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Diminished Function of the Somatotropic Axis in Older Reproductive-Aged Women

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ABSTRACT

Circulating GH and insulin-like growth factor-I (IGF-I) levels in adults generally fall with age. Studies in aging women have rarely controlled for menstrual cycle stage or status or body mass index. We hypothesized that GH and IGF-I levels in reproductive-aged women fall with age despite the stimulatory effects of endogenous estradiol (E_2). Eight older reproductive-aged women (aged 42–46 yr) with regular menses, of normal weight, and in good health were compared to a group of eight young control subjects (aged 19–34 yr). Daytime frequent blood sampling was performed in the early follicular phase of the menstrual cycle to characterize pulsatile GH and LH concentrations. Pooled samples were also analyzed for IGF-I, E_2, progesterone, and FSH levels. Older reproductive-aged women had lower 12-h integrated daytime GH concentrations (mean ± SE, 171 ± 35 *vs.* 427 ± 130 μg min/L; $P = 0.036$) than younger controls and a strong trend for lower IGF-I levels (22.7 ± 2.1 *vs.* 31.3 ± 3.5 nmol/L; $P = 0.055$) than younger controls despite having higher circulating E_2 on the day of sampling (368 ± 51 *vs.* 167 ± 20 pmol/L; $P = 0.002$). We conclude that older reproductive-aged women have lower daytime GH concentrations than younger controls despite having higher E_2 levels on the day of sampling and overall normal gonadal hormone parameters. (*J Clin Endocrinol Metab* **80:** 608–613, 1995)

THE MENOPAUSE is a well studied transition period marked by profound changes in sex steroid, gonadotropic, and somatotropic hormone profiles. Although it is clear that GH levels in men gradually fall in relation to increasing age, studies in women are dramatically affected by the acute loss of estradiol (E_2) that occurs at menopause. After the menopause, GH concentrations in women fall to levels comparable to those in age-matched male counterparts (1). These data have been interpreted to suggest a strong influence of E_2 on GH in women. There are also data to suggest that ovarian function begins to wane in a woman's forties (the perimenopause), well before the definitive decline that occurs about age 50 yr at menopause (2, 3). The behavior of the somatotropic axis during this transitional period has received little scrutiny and has not been acurately characterized to date.

Some of GH's effects are mediated through insulin-like growth factor-I (IGF-I), an anabolic peptide whose levels in the circulation primarily reflect hepatic production. Levels of IGF-I have generally, but not always, been shown to correlate with 24-h integrated GH concentrations (4). E_2 directly stimulates IGF-I secretion and significantly increases IGF-I levels. This effect occurs within 2–3 days and is not always linked to increases in GH (5). Androgens have little effect on IGF-I concentrations (5). If IGF-I levels are to be used as markers of overall somatotrophic activity, they must be evaluated in the context of the prevailing sex steroid environment.

There is general agreement that IGF-I levels in both sexes fall with increasing age after achieving adulthood (6–8). Given the fact that E_2 stimulates and adiposity inhibits somatotropic axis activity, interpolation of the overall decline in IGF-I levels noted in the general population specifically to the subset of cycling, normal weight, reproductive-aged women may not be justified (9, 10).

We undertook this study to test the following hypothesis. Somatotropic axis activity declines with aging in adult cycling women despite the presence of stimulatory levels of circulating E_2 from regular ovulation. To achieve this end, we evaluated the somatotropic and gonadotropic axes of older reproductive-aged women and compared them to a group of younger controls. In an attempt to control for the effects of the sex steroids on GH and IGF-I dynamics, daytime frequent blood sampling was performed during the early follicular phase of the menstrual cycle, during which time E_2 and progesterone (P) levels could be expected to be at their lowest and most comparable levels.

Materials and Methods

Subjects

Paid volunteer female subjects, aged 19–34 yr (young controls) or 42–46 yr (older reproductive age), were recruited for study. Study protocols were approved by our Institutional Review Board, and informed consent was obtained from all subjects. All women had a history of regular menses every 25–35 days and had recently been shown to be ovulatory by a midluteal serum P level greater than 22 pmol/L. All were in good health, had no history of pelvic pathology, had a hemoglobin concentration greater than 120 g/L, took no medications, and had not taken any hormone in the past 2 months. All women were required to be of normal weight with body mass indexes (BMIs) between 18–28 kg/m^2 (8). Subjects agreed to avoid the possibility of becoming pregnant

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during the period of study or had previously been surgically sterilized. No subject exercised excessively (*i.e.* no more than 1 h of aerobic exercise five times weekly).

Sampling techniques

Beginning within the first several days of a subject's menstrual cycle, daily blood samples were collected until the subsequent menses began. These samples were analyzed for gonadotropins and sex steroids to confirm that the study cycle in question was ovulatory. The day with the highest serum LH concentration (albeit from one venipuncture) was defined as the day of the midcycle LH surge. On or around menstrual day 3, the study subjects were admitted to a metabolic testing unit at approximately 0800 h for 12 h of frequent blood sampling. An indwelling iv catheter was placed in an arm vein, and 2-cc samples of blood were withdrawn every 10 min. Subjects consumed a regular diet and were free to move about their rooms. These meals consisted of the regular diet routinely served in the hospital. Breakfast was usually consumed between 0830–0900 h, lunch between 1230–1300 h, and dinner between 1700–1730 h.

Blood samples were collected into evacuated glass containers and allowed to clot at room temperature. The entire batch of samples was then centrifuged at 600 × *g* for 20 min, and the serum was decanted from the cells and stored at −20 C until analyzed. A pooled sample was also created from combined aliquots of each of the patient's 12-h frequent samples.

Assays

The daily and pooled day 3 serum samples were analyzed in duplicate for E_2 and P with commercially available RIAs (Direct Estradiol, Pantex, Santa Monica, CA; Coat-A-Count Progesterone, Diagnostic Products Corp., Los Angeles, CA). Both assays have interassay coefficients of variation (CV) less than 12% and intraassay CVs less than 5%. GH and LH concentrations were measured in duplicate at 10-min intervals with commercially available fluoroimmunoassays (DELFIA, Wallac, Gaithersburg, MD). The GH assay detection limit was 0.01 μg/L, with an intraassay CV of 7.1% and an interassay CV of 8.9% at GH concentrations of approximately 0.2 μg/L; the intraassay CV was 5.3% and the interassay CV was 6.5% at GH concentrations near 2 μg/L; and the intraassay CV was 4.0% and the interassay CV was 4.7% at GH concentrations near 15 μg/L. The test cross-reactivity with PRL, TSH, and FSH was less than 0.001%, and cross-reactivity with LH was less than 0.1%. The LH detection limit was 0.12 IU/L, with an intraassay CV of less than 5% and an interassay CV of less than 7%.

Pooled samples were also analyzed for FSH and IGF-I. FSH was assayed fluoroimmunometrically (DELFIA), with an assay detection limit of 0.05 IU/L, an intraassay CV of less than 4.8%, and an interassay CV of less than 4.3%. Serum concentrations of IGF-I were determined after acid-ethanol extraction to avoid interference of IGF-binding proteins (Nichols Institute, San Juan Capistrano, CA) (11). The intraassay CV was less than 6%, and the interassay CV was less than 9% in our hands and at the commercial lab.

Statistics

The frequent sampling GH data were analyzed to statistically compare somatotropic axis activities of the older reproductive-aged women and the young controls. Each subjects' 12-h GH concentration pattern was evaluated for the total area under the curve (AUC) by trapezoidal integration (12). These quantitative GH AUC values were then arranged by group and compared using the appropriate means comparison test.

GH and LH pulse patterns were analyzed objectively on a DELL 325sx computer with the pulse detection program PULSEFIT (13). The program is based on a dynamic model that reiteratively fits a differential equation to frequent sampling data by assuming that pulsatile hormone levels follow a pattern of rapid increase after an "injection event," followed by an exponential decay.

Pulse analysis was also performed using the modified technique of Santen and Barden (S+B), as previously described (14, 15). Briefly, this algorithm defines a pulse as two consecutive values more than 20% larger than a preceding nadir. The maximum GH pulse amplitude was defined as the highest GH level measured during the day. Average GH and LH pulse amplitudes were determined by averaging the values of each peak identified by PULSEFIT over the 12-h sampling period.

Overall patterns of daytime GH concentration were evaluated using a locally weighted and smoothing program, Lowess (Data Desk, version 4.0, Data Description, Ithaca, NY). This function, similar to a spline fit, is a robust method for revealing underlying patterns in large data series and is minimally affected by outlying or aberrant values (16). The curve is produced from a scatter plot composed of GH concentration data points from all 16 subjects at all time points. Briefly, the program computes a best-fit line through the data at every time point by assessing the "local environment" and weighting the surrounding data according to a Gaussian distribution; the width of this curve is determined by a "flexibility level," which is variable and adjusted by the investigator. A larger value creates a "stiffer" straighter line, whereas a smaller value results in a more "flexible" or wavy line. In general, the flexibility level must be iteratively adjusted until the Lowess smooth fit shows the amount of detail in the data that is desired.

Group means of normally distributed data were compared using the two-tailed Student's *t* test. Nonnormal distributions were identified with the Shapiro-Wilk (SW) test, and if identified, group comparisons were performed using the nonparametric Wilcoxon rank sum test. Simple linear regression and one-way analysis of variance were used to assess relationships between variables. Statistical significance was declared for differences found to have less than a 5% chance of type 1 error. Means comparisons, analysis of variance, SW tests, and linear regressions were performed with JMP 2.0.4 statistical software (SAS Institute, Cary, NC), and the graphs were created using Excel 3.0 software (Microsoft) or Data Desk 4.0 on a Macintosh LC II computer.

Technical difficulties resulted in less than complete frequent sampling data series in two study subjects. One individual did not have a time zero value, but the overall contribution of this point to the total GH AUC was trivial. To ascertain the effect of this absent data point on our conclusions, all statistics were recomputed with the first frequent sampling point of the remaining series removed to provide equivalent series lengths. No significant changes resulted, so we arbitrarily assigned a value of zero to this data point. A second subject in the younger group had to cut short her 12-h frequent sampling session after 8 and 2/3 h (*i.e.* after completing >70% of the normal sampling period) due to a low hemoglobin level (<11.08/dL) at that time. Her total GH AUC, GH pulse frequency, and LH pulse frequency were, therefore, adjusted by assuming that her truncated sampling was representative of the entire 12 h. These prorated values fell very close to the group averages. Because inclusion or exclusion of these data points from group means comparisons did not result in different statistical conclusions, this subject's prorated data points were included in the final computations.

Results

Comparisons of the gonadotropic and somatotropic axis parameters derived from the groups of younger and older women are displayed in Table 1. Older women had significantly higher E_2 and lower 12-h daytime integrated GH levels than their younger controls (Fig. 1). GH levels were always detectable. We found no significant correlation between IGF-I levels and 12-h GH AUC or maximum GH pulse amplitudes. However, we observed a trend for IGF-I to correlate with average GH pulse amplitudes ($r = 0.41$; $P = 0.11$). We also observed a decrease in the number of large GH peaks (*i.e.* pulse values >2 μg/L) in individuals as BMI increased ($r = -0.60$; $P = 0.014$). The GH AUC also tended to fall with increasing BMI ($r = -0.40$; $P = 0.12$).

Older reproductive-age women were found to have twice the early follicular phase serum E_2 concentration as their younger controls (mean ± SE, 368 ± 51 *vs.* 167 ± 20 pmol/L; $P = 0.002$) and a strong trend to higher average monthly E_2 levels (582 ± 76 *vs.* 406 ± 23 pmol/L; $P = 0.057$). In addition, the older group had almost twice the levels of FSH. The large

TABLE 1. Comparisons of gonadotropic and somatotropic axis parameters in younger and older women

	Young controls (n = 8; aged 19–34 yr)	Older women (n = 8; aged 42–46 yr)	*P* value
Average age (yr)	26 ± 1	44 ± 1	
BMI (kg/m^2)	21 ± 1	24 ± 1	NS
Somatotropic axis			
GH 12-h AUC (μg/min·L)	427 ± 130	171 ± 35	0.036
No. of GH pulses/12 h	4.9 ± 0.8	5.6 ± 0.8	NS
Maximum GH pulse amplitude (μg/L)	6.2 ± 2.5	3.0 ± 0.5	NS
Average GH pulse amplitude (μg/L)	2.8 ± 0.9	1.5 ± 0.4	NS
IGF-I (nmol/L)	31.3 ± 3.5	22.7 ± 2.1	0.055[a]
Gonadotropic axis			
No. of LH pulses/12 h	9.5 ± 0.7	6.9 ± 1.2	NS
Average LH pulse amplitude (IU/L)	3.3 ± 0.3	3.9 ± 0.7	NS
Pooled day 3 E_2 (pmol/L)	167 ± 20	368 ± 51	0.002
Average daily E_2 (month, pmol/L)	406 ± 23	582 ± 76	0.057[a]
Day 2 P (pmol/L)	1.8 ± 0.2	1.5 ± 0.2	NS
Day 3 FSH (IU/L)	5.9 ± 0.4	9.9 ± 2.6	NS
Cycle day of LH surge	14 ± 1	11 ± 2	0.031

All data are reported as the group mean ± SE.
[a] Differences of borderline statistical significance.

variance in this group precluded a statistically significant difference. The dynamics of pulsatile LH secretion were very similar in both groups; pulse counts and average pulse sizes were equivalent, and the data sets were normally distributed.

Daily blood samples taken throughout the menstrual cycle revealed that the group of young control women had LH surges between cycle days 10–16 (inclusive; average day 14). All but one older reproductive-aged woman produced a LH surge between days 7–13 (inclusive; average day 11.5 for all older subjects). The remaining older subject did not experience a surge until day 25. Investigation of the daily blood samples used to evaluate the study cycles revealed normal peak follicular E_2 and luteal P levels in this individual despite a prolonged follicular phase. Even with the delayed ovulation in this subject, the general shortening of the follicular phase in the group of older subjects was significant (day 11.5 ± 2.0 *vs.* 14.1 ± 0.8; $P = 0.031$).

Parallel GH secretion patterns throughout the day for the younger and older subjects are graphed in Fig. 2. The sum of the areas under individual curves was used to determine the GH AUC values for each group. The group of GH AUC values from the older subjects was distributed in a pattern suitable for parametric analysis. However, the group of GH AUC values from the younger women differred significantly from a normal distribution (by SW test, $P = 0.025$) due to the presence of an outlier that fell more than 3 SD above the mean (the truncated GH concentration curve derived from this subject is indicated with an *arrow* in Fig. 2). Common mathematical transformations failed to normalize the data. Comparison of the GH AUC between the two groups using all subjects revealed a significant decline in the older group (427 ± 130 *vs.* 171 ± 35 μg/min·L; $P = 0.036$, by Wilcoxon's rank sum test).

The PULSEFIT mathematical model idealizes pulses as narrow sharp peaks. LH pulses identified by the program correspond very well with both visually apparent peaks and those identified by the S+B method. In the case of GH, which has rather broad concentration peaks, PULSEFIT describes these wide peaks as a cluster of narrower pulses. PULSEFIT-identified GH peaks, therefore, were grouped together if they fell 20 min apart or less. As most published reports show that GH pulses occur at least 1–2 h apart, it is unlikely that this grouping will result in failure to identify some pulses (17). The pulse patterns deduced from this modified PULSEFIT analysis agree well with S+B and visually identified pulses. The slope of the regression line comparing the number of S+B peaks per subject to the number of PULSEFIT peaks per subject approached unity (slope = 1.03; r = 0.63; $P = 0.01$), confirming the close agreement of the two objective methods.

GH concentration patterns were also analyzed via a Lowess smoothing curve (Fig. 2). This curve provides a mathematically robust way to look for underlying patterns of GH concentration throughout the day and is affected little by outliers. A remarkably similar pattern of daily GH secretion is apparent among the 16 women as a whole and when the younger and older groups are analyzed separately. The entire group appears to have GH nadirs at approximately 1000, 1400, and 1930 h, and overall GH peaks occurred at approximately 1200 and 1500 h. Breakfast, lunch, and dinner were consumed (on the average) at 0840, 1250, and 1720 h, respectively. GH concentration nadirs generally occurred about 1–2 h after food consumption (although meal times, durations, and quantities were not regimented, and all snacks were not recorded). To statistically analyze this apparent association of falling GH concentrations with meal consumption, the actual times at which the subjects began to eat full meals were identified; 37 of these events were noted in the blood sampling logs. Average GH concentrations for the hour immediately preceeding each meal and during the second hour after beginning the meal were determined. The average GH level before eating fell, on the average, over 4-fold, from 0.86 μg/L before meals to 0.19 μg/L in the hour after eating ($P < 0.0001$, by paired *t* test). Similar analysis, comparing spot GH values at the time of initiating a meal and 1 h later, produced similar findings (0.78–0.24 μg/L; $P < 0.01$, by paired *t* test).

Discussion

Our study compared somatotropic and gonadotropic axis activities in younger and older normally cycling reproduc-

tive-aged women. This is the first study, to our knowledge, that has shown a decrease in GH and IGF-I levels in women over age 40 yr while controlling for cycle-related changes in sex steroids, cycle normalcy, and body habitus. We have thus been able to support the hypothesis that GH concentrations decrease throughout a woman's reproductive years, a period that is also associated with modest menstrual cycle and major fertility alterations.

Although the length of an adult woman's menstrual cycle shortens with aging, the characteristics of daily serum LH concentrations remain basically the same as long as ovulation occurs regularly (2, 3). Our findings confirm this notion. In contrast to LH, FSH levels rise despite regular menstrual cycles. A decrease in cellular FSH receptors in the follicular apparatus, a decrease in required trophic cofactors or gonadotropin inhibitors, or an accumulation of time-related damage to the primary oocyte or follicle may play a role in this phenomenon (3). The rise of FSH associated with a decrease in fecundity in older, regularly cycling women has been widely reported (18).

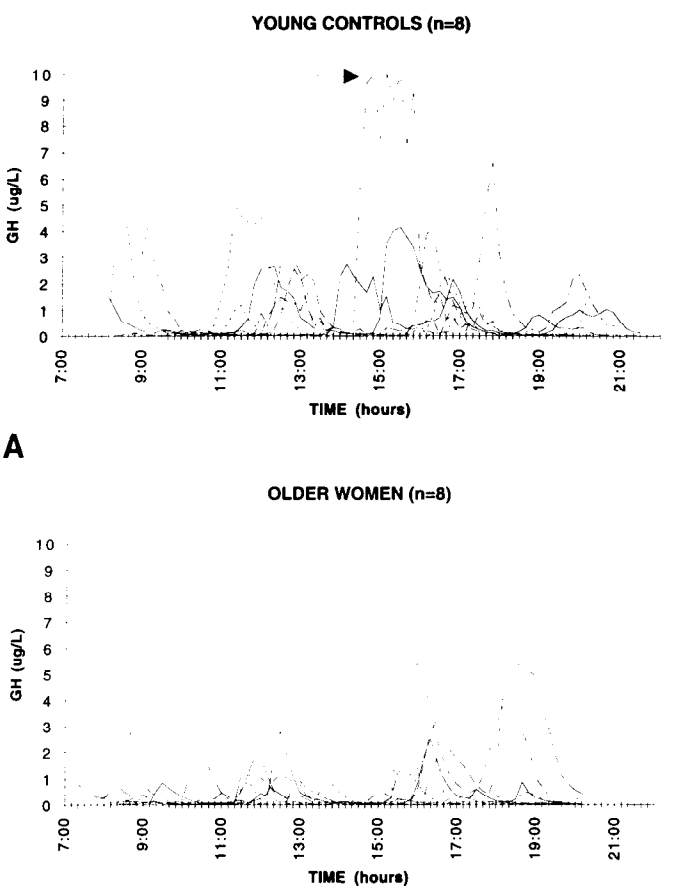

FIG. 1. A, The GH concentration curves of the young control subjects determined from frequent sampling performed for 12 h during the day in the early follicular phase of the menstrual cycle. The AUCs were quantified and used to evaluate somatotropic axis activity. The *arrow* identifies a very large GH peak (that measured 23 μg/L at its highest point), which was truncated to show greater detail of the remaining subjects' concentration curves. Some lines are dashed to aid in the visual differentiation of subjects. B, The daytime GH concentration curves of the older reproductive-aged women, also performed in the early follicular phase. The AUC is significantly less than that in the younger subjects (171 *vs.* 427 μg/min·L; $P = 0.036$). Note the apparent coincidence of the GH pulses that occurs around 1200 and 1600 h and the relative lack of pulsatile activity seen around 1000 and 1400 h in both groups.

In addition to FSH, a rise in early follicular E_2 levels in older reproductive-aged women has sometimes been observed (19). This rise probably occurs from increased FSH drive and the resulting earlier recruitment of ovarian follicles. Previous studies that characterized the changes in the menstrual cycle with aging have reported that follicular phase E_2 levels in perimenopausal women are significantly lower than those in younger controls (2, 3). Two reasons may explain the apparent discrepancies between our present study and the former ones. First, our subjects were 42–46 yr old, whereas the subjects in these aforementioned studies were 46–56 yr old. Secondly, we compared hormone levels on menstrual day 3, whereas the previous reports compared levels adjusted to equal time intervals from the midcycle E_2 peak.

The rise of endogenous E_2 in the late follicular phase as well as oral E_2 supplementation have both been associated with increased GH levels in women (17, 20, 21). Therefore, one might expect to see a higher GH AUC among the older female subjects in the context of their doubled E_2 concentrations in the early follicular phase. In fact, the opposite association was observed. It would appear that the stimulatory effects of endogenous E_2 in these subjects have been overridden by the effects attributable to aging alone.

We performed 12-h daytime frequent sampling for GH. Although it is known that children exhibit a significant increase in GH secretion during the night, especially during the early hours of sleep, the sizes of these sleep-associated GH peaks decrease with maturation into early adulthood (22, 23). Several studies have found that adults secrete equivalent amounts of GH during the day and night (24, 25). Women in the early follicular phase have specifically been shown to have no diurnal variation in GH concentrations (26). Twelve-hour daytime sampling might, therefore, be expected to yield a reasonable representation of an adult woman's 24-h concentration pattern. The fact that our sex steroid, gonadotropin, and IGF-I findings are also in agreement with observations from previous studies indicates that daytime sampling is adequate to monitor these parameters as well (2, 10). The data also confirm the well known inhibitory effect of adiposity (as reflected by BMI) on GH pulse sizes (8).

Although we had only 16 individuals in our study, we have shown with statistical likelihood that GH concentrations are lower in older reproductive-aged women than in younger-aged controls and that IGF-I concentrations fall with age. These findings indicate that somatotropic axis activity in adult women decreases with aging (as it does in men) independent of changes in the reproductive axis. Estrogenic influences during a woman's reproductive years appear to augment somatotropic activity, but these effects are not sufficient to override the progressive decrease in central activity due to the aging process.

Our findings clearly show that cycling women demonstrate active daytime GH pulsatility, which appears to be responsive to nutritional and/or circadian influences. Hy-

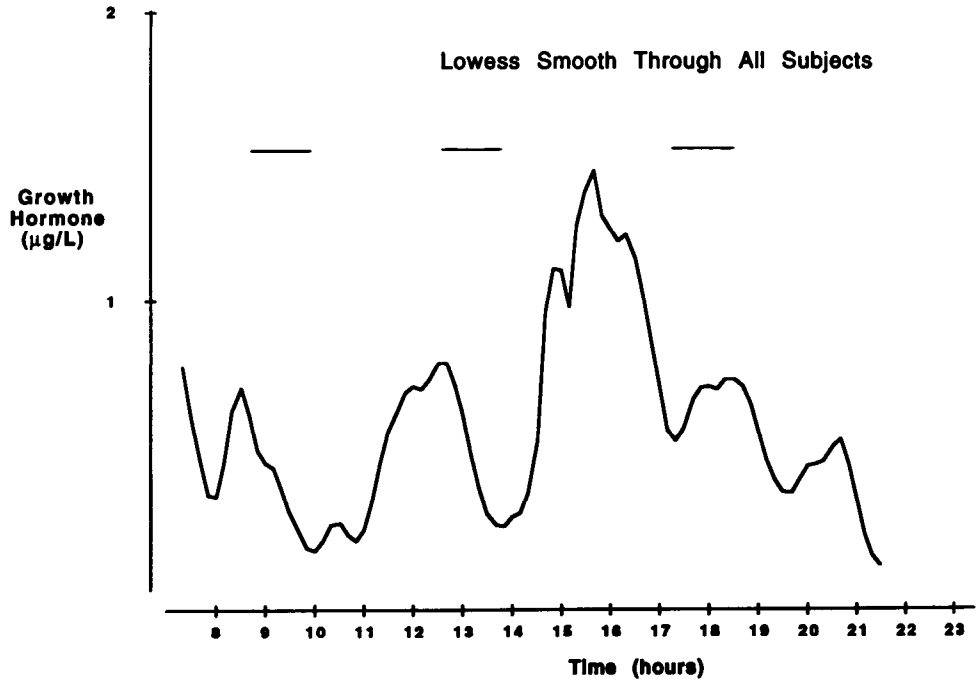

FIG. 2. This diagram depicts the Lowess smoothed fit through the scatterplot created from the entire GH concentration data set for all subjects. This group of ovulatory women in the early follicular phase demonstrates a remarkably consistent underlying pattern of GH secretion. GH nadirs occur at approximately 1000, 1400, and 1930 h, and GH peaks occur at approximately 1200 and 1530 h. Very similar patterns were observed when the Lowess function was plotted separately through the scatterplots of the younger or older group's data (not shown). The *left side of the horizontal bars above the curve* indicates the average times of the start of meal consumption (0840, 1250, and 1720 h; breakfast, lunch, and dinner, respectively). A fall in GH about 1–2 h after meals is apparent and is confirmed statistically (see *Results*).

perglycemia, for example, has long been known to suppress GH concentrations (27). Our observation of a general decline in GH concentrations after meals is consistent with this physiological response. It also appears that reproductive-aged women have active daytime GH concentration pulses; a very different situation from that seen in men, who show little daytime activity (28).

We have noted a slight trend for IGF-I levels to correlate with average GH pulse amplitudes ($r = 0.41$; $P = 0.11$). However, virtually no relationship was noted between IGF-I levels and maximum GH pulse values or GH AUC. This is consistent with previous work, which has shown that IGF-I is proportional to the amount of GH secreted as pulses (4). One would expect the GH AUC to have less correlation with IGF-I because this measurement incorporates between-pulse values of GH. Maximum GH pulse values do not necessarily reflect overall somatotropic axis activity either, because they may occur in relative isolation.

In summary, we have demonstrated in a prospective controlled study that older reproductive-aged women experience a fall in serum IGF-I levels and integrated GH concentrations compared to those in a group of young control subjects. Although we attempted to control for the effects of sex steroids, elevated E_2 levels among the older subjects may have obscured even larger underlying changes in somatotropic axis activity that could be attributable to the aging process.

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