Growth hormone secretion pattern is an independent regulator of growth hormone actions in humans

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PITUITARY GH SECRETORY PATTERN in humans and other species is highly pulsatile. In rats, this pattern is sexually dimorphic; males have regular high-amplitude pulses and relatively low interpulse growth hormone (GH) levels, and females have lower amplitude pulses and higher interpulse levels (11). These gender-specific patterns of GH secretion in rats are independent regulators of GH bioactivity. For example, pulsatile GH is more effective than continuous GH infusion in stimulating somatic growth (9, 16) and muscle and growth plate insulin-like growth factor (IGF) I mRNA (16, 17). In addition, the expression of some P-450 cytochromes (CYPs) is highly sexually dimorphic in rodents, and a gender-specific GH secretory pattern, not sex steroids, regulates many of these differences (7, 49, 50). For example, the male pattern in rats increases hepatic expression of the male-specific P-450 steroid hydroxylase CYP2C11, whereas the female GH pattern of more continuous GH release stimulates the expression of the female-specific CYP2C12 (50). In rats, GH secretory pattern also controlsapolipoprotein levels (34, 41), major urinary proteins (15), and hepatic carbonic anhydrase III (21). Similar gender-dependent effects of GH are found in mice (20, 22, 32) and hamsters (40, 42).

Whether the pattern of GH secretion plays a role in the regulation of human growth and metabolism is unclear. Equal daily doses of GH administered either as continuous infusion or as intravenous boluses every 3 h for 24 h were equipotent in inducing a rise in plasma IGF-I (24). Laursen et al. (29) reported that GH administered as a continuous subcutaneous infusion or intermittently had similar effects on serum concentrations of IGF-I, IGF-binding protein (IGFBP)-3, and lipoproteins and on insulin sensitivity (29). However, once a day, subcutaneous GH administration does not reproduce the highly pulsatile GH profiles found in normal men and women.

Parallels between human and rat physiology suggest that GH pulse pattern could play a role in regulating GH effect in humans. As is true in rats, humans have gender differences in the ability to metabolize drugs. Women metabolize caffeine (3-demethylation by CYP1A2) more slowly than do men (4, 36). The observations that GH deficiency results in a rise in the 3-N-demethylation of caffeine, as measured by the caffeine 13CO2 breath test (CBT), and that GH therapy
METHODS

The study was approved by the University of Michigan Institutional Review Board and the General Clinical Research Center (GCRC) Advisory Committee. All subjects signed informed consent documents before participation. Nine patients (4 men, 5 women) with GH deficiency were recruited from the University of Michigan Endocrinology and Metabolism Clinics. All had onset of GH deficiency before puberty, and none had been previously treated with GH. Each subject had documented GH deficiency during an insulin tolerance test of 0.0001. Mean daily GH during protocols 1–4, respectively (Fig. 2). By ANOVA, all GH concentrations were run on the same ELISA plate. The intra-assay coefficient of variation (CV) for each analyte was <10% at the measured concentrations.

Results of variance (ANOVA) with repeated measures was used to determine the effects of the GH treatment regimens on a measured parameter (SuperAnova; Abacus Concepts, Berkeley, CA). When a treatment effect was found in a primary analysis, post hoc testing was performed by contrasts. P values for all ANOVA and contrasts were corrected for the repeated measures by Huynh-Feldt adjustment factors. Data having unequal variance were logarithmically transformed before analysis. Results are reported as means ± SE, and P < 0.05 was considered statistically significant.

RESULTS

Figure 1 shows the mean 24-h GH profiles for nine subjects during the four stages of the study. Daily mean plasma GH concentration was 0.09 ± 0.05, 1.58 ± 0.11, 1.35 ± 0.12, and 1.83 ± 0.20 μg/l for protocols 1–4, respectively (Fig. 2). By ANOVA, all GH treatments increased mean plasma GH above baseline daily GH (P < 0.0001). Mean daily GH during protocol 4 was greater than during protocol 3.
The effects of the GH treatments on serum IGF-I and IGFBP-3 are shown in Figs. 3 and 4, respectively. By ANOVA, there was a treatment effect for IGF-I (P < 0.0001), and all GH treatment protocols significantly increased IGF-I above the baseline concentration (P < 0.005 for each). Moreover, serum IGF-I concentrations were higher in protocols 3 and 4 than in protocol 2.

Similarly, there was a treatment effect for IGFBP-3 (P < 0.001), and all treatments increased IGFBP-3 above the baseline concentration. Continuous GH (protocol 4) was significantly more efficacious than protocol 2 in increasing IGFBP-3 (P < 0.05).

The effects of the GH treatments on bone formation were measured by serum osteocalcin. Serum osteocalcin on day 1 of each protocol was similar (20.8 ± 4.2, 21.0 ± 3.1, 18.8 ± 3.7, and 22.4 ± 4.5 ng/l for protocols 1–4, respectively; P = 0.64). There was a treatment effect (P = 0.015) such that serum osteocalcin concentrations on day 7 of each GH administration protocol were higher than serum osteocalcin at the end of the baseline study. Although the order of the three GH treatment protocols was randomized, and serum osteocalcin on day 1 of protocols 2–4 was similar, there was concern for carryover effect from previous GH treatment (3). To adjust for this potential confounding factor, an “adjusted osteocalcin” was determined by subtracting serum osteocalcin concentration on day 1 from the concentration measured on day 7 of each study (Fig. 5). Repeated-measures ANOVA again demonstrated a treatment effect (P = 0.016). During the baseline study, there actually was a decline in serum osteocalcin between days 1 and 4. In contrast, during the GH treatment protocols, there was no decrease in
osteocalcin during each study, and the adjusted osteocalcin was significantly greater in protocols 2 and 3 than at baseline.

Figure 6 shows the effects of treatment on Dpd, a marker of bone resorption. Again, there was a treatment effect (P < 0.05). The urinary Dpd-to-creatinine ratio was similar in protocols 1 and 3 but increased and decreased by ~25% above and below baseline in protocols 2 and 4, respectively. The difference between the Dpd-to-creatinine ratio for protocols 2 and 4 was the driving force for the observed treatment effect in the ANOVA.

GH treatment also significantly affected CYP1A2 and CYP3A4 activities. As shown in Fig. 7, there was a treatment effect on CBT (P < 0.05), and all GH treatments tended to decrease CBT below the baseline value. This difference was significant for protocols 2 and 3, and there was a trend to lower CBT in protocol 4 (P = 0.10). The effect of GH ERMBT is shown in Fig. 8. There was an overall treatment effect (P = 0.01), and, again, the effect of GH was dependent on the pattern of administration. There were trends for lower ERMBT during protocols 2 and 3 compared with baseline (P = 0.1 and 0.2, respectively). Continuous GH (protocol 4) resulted in significantly higher ERMBT than at baseline (P = 0.03) or during either of the two protocols in which pulsatile GH was administered (P = 0.002 and 0.006 for protocols 2 and 3, respectively). This corresponded to an average increase in ERMBT of 51 and 35% for protocols 2 and 3, respectively, vs. protocol 4.

By unpaired t-tests, there was no gender-related difference in any parameter during protocol 1 (baseline). Repeated-measures ANOVA with one between-group factor (gender) was performed on each of the data sets to determine whether sex steroids made a difference in the treatment group responses. No gender effects were found.

DISCUSSION

Gender-specific GH secretion is an important determinant of the metabolic and somatic actions of GH in rodents. The present study demonstrates for the first time that several GH-sensitive end points in humans are regulated by different components of GH exposure. These observations have important implications with regard to our understanding of human GH physiology and replacement therapy.

After the groundbreaking animal studies demonstrating the importance of GH pulse pattern as a tissue-specific regulator of IGF-I biosynthesis and of hepatic cytochrome P-450 enzymes, several attempts...
were made to assess whether the same was true in humans. Jorgensen and colleagues (23, 24) could not find a difference between the effects of continuous GH infusion or eight intravenous boluses on IGF-I, IGFBP-3, or serum lipids. More recently, Laursen et al. (29) reported that 6-mo treatment with continuous or daily subcutaneous GH had similar effects on IGF-I and IGFBP-3, bone metabolism, body composition, insulin sensitivity, and lipoproteins. Thus it is currently assumed that the pattern of GH pulsatility, whereas important in rodents, is not important in humans. Our study, however, proves that GH pattern does play a role in determining GH effect in humans.

There are several simple explanations why previous studies failed to detect the differences. The first reason is the duration of some of the previous negative studies. In the studies from Jorgensen and colleagues (23, 24), GH-deficient patients received GH for only 20 h. An increase in serum IGF-I concentration occurred within 6 h of beginning GH treatment, and IGF-I was still increasing when the GH treatments were stopped. The effect on IGFBP-3 was much slower, and a measurable increase in IGFBP-3 did not occur until ~20 h after GH treatment was begun. In contrast, our subjects were treated with GH for 1 wk. We achieved steady-state GH concentrations with the GH infusion, which was not the case in the earlier study. One week of treatment should also have allowed us to achieve IGF-I and IGFBP-3 steady-state concentrations.

Steady-state effects were reached in a recent study that compared the metabolic effects of 6-mo treatment, using continuous GH, with daily subcutaneous GH (29). The authors concluded that GH effects in humans were not dependent on a pulsatile pattern. This conclusion, however, is based on the assumption that daily subcutaneous GH administration is an appropriate way to approximate endogenous GH secretion. The GH concentration profiles obtained with subcutaneous GH are relatively flat and of long duration (25), rather than the normal, highly pulsatile pattern found in normal men and women (18). Moreover, the nadir concentrations of GH in the study by Laursen et al. (29) were not formally reported; they did not appear to reach the very low interpulse GH concentrations obtained with our intravenous boluses. Therefore, although this study was of adequate duration, the protocol used did not truly address the selective effects of GH pulse pattern, since subcutaneous GH injections are a better approximation of continuous GH exposure than of endogenous GH pulses.

A third and most important limitation of the earlier studies was the primary end point measured. In rats, both continuous GH and pulsatile GH are equally efficacious in increasing hepatic IGF-I mRNA and protein (16, 17). Therefore, failure to see an effect on systemic IGF-I in either earlier or the present studies does not indicate that GH pulse patterns play no role in human physiology. Rather, it suggests that the rat is an appropriate model for humans with regard to GH effects on hepatic IGF-I production. Other GH-sensitive end points could be responsive to pulse pattern.

In contrast to the pulse pattern-independent effect on hepatic IGF-I production, several constitutively activated CYPs in rats are regulated by GH pulse pattern (33). In this study, we examined the role of GH pulse pattern on human CYP1A2 and CYP3A4 activity. These enzymes are central to the metabolism of a large number of xenobiotics and to the bioactivation of procarcinogens (28). Differences in their levels of expression might contribute to gender differences in susceptibility to the toxic effects of many medications (2). As discussed earlier, limited data have implicated a role for GH in the regulation of CYP1A2 and CYP3A4 activity.

All GH treatments decreased CBT, although the effect tended to be greatest with pulsatile GH administration. This fall in CBT during GH treatment is consistent with the decrease in CBT at puberty (26). Whether the small difference in CBT between pulsatile GH (protocols 2 and 3) and continuous GH (protocol 4) is of physiological significance is uncertain. In normal adults, CYP1A2 activity is higher in men than in women (2, 28). As discussed below, protocol 3 is likely to be more representative of normal GH secretion in men, whereas protocol 4 represents GH secretion in normal women. Therefore, CBT might be expected to be higher in protocol 3 than in 4. Although it is clear that GH per se decreases CBT, more studies are needed to determine whether the patterns of either exogenous GH administration or gender-specific GH secretion differentially regulate CYP1A2 levels.

The ERMBT data provide compelling evidence for the importance of GH pulse pattern in the regulation of other important metabolic pathways. Only the continuous GH infusion (protocol 4) increased ERMBT, whereas pulsatile GH in protocol 2 tended to decrease ERMBT. Although changes in ERMBT could reflect an effect on P-glycoprotein (27), we believe that it most likely indicates changes in CYP3A4 regulation (31, 39). Because ERMBT decreases with increasing erythromycin volume of distribution, and GH treatment of GH-deficient adults increases total body water (8), the difference between baseline and protocol 4 ERMBT likely underestimated the magnitude of the GH effect on CYP3A4 activity.

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**Fig. 8. Effect of GH pattern on erythromycin breath test (ERMBT).** Data are presented as means ± SE. Statistical analyses are as described in METHODS and Fig. 2. *P < 0.05 vs. protocol 1. †P < 0.05 vs. protocol 2. #P < 0.05 vs. protocol 3.
We had previously reported that ERMBT was higher in women than in men (47) and that ERMBT was high in acromegaly patients (48), a state of continuous GH exposure. In addition, we noted that ERMBT increased in middle-aged men treated with GHRH every 2 h for a week (48). Because plasma GH concentrations during GHRH treatment never fell below 0.5 µg/l (19), the subjects effectively had continuous GH exposure. This last explanation fits best with the data from the present study. Similarly, the demonstration that subcutaneous GH treatment increased antipyrine metabolism (5) suggests that subcutaneous GH administration approximates tissue exposure to continuous, not pulsatile, GH.

Presumably, this differential regulation in humans is similar to what occurs in rats. Regulation of constitutively expressed rat CYP by GH pulse pattern has been extensively investigated (33), and GH-mediated control of the signal transducer and transcriptional activator STAT5b is central to many of these gender-specific GH effects (6, 10, 35, 43). Pulsatile or continuous GH increases or decreases STAT5b activation, respectively (12, 13, 50). Whether gender-specific GH patterns are involved in STAT5b regulation of human CYPs has not been studied.

The pattern of GH administration also differentially affected markers of bone metabolism, with parameters of both bone formation and resorption increasing more after pulsatile than after continuous GH infusion. These results agree with data demonstrating that pulsatile GH is more potent than continuous GH in stimulating IGF-I message and growth in cartilage and bone growth in rats (9, 16). The reason for a decrease in serum osteocalcin between the first and last days of protocol 1 is not clear. Potentially, this fall is related to a relative decrease in physical activity, as the subjects were at bed rest for much of the day. It will be important to verify this decrease in subsequent studies and interpret data from periods of GH treatment accordingly.

In clinical practice, subcutaneous GH administration, which does not give a sharp serum GH pulse, accelerates linear growth in GH-deficient children. However, many of these children fail to achieve their target midparental height. This could be due to an irreversible loss of growth potential resulting from late diagnosis of GH deficiency, an inadequate daily GH dose, or non-GH causes, such as sex steroid deficiency. Alternatively, the manner of GH delivery to the tissue might be important. In support of this, positive growth in normal children correlated best with GH peak height (1). Our data suggest that pulsatile pattern of GH treatment might be optimal for bone development and subsequent growth. Therapeutic modalities producing rapid, transient, and high GH pulses might be more efficacious in restoring linear growth in GH-deficient children.

A major aim of the study was to determine whether gender-specific GH secretion patterns resulted in different peripheral effects of GH. Men have small day-time pulses with pronounced nocturnal augmentation of GH, whereas GH pulses in women are more uniform in size, and nocturnal augmentation of GH secretion is less extreme (18). Perhaps most importantly, we (18) and others (14, 51) have shown that tissue exposure to physiologically effective concentrations of GH is more constant in women than in men. GH concentrations in normal women fall below a cutoff of 0.25 µg/l for only 20% of the day, whereas they are below this value for over 50% of the time in men (18). On the basis of data from GH-deficient subjects, GH concentrations below 0.25–0.5 µg/l are likely to be minimally effective (37). Our patterned GH treatments resulted in GH concentrations below this cutoff 89, 78, 54, and 0% of the day for protocols 1–4, respectively. Protocols 2 and 3 were designed to represent the idealized versions of male and female GH patterns, respectively. However, on the basis of the duration of time during which GH concentrations fell below the 0.5-µg/l cutoff, the true male pattern is likely closer to protocol 3, whereas the true female pattern is closer to protocol 4. Moreover, the nocturnal GH pulse in protocol 2 reached peak amplitudes of >80 µg/l, which was clearly beyond peak concentrations in normal adult men (18). In contrast, peak GH concentrations in protocol 3 were more physiological. Again, this supports protocol 3 over protocol 2 as more representative of normal physiology in men.

An alternative explanation for the measured differences in GH effect is gender-specific effects of sex hormones. However, separately analyzing the data in men and women did not change the conclusions. For example, there was a treatment effect (P < 0.05) on ERMBT in men, with the highest ERMBT occurring in protocol 4. In women, there was a strong trend for treatment effect (P = 0.07), and, again, the highest ERMBT was observed in protocol 4. Repeated-measures ANOVA that included gender as a between-groups effect found no differences across gender. Admittedly, after stratification of the data by gender, the numbers of subjects in each group were small, so that it was possible that the study was underpowered to observe sex hormone-mediated differences. However, the fact that we still observed differences across GH treatments suggests that even if sex steroids play a role, their effects are likely of a lesser magnitude than that of GH.

Another potential limitation of this study is that assignment of biological importance to these data is uncertain. Although tissue- and GH pattern-specific effects were found, some of these differences were relatively small. For example, continuous GH (protocol 4) compared with pulsatile GH treatment (protocol 2) resulted in a 51% average increase in ERMBT. This is smaller than many of the GH-regulated effects on hepatic enzyme levels observed in rodents. Yet, changes in ERMBT of a magnitude similar to that observed in our study result in clinically important effects on CYP3A4 (47). Further studies are needed to clearly define the relative biological relevance of each of our observations.

Even with these limitations, our data clearly support our hypothesis that GH delivery pattern results in
tissue-specific effects in humans. Although protocol 2 was less effective with regard to IGF-I and IGFBP-3, pulsatile GH was more effective in inducing bone metabolism and trended to be more effective in suppressing CYP1A2. In contrast, only continuous GH exposure increased CYP3A4 activity, which is consistent with GH and CYP3A4 differences in normal men and women.

In conclusion, this study demonstrates for the first time the tissue- and pattern-specific effects of GH secretion in humans. The interpulse GH levels are the primary determinants of hepatic IGF-I, IGFBP-3, and CYP3A4. In contrast, bone effects of GH are pulse amplitude sensitive. CYP1A2 is regulated largely by the prevailing daily GH concentration, although GH pulse amplitude might also play a role. These data shed light on the known gender-specific differences in drug metabolism and linear growth. Pattern-specific delivery protocols for GH treatment might bring about selective effects on linear growth and hepatic CYP expression.

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