Short-term fasting abolishes the sex-related difference in GH and leptin secretion in humans


Short-term fasting abolishes the sex-related difference in GH and leptin secretion in humans. Am J Physiol Endocrinol Metab 279: E411–E416, 2000.—We studied growth hormone (GH) and leptin secretion in eight male (age 29.3 ± 1.2 yr, body mass index 22.2 ± 0.5 kg/m²) and seven female normal subjects (28.0 ± 0.8 yr, 20.1 ± 0.7 kg/m²) before and after 36 h of fasting. In the fed state, 8-h mean GH and leptin concentrations were higher in females (P < 0.05 and P < 0.0001, respectively). Fasting increased GH and decreased leptin in both sexes. There was significant interaction between gender and fasting (P < 0.05 for GH and P < 0.005 for leptin). Females showed a slighter increase in GH but a more marked decrease in leptin, so that there was no significant gender-related difference in GH and leptin after fasting. Fasting did not modify insulin-like growth factor (IGF) I, IGF binding protein (IGFBP)-3, acid-labile subunit, or GH binding protein; increased IGFBP-1 and free fatty acids (P < 0.0001) but decreased glucose (P < 0.001) and insulin levels (P < 0.05) in the fed state and underwent deeper reduction after fasting (interaction P < 0.03). In conclusion, GH and leptin secretions are higher in women than in men in the fed but not in the fasting condition, which abolishes these gender-related differences in humans.

gender; insulin-like growth factor I; insulin-like growth factor binding proteins; insulin; glucose

GROWTH HORMONE (GH) is secreted in pulsatile fashion in both animals and humans (13, 15, 23, 43), and GH pulsatility is mainly dependent on the tight interaction between the hypothalamic hypophysiotropic neurohormones GH-releasing hormone and somatostatin, although several neurotransmitters, peripheral hormones, and metabolic fuels also play an important role in the control of somatotroph secretion (8, 13–15, 18, 23, 43).

GH secretion is also dependent on gender, at least in adulthood (15, 19, 53). In fact, higher mean GH concentrations due to greater GH secretory burst mass in women than in men have been shown (19, 53). Gonadal steroids have an important positive influence on GH secretion. Estradiol levels directly correlate with 24-h integrated somatotroph secretion and GH pulse amplitude (19), and both estradiol and aromatizable, but not nonaromatizable, androgens increase GH pulsatility at puberty and in hypogonadal individuals (27, 51, 52). Interestingly, androgens possess a stimulatory influence on insulin-like growth factor I (IGF-I) secretion, which in turn is decreased by estrogens, depending on dose and route of administration (12, 19).

Other variables, such as age, body composition, and nutritional status, play a major influence on GH secretion (8, 14, 15, 48, 53).

Fasting strikingly stimulates somatotroph secretion in humans as a consequence of combined amplification of GH secretory burst frequency and amplitude without any change in estimated GH half-life (17, 21). Noteworthy short-term fasting-induced GH hypersecretion occurs even before IGF-I reduction (17); on the other hand, prolonged fasting as well as caloric restriction leads to peripheral GH resistance and impairs IGF-I synthesis and release (25, 44), thus reducing the negative IGF-I feedback action (16).

Leptin, the protein hormone produced mainly in the adipose tissue, is known as an anti-obesity hormone; however, above all, it appears to be a signal of energy deficiency and an integrator of neuroendocrine functions (4, 9, 11, 31). In fact, leptin synthesis and release are markedly inhibited by fasting and caloric restriction, although leptin levels do not begin to decrease before 12–14 h of fasting in humans (3, 10, 28).

Leptin levels show a diurnal rhythm in both sexes but are always higher in women than in men (32, 37). After adjustment for age, waist-to-hip ratio, fat mass, and insulin levels, estradiol, and testosterone are directly and inversely correlated, respectively, with lep-
SUBJECTS AND METHODS

Fifteen normal adult volunteers matched for age and weight (eight men, age 29.3 ± 1.2 yr, body mass index (BMI) 22.2 ± 0.5 kg/m²; and seven women, age 28.0 ± 0.8 yr, BMI 20.1 ± 0.7 kg/m²) were studied. All subjects had stable weight in the last 6 mo before entering the study protocol and were on a classical Mediterranean diet (~50% carbohydrates, 30% lipids, and 20% proteins). All female subjects had regular menses and were studied in their early follicular phase.

The study protocol had been approved by our ethical committee, and all subjects had given their informed consent to participate in the study.

All subjects entered the Department at 6:30 AM on the day of the test after overnight fasting from 8 PM of the evening before. From 8 AM to 4 PM, blood samples were taken every 30 min from an antecubital vein of the forearm kept patent by slow infusion of isotonic saline for GH, leptin, insulin, and glucose assays. IGF-I, IGFBP-3, ALS, IGFBP-1, FFA, and estradiol (in women) or testosterone (in men) levels were measured at 8 AM, 20.1 ± 0.8 yr, BMI 1.2 yr, body mass index (BMI) 0.8 yr, BMI 20.1 ± 0.7 kg/m²; and seven women, age 28.0 ± 0.8 yr, BMI 20.1 ± 0.7 kg/m²) were studied. All subjects had stable weight in the last 6 mo before entering the study protocol and were on a classical Mediterranean diet (~50% carbohydrates, 30% lipids, and 20% proteins). All female subjects had regular menses and were studied in their early follicular phase.

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Serum GH levels (μg/l) were measured in duplicate at each time point by two immunoradiometric assay (IRMA; Human GH, CTK, Sorin, Saluggia, Italy) and immunofunctional assay (IFA; in-house method, Neuroendocrine Unit laboratory, Munich, Germany) because of evidence that the latter method could better evaluate the GH bioactivity (41). The sensitivity of the IRMA method was 0.15 μg/l; the inter- and intra-assay coefficients of variation (CVs) were 5.1–7.5 and 2.6–5.4%, respectively, at GH levels 2.9–42.4 and 2.8–41.2 μg/l, respectively. The sensitivity of the IFA method was 0.05 μg/l; the inter- and intra-assay CVs were 12.8, 9.4, 7.9, and 8.5, 7.3, and 6.1% at GH levels 0.43, 5.3, and 19.2 μg/l, respectively.

Serum leptin levels (μg/l) were measured in duplicate at each time point by RIA using commercial kits (Human Leptin RIA, Linco Research, St. Charles, MO). The limit of sensitivity was 0.5 μg/l; the intra-assay CV was 8.3, and the inter-assay CV was 6.2%.

Serum insulin levels (μU/ml) were measured in duplicate by the RIA method (INSIK-5, Sorin). The sensitivity of the assay was 4.0 μU/ml. Inter- and intra-assay CVs were 5.9–6.3 and 3.5–8.6%, respectively.

Plasma glucose levels (mg/dl) were measured by the glucose oxidase colorimetric method (Menarini Diagnostics, Firenze, Italy).

Serum IGF-I levels (μg/l) were measured in duplicate by the RIA method (Nichols Institute of Diagnostics, San Juan Capistrano, CA) after acid-ethanol extraction to avoid interference by binding proteins. The sensitivity of the method was 0.1 μg/l. The inter- and intra-assay CVs were 8.8–10.8 and 5.0–9.5%, respectively, at IGF-I levels 79.6–766.4 and 79.4–712.5 μg/l, respectively.

Serum ALS levels (mU/ml) were measured in duplicate by the sandwich immunometric assay using monoclonal antibodies directed against specific NH₂- and COOH-terminal oligopeptides (40). To optimize immunorecognition, samples were pretreated with 3 M urea and 0.05% SDS. A serum pool of healthy male volunteers was used for calibration and assigned 1 U/ml. The assay range is 50–5,000 mU/ml, and the intra- and interassay CVs were <9%.

Serum IGFBP-3 levels (mg/l) were measured by a ligand IFA with a monoclonal anti-GHBP antibody (clone 10B8) (34). The whole assay CV was 3.4% at 115 pM and 5.9% at 1,550 pm. Between-assay CVs were 8.5 and 10.9%, respectively.

Serum IGFBP-1 levels (mg/l) were measured in duplicate by the IRMA method (Diagnostics Systems Laboratories, Webster, TX). The sensitivity of the assay was 0.33 mg/l. Inter- and intra-assay CVs were 3.5–6.0 and 2.7–5.2%, respectively.

Serum FFA levels (mg/l) were measured by the enzymatic colorimetric method (Wako Chemicals, Richmond, VA).

Serum estradiol levels (pg/ml) were measured in duplicate by the RIA method (Diagnostic Products). The sensitivity of the assay was 1.4 pg/ml. Inter- and intra-assay CVs were 5.2–9.1 and 5.5–10.5%, respectively.

Serum testosterone levels (ng/ml) were measured in duplicate by the RIA method (Diasorin, Saluggia, Italy). The sensitivity of the assay was 0.05 ng/ml. Inter- and intra-assay CVs were 11.3–13.7 and 3.81–8.07%, respectively.

All samples from an individual subject were analyzed in one assay to avoid between-assay variation. The statistical analysis of the data was performed by a two-way ANOVA: between-subject (gender) and within-subject (fasting) factors and their interaction were considered; prefast and 36-h-fasting values for each variable were taken as dependent variables (repeated-measure analysis). The unpaired Student’s t-test was performed on percent changes of GH and leptin levels. Simple linear correlation (Pearson r) was also employed to analyze the association between variables.

Results (means ± SE) are expressed as absolute values and percentages. Mean GH, leptin, insulin, and glucose con-
concentrations were expressed as means of the values measured at each time point over the 8 h.

RESULTS

Before-fasting levels of IGF-I, IGFBP-3, ALS, GHBP, IGFBP-1, glucose, and FFA were similar in men and women; insulin levels were higher ($P < 0.05$) in men (Fig. 1).

When assayed by IRMA, mean GH concentration in men ($0.5 \pm 0.4 \mu g/l$) was lower ($P < 0.05$) than in women ($2.3 \pm 0.4 \mu g/l$). When assayed by IFA, GH levels were generally lower by $\sim50\%$ in both groups, so that mean GH concentration in men was again lower than in women ($0.3 \pm 0.2 \mu g/l$ vs. $1.0 \pm 0.3 \mu g/l$, $P < 0.05$). Independently of the assay method, mean GH concentration from 8 to 12 AM (men vs. women: $0.3 \pm 0.2 \mu g/l$ vs. $3.2 \pm 0.5 \mu g/l$, $P < 0.001$) accounted for this gender-related difference (Fig. 1).

Mean leptin concentration in men ($2.7 \pm 0.5 \mu g/l$) was lower ($P < 0.0001$) than in women ($5.9 \pm 0.5 \mu g/l$; Figs. 2-3).

After 36 h of fasting, IGF-I, IGFBP-1, and FFA increased ($P < 0.001$) independently of gender, whereas IGFBP-3, ALS, and GHBP were unchanged (Fig. 1). Mean glucose levels decreased ($P < 0.001$) to the same extent in both groups, whereas insulin decreased in men more than in women (interaction between gender and fasting: $P < 0.03$); thus, after fasting, men and women had similar mean insulin concentration values ($2.8 \pm 0.9$ and $3.8 \pm 0.8 \mu U/ml$; Fig. 1).

Fasting increased GH and decreased leptin in both sexes. There was significant interaction between gen-

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**Fig. 1.** Before (open bars) and after fasting (hatched bars), mean (and range) levels of insulin-like growth factor I (IGF-I), IGF binding protein (IGFBP)-3, acid-labile subunit (ALS), GH binding protein (GHBP), IGFBP-1, glucose, insulin, and free fatty acids (FFA) in male (M) and female (F) individuals. *$P < 0.05$ vs. before fasting.

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**Fig. 2.** Mean ($\pm$SE) circulating growth hormone [GH, assayed by immunoradiometric assay (IRMA)] and leptin levels from 8 AM to 4 PM before (A) and after fasting (B) in male and female subjects.
and fasting ($P < 0.05$ for GH and $P < 0.005$ for leptin).

Females showed a lower GH increase ($P < 0.05$ in terms of percent increase above baseline) than males independently of the assay used (males vs. females: IRMA, $6.2 \pm 1.1$ vs. $5.4 \pm 1.2 \mu$g/l; IFA, $3.3 \pm 0.7$ vs. $2.7 \pm 0.5 \mu$g/l). In fact, after-fasting mean GH concentration values in females and males were similar.

On the other hand, females showed a higher leptin decrease ($P < 0.05$ in terms of percent decrease below baseline) than males (males vs. females: $0.3 \pm 0.2$ vs. $3.2 \pm 0.5 \mu$g/l, $P < 0.001$). In fact, after-fasting mean leptin concentration values in females and males were similar.

Estradiol in women ($55.0 \pm 3.5$ vs. $53.6 \pm 4.2$ pg/ml) and testosterone levels in men ($8.7 \pm 3.1$ vs. $9.1 \pm 4.1 \mu$g/l) were not modified by fasting. Both before- and after-fasting mean GH and leptin concentrations were not associated with testosterone or estradiol levels in men and women, respectively.

Both GH and leptin levels were not associated with BMI, before and after fasting.

**DISCUSSION**

The results of the present study show that mean GH and leptin concentrations are higher in women than in men in the fed but not in the fasting condition. In fact, short-term fasting stimulates GH less and inhibits leptin more in women than in men before any decrease in total IGF-I, as well as in IGFBP-3 and ALS levels. Thus our results demonstrate that the gender-related differences in GH and leptin levels in humans are no longer detectable after fasting.

There is clear evidence to show that, although IGF-I levels are generally similar in men and women, mean GH concentrations in women are higher than in men due to greater GH secretory burst mass (15, 19, 27, 53, and present results). In the present study, the mean GH concentration in the morning hours accounted for most of the difference between sexes. Gonadal steroids, namely estradiol, have an important positive influence on GH secretion (27, 51). Estrogens as well as testosterone, but not dihydrotestosterone, increase GH pulsatility in peripubertal children and in hypogonadal subjects (27, 51, 52). Also, in normal adults, sex steroids increase GH secretion: androgens increase somatotroph secretion in men (47), whereas estrogens have a positive effect in both sexes (20, 54).

Similarly, gender is a factor in predicting leptin levels (4, 9, 11, 31, 32, 37, and present results). In fact, even after adjustment for body mass, total fat mass, and age, women have leptin levels higher than men (32, 37). It has been suggested that the sexual dimorphism in leptin levels is due to the inducing effects of estrogens or progesterone and/or to the suppressive effect of androgens on leptin synthesis and release (4, 9, 11, 24, 30, 31). Other potential explanations of the gender-related difference in leptin levels include 1) influence of gonadal steroids on leptin action to produce relative leptin resistance (26); 2) peculiar sensitivity of female adipose tissue to hormones, such as insulin and glucocorticoids, or other substances stimulating leptin production (46); 3) evidence that subcutaneous gynoid fat produces more leptin mRNA than visceral android fat (37).

Among other variables influencing GH and leptin secretion, body composition and nutritional status play a major role (4, 8, 11, 14, 15, 18, 23, 31, 48, 53). In fact, prolonged caloric restriction and fasting stimulate somatotroph secretion in humans as a consequence of combined amplification of GH secretory burst frequency and amplitude without any change in estimated GH half-life (17, 21). Noteworthy, short-term fasting-induced GH hypersecretion occurs even before reduction of total IGF-I levels (17, and present results), suggesting that the lack of negative IGF-I feedback is not the cause of fasting-induced early amplification of GH pulsatility. In agreement with this assumption, the fasting-induced GH hypersecretion is inhibited by recombinant human IGF-I administration, as well as by eating, well before normalization of IGF-I levels (16, 17). Thus the early fasting-induced amplification of GH secretion mainly reflects central nervous system (CNS).
mechanisms, likely including GHRH hyperactivity and somatostatin hypoactivity (17, 21, 45).

In humans, significant reduction of energy intake or fasting suppresses leptin levels to a much greater extent than would be expected on the basis of changes in adipose tissue mass (3, 9, 10, 28, 36, and present results). At least 12 h of fasting are needed to inhibit leptin levels in humans (28). Thus leptin can be assumed as an afferent signal to the CNS for modulating both long-term energy balance and/or short-term energy imbalance (2, 11, 31, 39). The mechanisms underlying the inhibitory effect of fasting on leptin are, however, still unclear. It has been suggested that insulin and/or carbohydrates may mediate the leptin response to fasting (3, 4, 11, 31). Alternatively, fasting-induced changes in ketones, catabolic lipid autacoids, or the autonomic nervous system could play an important role (28).

Our present data show that the gender-related difference in GH and leptin levels is no longer apparent after the short-term fasting that stimulated GH to a lesser extent and inhibited leptin to a higher extent in women than in men. These findings allow some important considerations, questioning assumptions about the control of GH and leptin secretion. Because no change was found in gonadal steroid levels after 36 h of fasting, the gender-related difference in GH and leptin response to fasting in humans is unlikely to be dependent on gonadal steroids. In fact, in agreement with previous data, 1) at least 3.5 days of fasting are needed to reduce gonadal steroid levels, whereas GH secretion is already enhanced after 24 h in young adults (1, 17); 2) in both normal and overweight subjects during caloric restriction, leptin levels decrease more in women than in men (10). Early metabolic changes could account for the disappearance of gender-related difference in GH and leptin concentrations after short-term fasting, although, in the present study, 36 h of fasting had the same effect on glucose and FFA levels, as well as on total IGF-I, IGFBP-3, ALS, IGFBP-1, and GHBP levels in both sexes. On the other hand, in agreement with other authors (10), we found that the fasting-induced decrease of insulin levels was more pronounced in men than in women. Taking into account evidence indicating that insulin per se plays an inhibitory and a stimulatory role on GH and leptin, respectively (4, 11, 31, 55), our present findings do not seem fully explained by the gender-related difference of the insulin response to fasting.

Our present results also make unlikely the hypothesis that leptin positively influences GH secretion in humans, differently from what has been shown in rodents (6, 35, 42, 49).

In conclusion, this study shows that, in humans, at least young and lean individuals, gender-related differences in GH and leptin levels are no longer apparent after short-term fasting despite no acute change in gonadal steroid levels. The loss of the sexual dimorphism of GH and leptin secretions could theoretically be placed in the context of sex-related regulation of energy metabolism (22).

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