and quantitative changes in the gonadotrophins occurring at the menopause (146). Studies of transdermal administration of estrogen to postmenopausal women, at $E_2$ levels comparable to those in the present study, were found not to influence the GH secretion (79, 81) while larger doses decrease IGF-I and increase both basal and 24-hour GH secretion (82). The findings in the present study indicate that the decrease with age in the morning GH levels in the women is not a direct effect of estrogen alone.

Influence of gender on the effects of short-term administration of GH in healthy young men, women, and women taking OCs (Paper IV)

Influence of GH administration on hormones and SHBG

In the present study there was no gender difference in IGF-I concentrations prior to treatment. The IGF-I levels increased during the first week both in men and in women not taking OCs. The increase in IGF-I levels from baseline to day 14 was 86% in the men, 52% in the women without OCs and 16% in the women taking OCs (Fig 7a). The testosterone concentrations did not change in any of the groups, whereas the SHBG level decreased in the men, resulting in an increase of borderline significance.
in the testosterone/SHBG ratio at day 14 ($P = 0.06$). After the first week, the insulin level had increased in the men and in the women without OCs. From baseline to day 14 the increase was 122% in the men, 111% in the women not taking OCs, and 47% in the women taking OCs (Fig 7b) (Table 1 in Paper IV).

**Influence of GH administration on lipids**

Upon GH administration, the total cholesterol (Fig 7c) and LDL cholesterol levels decreased in the men which resulted in a decrease in the LDL/HDL ratio (Fig 7d). The concentration of triglycerides increased in the men and in the women without OCs after 2 weeks of treatment (Table 2 in Paper IV).

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**Fig 7.** Changes in serum levels of IGF-I (a), insulin (b), total cholesterol (c), and in LDL/HDL ratios (d) from baseline (0% at day 0) to day 3, 7, 10, and 14 during 2 weeks of GH administration in six men, six women, and six women taking OCs.

- men; O women; ● women taking OCs.

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ at day 7 or 14 vs. baseline.
**Influence on markers of bone formation**

At baseline, the concentrations of osteocalcin and bALP were lower in the OC women than in the women without OCs. When GH was given, osteocalcin increased both in men and in women without OCs (Table 3 in Paper IV). The serum concentration of bALP decreased in the men, and that of PICP increased both in men and women without OCs.

**Influence on markers of bone resorption**

At baseline, the concentrations of ICTP were lower in the women taking OCs than in the women not taking OCs. Upon GH administration ICTP values increased in the men and in the women taking OCs, while the U-Dpyr/cr ratio increased in the men only (Table 3 in Paper IV).

**Comments**

In the present study in young adults, we observed a clear gender difference in the response to short-term administration of GH, with the largest effect in the men and the smallest in the women taking OCs. At the time of our study, there were few reports addressing gender differences in response to GH administration in healthy adults. In one study, after a single dose of GH (0.1 mg/kg), both peak levels in IGF-I and the change in IGF-I measured after 24 hours were higher in young men than in young women (147). In another study, no effect on either IGF-I or insulin over a period of 5 hours was found after a single GH pulse (200 µg), whereas more marked lipolysis was seen in the men (46). Our results were recently supported by the GH-2000 study group (148) which investigated 99 healthy young subjects divided into 3 groups (GH doses 0.1 IU/kg or 0.2 IU/kg, or placebo) in a 4 week double-blind, placebo-controlling trial. IGF-I increased in the men with no significant difference between the two doses, and in the females, a lower absolute IGF-I response was found than in the men. In line with the present data, a relative GH resistance in women has recently received support from a study in which the minimum GH dose needed to elicit an IGF-I response was higher in women than in men (5.0 µg/kg and 2.5 µg/kg, respectively) (149).
In the GH-2000 study, osteocalcin and PICP increased in both men and women as well (150). The men had higher responses in ICTP, which confirms our findings. We did not find a gender difference in ostrocalcin and PICP at baseline in contrast to the GH-2000 study, in which higher levels in the men were found. This could be due to the smaller number in our study.

The increase in triglycerides that we observed in healthy young adults differ from the results seen in GHD patients, in whom no effect of GH therapy on triglycerides has been reported (101, 151), whereas others have observed increases in triglyceride concentrations after one week of GH administration in non-GHD adults (152, 153). Probably, a steady-state situation had occurred in the GHD patients. Alternatively, similar to the situation in acromegaly, GH administration in healthy subjects is likely to confer a state of insulin resistance known to be accompanied by hypertriglyceridemia, whereas in patients with GHD a replacement dose of GH merely elevates insulin from low to normal levels. The decrease in total cholesterol and LDL cholesterol levels that we noted in the men was also seen in other studies (101, 105, 151).

The inhibitory influence of contraceptives in the present study underlines the role of sex steroids in modulating the susceptibility to GH. No study had investigated the effects of GH administration in healthy women taking OCS. In postmenopausal women on oestrogen replacement therapy a single GH pulse increased the IGF-I level less than in young women in the follicular phase of the menstrual cycle. The largest effect was seen in older women without oestrogen therapy (147). Amongst healthy, elderly women treated with GH for 6 months, smaller increases in IGF-1 and osteocalcin were found in women treated with oestrogen than in those without (154).

A striking finding was the lower levels of osteocalcin, bALP, and ICTP prior to treatment in the women taking OCS compared to the women without OCS. Conclusive data regarding the effects of OCS on bone metabolism and bone density in eugonadal women are lacking. It has been suggested that OCS have no significant effects on bone metabolism during the childbearing years but may be beneficial in inhibiting the activation of bone turnover in pre- and postmenopausal women and in women with ovulatory disturbances (155, 156).
Influence of gender on the effects of long-term treatment with GH on bone and bone metabolism in men and women with GH deficiency (Paper V)

**Gender differences in response to GH treatment**

During the open study phase the dose of GH was adjusted according to side-effects and to maintain IGF-I within the age-related reference interval, which resulted in doses almost twice as high in the women compared to the men (1.9 ± 1.1 U vs. 1.0 ± 0.6 U) after 33 months of treatment (Fig 8). The increase in IGF-I compared to baseline was then similar in men and women.

![Graph showing changes in dose of GH, serum levels of IGF-I, and osteocalcin during the study period](image)

**Fig 8.** Changes in dose of GH (*top*), in serum levels of IGF-I (*middle*), and osteocalcin (*bottom*) during the study period in men (*open circles*) and women (*filled squares*) with GHD, compared to pretreatment values. *P<0.05, **P<0.01 (significant difference between genders).*  
*Symbols and vertical bars* denote the mean ± SEM.

The serum concentrations of osteocalcin increased more in the men during the first part of the study, but to the same extent in men and women when adjusted GH doses were used (Fig 8). The serum markers of bone formation (PICP) and bone
resorption (ICTP) also increased in a similar way during the open study phase (data not shown).

In the entire group of patients, total body BMC increased after 45 months of treatment compared to baseline. BMD, BMC, and the area of the femoral neck increased from 15 to 45 months and BMD, BMC, and the area of the L2-L4 vertebrae were increased after 33 and 45 months compared to the values at 15 months. Separated for gender after 33 months of treatment, i.e. 24 months after individual dose adjustments, it was found that the total body BMC, femoral neck BMD and BMC, and spine BMC had increased significantly in the men only.

Comments

In the patients of the present study, the basal GH secretion, as well as the peak GH response to provocative stimuli did not differ between the sexes. In spite of this the men had higher IGF-I levels than the women prior to treatment, indicating a greater responsiveness to GH in the periphery among men. When these patients had been treated with a similar dose of GH (corrected for body surface area) during 9 months, men had shown a more marked response with respect to IGF-I, body composition, lipids and lipoproteins and serum markers of bone metabolism (105). The present study shows that women need approximately two times higher doses than men to obtain similar peripheral effects, as reflected in a similar increase in serum IGF-I and in osteocalcin.

In another study in GHD patients treated with GH for two years (104) a more marked increase in women than in men was reported for total body BMD. This finding could be questioned since total body BMD was not significantly altered from baseline, and the difference seems to be due mainly to an initial reduction in total body BMD in the male patients. Our results have recently been confirmed in a study in 44 men and 27 women with GHD where responses to GH treatment on the BMD in the lumbar spine, femoral neck and trochanter were more marked in men than in women after 42 months of treatment (107).
The effects of GH on bone and cartilage may be due to a direct effect of GH or may be mediated by GH-induced local production of IGF-I or by circulating IGF-I (157, 158). It is unknown whether there also are gender differences in the bone in response to locally produced IGF, or to circulating IGF-I.

Clinical investigations suggest that androgens may potentiate the effect of GH (88, 90). In experimental studies in castrated rabbits, the expression of GH receptor messenger ribonucleic acid in both the liver and the growth plate has been shown to be increased by testosterone and decreased by estradiol (89). This could be a mechanism responsible for the gender difference in IGF-I response to GH treatment and could be of relevance for the greater gain in bone mass in the GHD men. On the other hand, other animal studies have shown discrepant results (159-162) which could be due to tissue or species differences.

We did not detect any influence of estrogen replacement on the response to GH treatment. However, only eight women (n = 4, oral; and n = 4, transdermal) were on estrogen therapy, whereas all men were on testosterone replacement therapy. Similar to us, Johannsson et al (163) did not observe an influence of sex hormone replacement therapy. In contrast, others (164-166) have all reported that peroral estrogens reduce IGF-I and, accordingly, is accompanied by a need for higher GH doses in GHD women. Whether this also is true for the effects on bone seems likely but remains to be established.

**Influence of gender in response to treatment with octreotide in men and women with acromegaly (Paper VI)**

**Effect of short-acting octreotide on GH levels**

The median GH value decreased from 13.2 mIU/L (3.3-420) to 4.5 mIU/L (0.4-69.5) (mean of 1-7 hour GH values) after injection of 100 µg octreotide in the male patients and corresponding values in the female patients were 14.6 mIU/L (3.8-192) and 3.9 mIU/L (0.5-39.0), respectively. The mean suppression was 67% (± 3.5%) in the men and 65% (± 5.2) in the women.
**Effect of slow-release formulation of octreotide on IGF-I levels**

There was no gender difference in mean GH levels at the onset (when switching from ongoing daily octreotide injections to monthly injections) or after 3, 9, 15, and 21 months. In contrast, throughout treatment the mean IGF-I values were significantly higher in the men which resulted in lower GH/IGF-I ratios (Fig 9). Similar doses of octreotide were given to men and women during the study (Table 1 in Paper VI).

![Figure 9](image)

**Fig 9.** Serum GH (mIU/L) and IGF-I (µg/L) levels in 11 men (open circles) and 12 women (filled circles) with acromegaly after 21 months of treatment with long-acting octreotide.

**Comments**

Few studies have investigated gender differences in acromegaly. The sellar volume was reported to be greater in men with acromegaly (167), while others have reported no difference in tumor size between men and women (168). In the study by Jadresic et al (167) no difference in GH levels at diagnosis was observed, while higher IGF-I values were found in the men in a study of 22 patients by Maffei et al (169). In this study the men also had more marked electrocardiogram abnormalities which was not found in the report by Jadresic et al (167).
In our study, we found a similar suppression of GH levels in men and women in response to short-acting octreotide. As there was no gender difference in GH levels prior to treatment we concluded that the sex difference in response to octreotide is not at the level of the pituitary, but rather is explained by an increased peripheral response to GH in the males. One previous report has shown that the GH release was more suppressed in reaction to octreotide in elderly men (170) whereas in a recent study no difference was seen in men and women treated with lanreotide (171).

In the follow-up study of patients on long-term treatment with octreotide, the men continued to have higher IGF-I values than women despite equal doses of the drug. This is a new observation with clinical implications and indicates that, if dosing is based on GH levels, there is a need for gender related normative data, and that analyses of serum IGF-I should be used in addition to GH measurements in the management of acromegaly. The finding also underlines, that similar to the situation in GHD patients, gender should be considered in the treatment of acromegaly with somatostatin analogues, and that men with acromegaly will need higher doses than women to normalize IGF-I.

An early study in acromegaly reported beneficial effects of estrogen treatment, indicating a role of sex steroids in regulating the peripheral response to GH. When GH was administered to acromegalic patients, serum concentrations of bioassayable somatomedin were reduced, and the clinical status was improved without decreasing GH concentrations (172). In the large cohort of acromegalic patients (Parkinson, Abstract P433, 11th International Congress of Endocrinology, Sydney, 2000) lower IGF-I values were seen in women on estrogen replacement therapy. This could be due to the well known effect of oral estrogens in reducing the hepatic production of IGF-I described elsewhere in this summary. In our patients, we did not see any difference in response to octreotide between women with and without estrogens. This was probably due to the small numbers of women on estrogen replacement therapy and the fact that both oral and transdermal estrogens were given.
GENERAL SUMMARY

The median GH value was found to be 80- to 100-fold higher in young women than in young men in sera drawn in the ambulatory state in the morning after an overnight fast. Women taking oral contraceptives (OCs) had higher GH values than women not on OCs. This suggests that intake of ethinyl estradiol together with progestogens contributes to the higher GH concentrations in these women.

Ambulation in the morning was found to influence fasting GH values in different ways in young men and women; in the men, the values were lower compared to the resting state, whereas they were higher in the women, indicating a gender difference in the secretion of GH in response to mild physical activity.

Young men had lower GH concentrations and larger amounts of epinephrine in urine during the day than young women, whereas there were no differences at night. Women taking OCs were found to have lower epinephrine and higher GH values than women not taking OCs. The finding of a reciprocal relationship between GH and epinephrine during the day might reflect a gender difference in utilization of substrates for energy production regulated by these hormones.

The gender difference in median ambulatory morning GH values decreased from 100-fold in young individuals to 12-fold in the elderly, due to an increase in the men and a decrease in the women. In men there was an inverse correlation between GH and free androgen index (testosterone/SHBG) and a direct correlation between GH and SHBG (values adjusted for age). Treatment of postmenopausal women with implants of 17β-estradiol did not raise the morning GH values compared to an untreated control group, indicating that the decrease with age in the morning GH levels in the women is not an effect of lower estrogen levels alone.

Short-term administration of GH given to healthy young adults resulted in larger effects in the men than in the women on IGF-I and other key metabolic parameters. The smallest response was noted in women taking OCs underlying the role of sex steroids in modulating the susceptibility to GH.
After long-term treatment with GH to patients with GHD similar increases in serum levels of IGF-I and bone biomarkers were seen in men and women despite therapy with two-fold higher doses of GH in the women. A more marked gain in bone mass was seen in the men. The results demonstrate a gender difference in GH responsiveness, with women being less sensitive than men, an observation which should be considered in the treatment of GHD patients.

Short-acting octreotide was found to reduce the GH level to a similar extent in men and women with acromegaly. After long-term treatment with equal doses of the drug in men and women with a slow-release formulation of octreotide, the IGF-I level remained significantly higher in the men. This indicates that men with acromegaly need higher doses of octreotide than women to normalize IGF-I, a finding of clinical importance.
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