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Elements in the Pathophysiology of Diminished Growth Hormone (GH) Secretion In Aging Humans

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Remarkable decreases in growth hormone (GH) secretion accompany healthy aging. The pathophysiology of this hyposomatotropism is confounded by concurrent changes in body composition (with increased visceral fat), physiological declines in estrogen and androgen concentrations, differences in gender responses to aging, and alterations not only in the quantity of GH secreted, but also (as more recently evident) in the orderliness or regularity of the GH release process (e.g., as assessed by approximate entropy). In addition, physical fitness or aerobic capacity also positively modulates GH secretion. Lastly, confounding variables such as altered sleep patterns and nutritional state may contribute to overall regulation of the GH axis in aging. Despite confounding variables, available human experiments suggest partial growth hormone-releasing hormone (GHRH) deficiency in healthy older individuals, presumptively combined with somatostatin excess, and disruption of the moment-to-moment pattern of coordinated and orderly GH release. Here, the authors review these selected facets of recent experimental evaluation of the human GH insulin-like growth factor-I (GH-IGF-I) feedback axis in aging humans.

Introduction

Since the advent of radioimmunoassay, and in a more compelling way immunoradiometric, immunofluorometric, and recent chemiluminescence assays, profound physiological variations in the secretory activity of the growth hormone-insulin like growth factor (GH-IGF-I) axis have been documented in experimental animals and in the human *(1–34)*. Such changes are remarkable quantitatively, reflecting extremes as large as 30-fold within the human life span. Thus, in the later stages of puberty during maximal linear growth, healthy boys secrete approx 1–1.5 mg of GH daily, whereas some healthy older adults and/or obese middle-aged individuals produce as little as 50–300 μg GH daily *(24,26,27,29,35)*. The regulatory mechanisms that underlie such prominent discrepancies in daily GH secretion rates during human aging embrace at least (growth hormone-releasing hormone) GHRH and somatostatin actions, possible reciprocal interactions between GHRH and somatostatin, and/or the feedback impact of IGF-I or GH itself on the hypothalamo-somatotroph unit *(36)*. Definitive knowledge of the dominant and subordinate roles of specific control loci within the aging GHRH-somatostatin/GH/IGF-I axis is not yet available in the human, but important and novel experimental insights have been gained recently via physiological experiments carried out in the aging human adult male and female. Such experiments are often challenging to interpret, given multiple confounding factors that attend aging and that strongly influence neuroendocrine activity of the GH-IGF-I axis *(37,38)*. Evident confounding variables include (but are not limited to) an increase in visceral fat accompanying changes in body composition during healthy aging *(39)*; varying sex-steroid hormone concentrations, with falling testosterone in older men and diminished estrogen production in postmenopausal women *(26,27,35,40,41)*; unequal physical fitness or aerobic capacity in young and older individuals *(28)*; differences in the quality and quantity of sleep in older subjects, which may modulate GH secretion that occurs predominantly during stages III and IV of normal sleep *(42)*; disparate nutritional status, since nutrition is a strong regulator of IGF-I-directed negative feedback *(43)*; and, various interactions among the foregoing factors. Indeed, recent studies indicate that age and adiposity exert individually significant negative effects on secretory output of the GH axis, and also show evidence of a strongly interactive (negative) impact *(26,27,29,35)*. Here, the authors will review the primary pathophysiological issues briefly, evaluate existing experimental evidence in the human for GHRH deficiency and/or somatostatin excess in healthy aging, and discuss important ongoing and new studies that will be required to clarify these important issues.

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Primary Pathophysiological Issues Concerning Mechanisms of Diminished GH Secretion in the Aging Human

Body Composition

Compelling evidence now exists that a dominant physiological variable that negatively modulates secretory activity of hypothalamo-somatotroph axis is adiposity, and more particularly, visceral fat *(26,27,29,35,39)*. For example, as shown in Fig. 1, in healthy men stratification by percentage adiposity as determined by underwater weighing strongly modulates the negative impact of age on GH secretion. In particular, as noted in Fig. 1A, individuals who are moderately to markedly obese have profound suppression of mean 24-h serum GH concentrations measured by chemiluminescence assay across all ages, with relatively little additional discernible suppressive effects of age *(29)*. In contrast, adults with a lower percentage of total body fat exhibit strong age-dependent declines in daily GH secretion rates. Moreover, body fat percentage interacts not only with age but also with the positive effect of testosterone on GH secretion (Fig. 1B). In particular, within the healthy adult male population, individuals with a higher percentage of total body fat show reduced GH secretion at all serum testosterone concentrations, whereas lean individuals manifest a strongly positive correlation between serum total (or free) concentrations of testosterone and the daily GH secretion rate *(29)*. Thus, an important contemporary concept is that body fat percentage in the healthy adult human population strongly masks the negative effect of age on daily GH release, and markedly attenuates the otherwise positive association between plasma androgen concentrations and daily GH secretion. Clearly, clinical investigation of the GH axis must, therefore, recognize at least the three primary variables, age, percentage body fat, and serum androgen (or estrogen in women) concentrations, as well as other confounders discussed here.

The topographic distribution of total body fat appears to be a critical determinant of the impact of relative adiposity on the GH axis. Thus, recent studies using computerized axial tomography to evaluate visceral fat deposition quantitatively show increases in the elderly *(44)* and reveal a strongly negative correlation between visceral fat content and daily GH secretion in a cohort of men and women spanning a range of ages *(39)*. Indeed, the primary discriminative variable in multiple regression analysis in this population was visceral fat, which strongly controlled the daily GH secretory rate as estimated by deconvolution analysis, and/or the mass of GH secreted per burst. Indeed, the gender difference in GH secretory rates (discussed further below) was accounted for substantially by differences in visceral fat deposition in men and women. Thus, a plausible argument is that visceral fat represents a dominant variable in the feedback regulation of the GH axis *(39)*. Whether this is mediated specifically or exclusively via leptin or other fat-cell-derived regulatory molecules is not known. However, leptin correlates negatively with GH secretion.

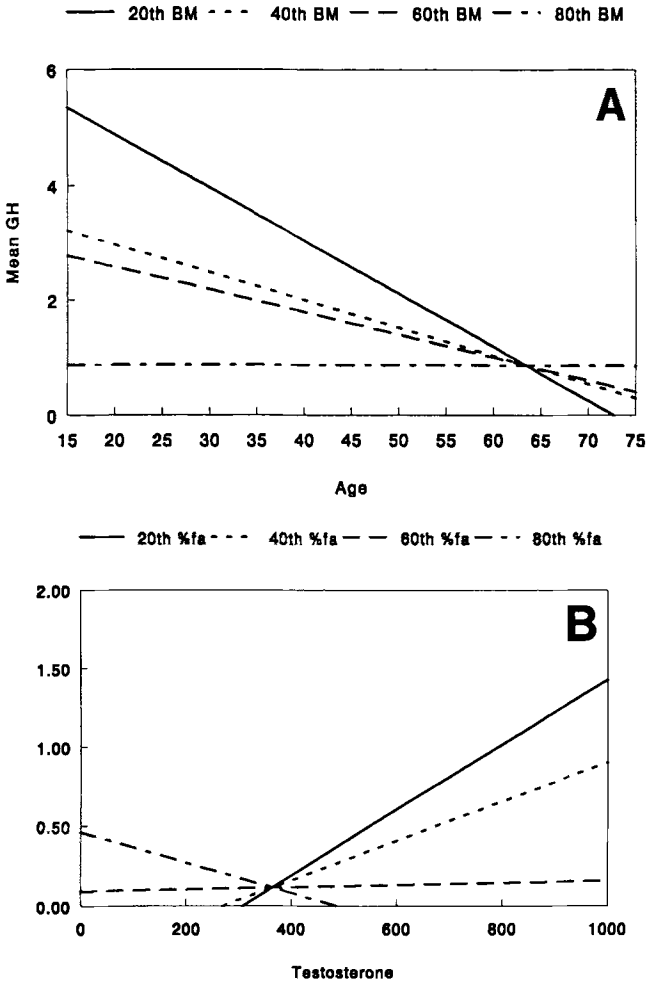

Fig. 1. **(A)** Confounding impact of relative obesity on the age-associated decline in mean (24-h) serum GH concentration in a population of healthy men. The individual lines denote regression-defined relationships between 24-h mean serum GH concentrations and age for individual strata of relative obesity *(29)*. Thus, individuals with lower body fat (uppermost curve) show a strongly negative impact of age on GH release over 24 h, whereas men with more body fat (lower curves) exhibit significantly reduced GH release at all ages. **(B)** Relationship of serum total testosterone concentrations to mean 24-h serum GH concentrations in a group of healthy men stratified according to relative adiposity. Note that in lean individuals (uppermost curve) testosterone bears a strongly positive correlation with serum GH concentration. This correlation is lost with increasing body fat. (Adapted with permission from ref. 29.)

With advancing age, the decline in serum testosterone concentrations, and the increase in percentage body fat (or visceral fat deposition, more particularly) are accompanied by greater quantifiable disorderliness of the GH release process, as estimated recently by the approximate entropy (ApEn) statistic *(30,45–47)*. As shown in Fig. 2, the absolute ApEn value of 24-h serum GH concentration profiles increases significantly in a healthy aging male population *(29)*, where increased ApEn values denote

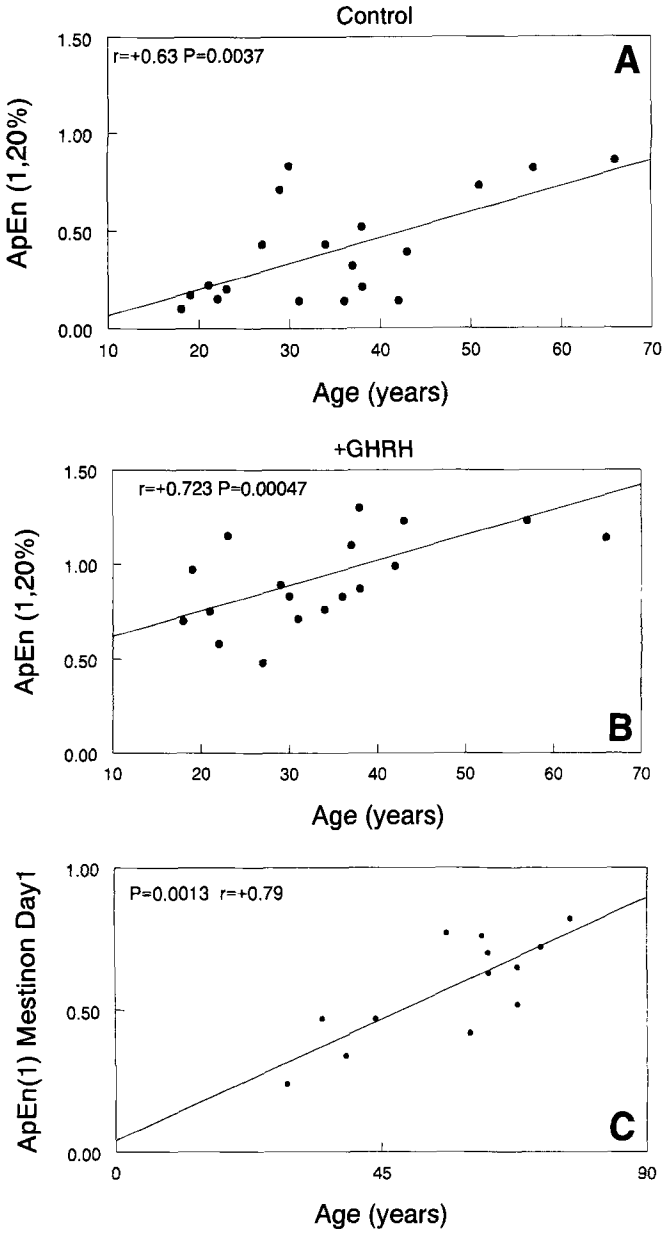

Fig. 2. Relationship between age and the disorderliness of the GH release process, as quantified by the approximate entropy statistic (ApEn). ApEn is a statistic that quantifies the relative disorderliness or irregularity of hormone release with higher ApEn values denoting greater disorderliness or irregularity of GH release *(29,46)*. **(A)** Shows the effect of age on GH ApEn under baseline (control) conditions; **(B)** relates ApEn to age during an exogenous fixed GHRH clamp imposed by iv GHRH pulses every 90 min for 3 d; and **(C)** relates ApEn to age during Mestinon (pyridostigmine) treatment to reduce presumptively endogenous somatostatin tone. The persistence of increased disorderliness of GH release with age independently of provision of fixed impulses of GHRH by exogenous injection, or partial withdrawal of somatostatin via pyridostigmine treatment, suggests age-related loss of coordination of feedback control within the GHRH-somatostatin-GH axis, which can be viewed as a multivalent servocontrol feedback system *(48)*. (Adapted with permission from refs.*33* and *53*).

greater process randomness or reduced orderliness of GH secretion. This estimate is complementary to traditional hormone pulse analysis and deconvolution quantitation of secretion, since ApEn measures the reproducibility of subpatterns within the hormone profile. Such subpatterns are believed to reflect network integration, or the coordinate feedback control that characterizes an intact neuroendocrine axis *(30,45–48)*. The greater disorderliness of 24-h GH release observed in healthy aging men is perhaps a distinct marker of the aging process *per se*, since increasing irregularity of hormone release has now been demonstrated also for lutein-izing hormone (LH), testosterone, and insulin *(29,49,50)*. Thus, the authors would propose that one pathophysiological feature of the hyposomatotropism of aging is disruption of normal minute-to-minute feedback control of, and orderly interactions within, the GH-IGF-I axis, perhaps reflecting a more general decline in neuroendocrine servomechanistic control also recognized for ACTH-cortisol, LH-testosterone, and more recently, insulin *(49–52)*. Recent studies indicate that greater disorderliness of GH release in aging is not obviated by fixed exogenous pulses of GHRH for 3 d *(33)*, or presumptive somatostatin withdrawal via pyridostigmine treatment for 2 d *(53)*: Fig. 2. These observations strongly suggest disturbance(s) in GHRH-somatostatin interactions in aging, rather than a singular defect at either locus alone.

Confounding by Gender or Sex-Steroid Hormones

As highlighted in Fig. 3, the negative impact of age on 24-h mean serum GH concentrations is approx two-fold more evident in men than in (premenopausal) women *(28)*. In particular, in a study of 32 premenopausal women and 12 men whose ages span a similar range, the authors observed that the standardized coefficient (slope) of the negative regression of 24-h serum GH concentration on age in men is approximately twice that in women. This difference is highly significant statistically, and suggests that the premenopausal female manifests some protective mechanism(s) (possibly via estrogen) that can limit the rate of decline of daily GH secretion with age, at least before the menopausal years. Available studies indicate that this gender contrast applies not only to the effect of age on GH secretion, but also to the positive impact of physical fitness and the negative impact of increasing percentage body fat *(28)*. Indeed, in all three circumstances (namely, age, aerobic fitness, and percentage body fat), women show an approx a 2-fold lower impact than men on daily GH secretion, as defined by the standardized coefficients of linear regression *(28)*.

Of considerable further interest by way of gender differences, women exhibit a significantly greater ApEn of GH release, signifying quantifiably greater irregularity or disorderliness of GH secretion over 24 h *(30)*. This strong gender contrast is also observed in the rat, as quantified by the new regularity statistic, ApEn *(29)*. Sex-steroids appear to condition this gender difference, since the administration of testosterone to boys with constitutionally delayed puberty, or estrogen to prepubertal girls with Turner's syndrome, will increase ApEn over intervals of 1–6 wk *(30,48,54)*.

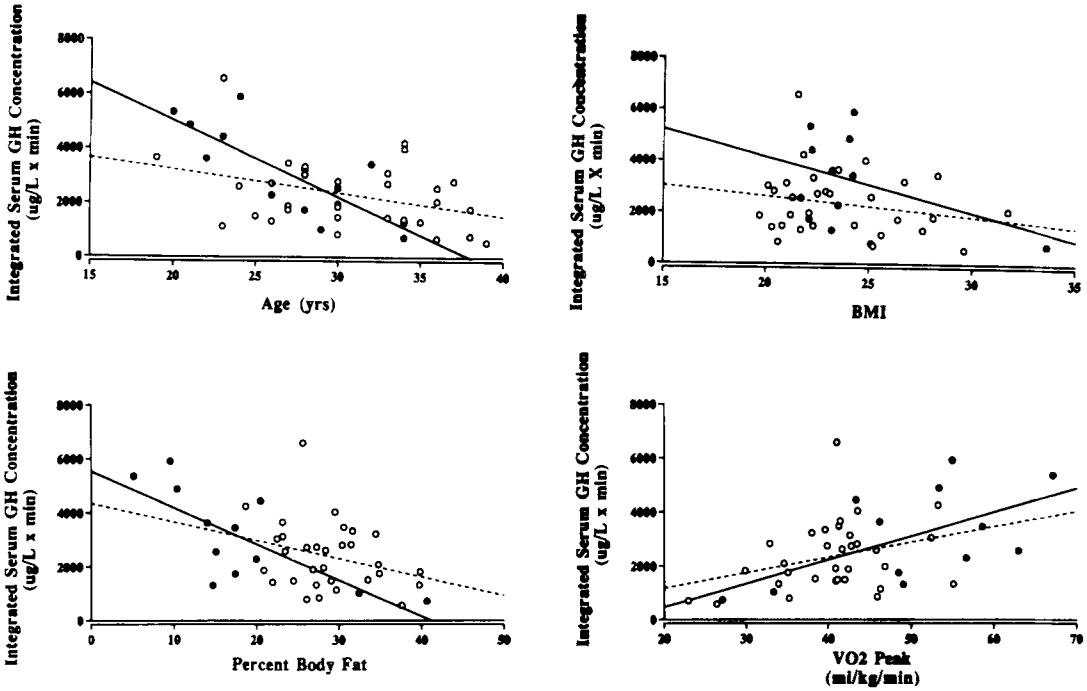

Fig. 3. Impact of gender on the negative effect of age, percentage body fat, and body mass index versus the positive impact of physical fitness, on 24-h mean serum GH concentrations in men ($N = 12$) and women of premenopausal age ($N = 32$). (Adapted with permission from ref. *28.*)

Indeed, treatment with very small amounts of testosterone (25 mg im once) will alter the orderliness of the GH release process within 2 wk in prepubertal boys with isolated GnRH-LH deficiency *(54)*. The effects of testosterone and estrogen are not mimicked by the 5-α reduced androgen, 5-α dihydrotestosterone *(48)*. Hence, in the human, a plausible current hypothesis is that testosterone acting via aromatization to estrogen, and estrogen *per se*, regulate feedback coordination within the GHRH/somatostatin-GH-IGF-I axis, such that the minute-to-minute orderliness of GH release, in addition to total GH secretion, is governed differentially in the male and female.

Available studies also show a strongly positive relationship in larger populations of men and women of various ages between serum total or free estradiol concentrations, and the pulsatile mode of GH release *(40)*. Estrogen administration in adequate amounts increases GH secretion in older women *(55–57)*. Moreover, in cohorts of healthy men or boys studied at various stages of puberty, serum total and free testosterone concentrations are strongly correlated with GH secretory burst mass and the daily GH production rate *(24)*. Recent clinical investigations indicate that testosterone administration to men treated with a gonadotrophin-releasing hormone (GnRH) agonist to downregulate the LH-testosterone axis stimulates increased GH secretory burst mass *(58)*. Other studies indicate that increased secretion of GH in this context either in normal men or men with primary gonadal failure is antagonized by an antiestrogen *(59,60)*. This is illustrated in Fig. 4 in experiments in which leuprolide was used to reduce endogenous androgen secretion, or exogenous testosterone enanthate was injected to increase serum total and free testosterone concentrations and thereby clamp the androgen milieu *(58)*.

The authors infer that gender differences in the negative impact of aging on the GH axis exist in the premenopausal age group, and are likely conditioned by estrogen. In addition, the fall of daily GH secretion in postmenopausal individuals is imposed to an important degree by relative estrogen deficiency. This inference, and whether the physiological decline in testosterone production with aging in men is causally linked to the parallel decline in GH secretion (as inferred indirectly by regression analysis), both require further study.

Confounding by Physical Fitness or Aerobic Capacity

As intimated earlier, physical fitness, as judged for example by the maximal oxygen consumption rate, is a strongly positive correlate of the mean 24-h serum GH concentration *(28)*. Of considerable interest, this positive association is diminished approx two-fold in strength in women compared to men, thus illustrating a strong gender difference in the impact of physical fitness upon GH neurosecretory activity. Since diminished maximal oxygen consumption, reduced muscle mass, and declining physical stamina occur during the healthy aging process, the impact of aerobic exercise and strength training on the GH axis will be important to assess further in the elderly. In young adults, endurance training by way of long distance running in young women increased 24-h serum GH concentrations by approx twofold, in the subset of women who exercised

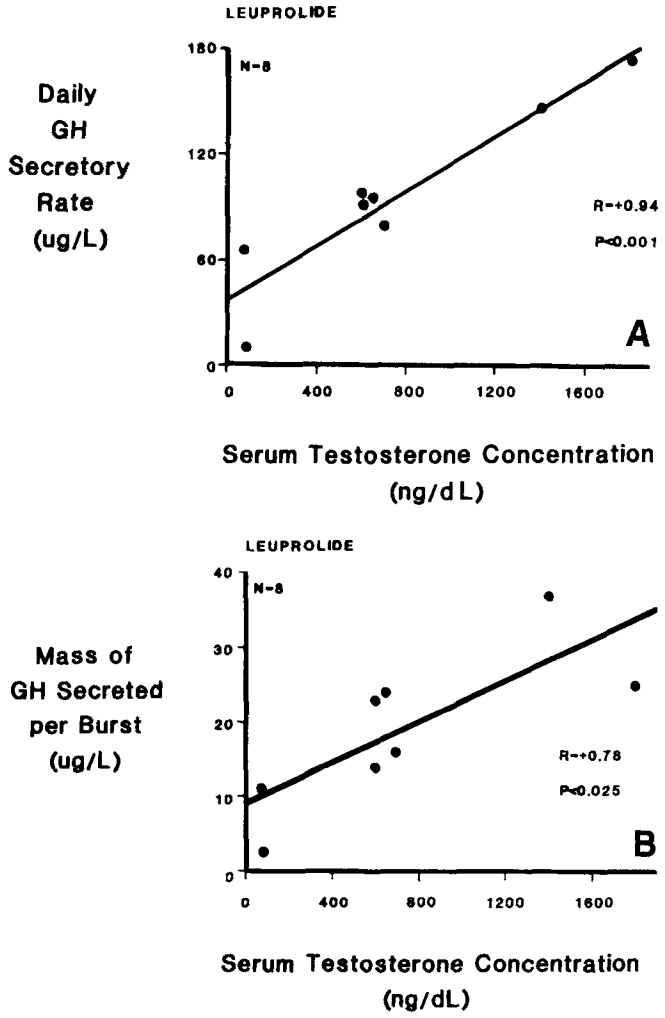

Fig. 4. Relationship between serum testosterone concentration and the daily GH secretory rate **(A)** and GH burst mass **(B)** in eight healthy young men administered either the GnRH agonist, leuprolide, to reduce serum testosterone concentrations, or testosterone enanthate im to produce high-physiological serum testosterone concentrations compared to normal midrange serum testosterone concentrations in healthy young adults. (Fryburg, D. and Veldhuis, J. D., unpublished observations.)

consistently above the lactate threshold, but not in a matched subgroup who exercised for the same total training volume and duration but at or below their individual lactate thresholds *(61,62)*. The extent to which exercise training and improved physical fitness can influence the aging GH axis is under intensive study *(3,63–65)*. Other potential confounding variables in the evaluation of the GH-IGF-I axis in aging include changes in sleep quality and quantity, nutrition, exogenous sex-hormone replacement, the use of medications that may influence the output of the GH axis, and so forth.

Neuroendocrine Network Mechanisms

The GH axis is under primary control by both a stimulating and inhibitory hypothalamic peptidergic system, namely somatostatin and GHRH *(37,66)*. In addition, existence of a possible natural ligand for the GHRP (growth hormone releasing peptide) receptor has been postulated, but not yet documented. Regulated release of the GHRH and somatostatin, and responsiveness of the somatotroph population, are believed jointly to coordinate the pulsatile secretion of GH, and influence the absolute amounts of GH stored and secreted. Thus, various clinical experiments have been carried out to identify possible GHRH deficiency, and/or somatostatin excess, in the pathophysiology of healthy aging.

To this end, the authors have injected GHRH at a dose of 0.3 μg/kg iv every 90 min for 3 d in men of varying ages and body compositions *(33)*. At baseline, as shown in Fig. 5, the relative capability of endogenous GH pulses to increase plasma IGF-I concentrations (normalized to GH secretory burst mass) was actually enhanced or not reduced respectively as the percentage body fat or age increased. Whereas the absolute increases in plasma IGF-I and GH concentrations during the GHRH clamp in older (and obese) individuals failed to equal those of the younger (leaner) counterparts, the fractional increases (percentage rise above baseline) were accentuated in the elderly (and obese) subjects. Twice-daily or longer-term (2 wk) continuous GHRH treatment was also shown to significantly increase plasma IGF-I levels in older men *(19,67)*. Thus, a partial GHRH deficiency is suggested in aging men, but this deficiency alone will not account for the total decrease in GH and IGF-I production *(9,33)*.

Other interpretations of the pathophysiology of aging include increased somatostatin inhibitory tone *(53)*, possible increased negative feedback by GH or IGF-I, diminished somatotroph biosynthetic or storage capacity, and so forth, and/or reduced tissue responsiveness to GH *(29,68)*. Of interest in relation to IGF-I's negative feedback, fasting-induced IGF-I deficiency in older volunteers increases GH secretion significantly, albeit not equivalently to that in young adults studied in the same paradigm *(11)*. In addition, combined administration of L-arginine to reduce endogenous somatostatin inhibitory input and GHRH or GHRP (a hexapeptide that synergizes with GHRH) or insulin injection will evoke substantial acute GH release in older individuals, supporting a view of combined GHRH and somatostatin dysregulation *(13,18,69–74)*. On the other hand, available data do not indicate whether GH autonegative feedback varies with age. Studies do show that GH clearance does not appear to change greatly with age *(34,75)*.

Use of the indirect cholinergic agonist, pyridostigmine, to putatively reduce hypothalamic somatostatin release, and possibly concurrently increase rebound endogenous GHRH secretion, unmasks a significantly negative impact of body mass-index on the (pyridostigmine-) stimulated mass of GH secreted per burst, and the daily GH secretion rate (Fig. 6). In contrast, in this cohort of 13 men, age did not impose any inhibition on pyridostigmine's stimulatory

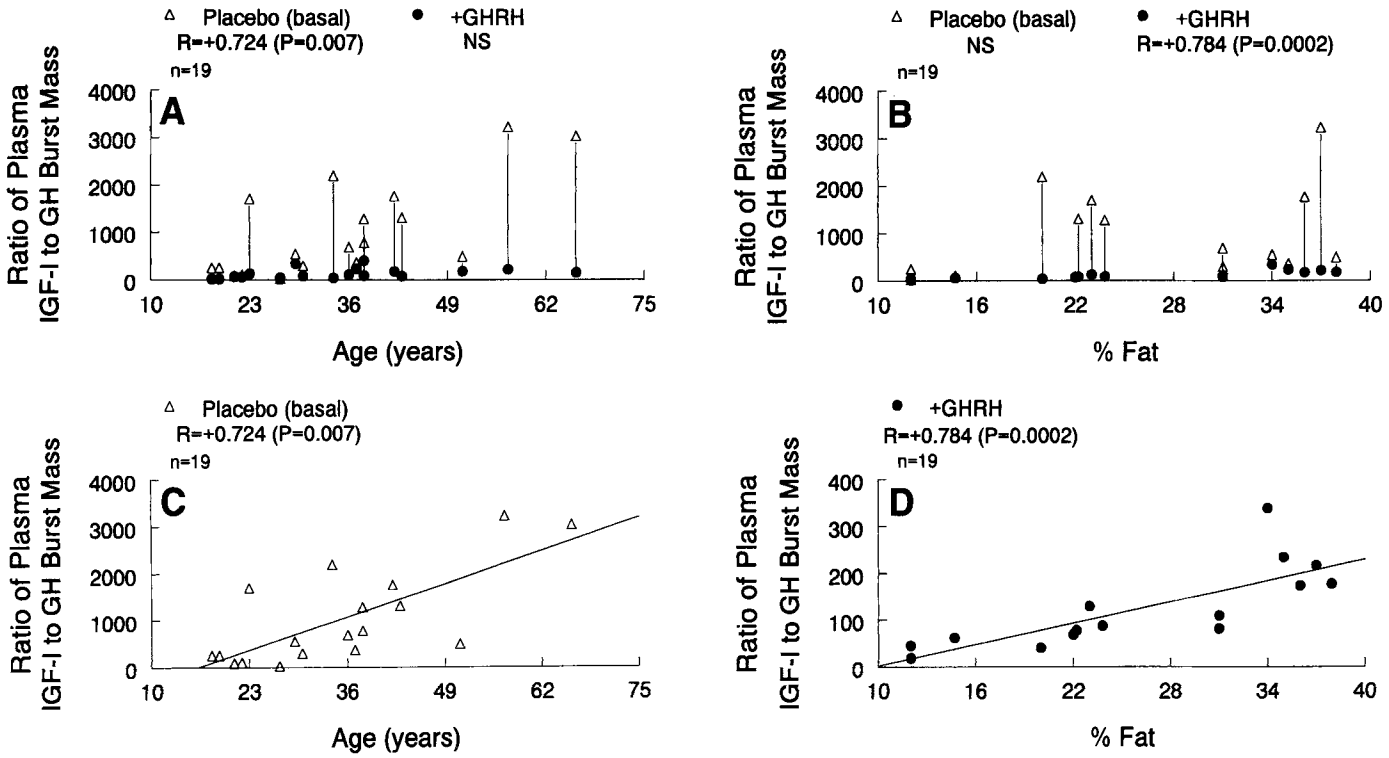

Fig. 5. Impact of age **(A)** and relative adiposity **(B)** on the plasma IGF-I response per unit GH secretory burst mass basally (open triangles) and following 3 d of iv GHRH (0.33 μg/kg every 90 min) clamping (closed circles) in 19 men of varying ages and body compositions. Expanded view and linear regression analysis of GHRH-treated responses of IGF-I versus age **(C)** or basal IGF-I/GH burst mass versus percentage body fat **(D)**. The plasma IGF-I concentration was measured in, and GH secretory burst mass was calculated by deconvolution analysis of, 24-h 10-min blood sampling after placebo versus during an exogenous GHRH clamp. Percentage body fat was determined by underwater weighing. (Adapted with permission from ref. *33*.)

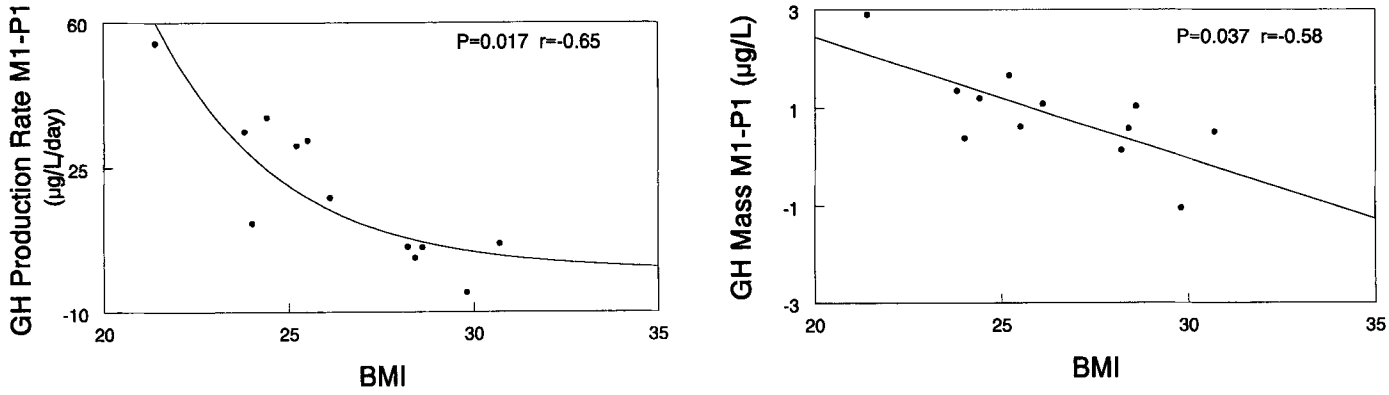

Fig. 6. Negative impact of BMI on pyridostigmine-stimulated 24-h GH secretion in 13 healthy men. **(Left)** Incremental daily GH production rate. **(Right)** Incremental mass of GH secreted per burst. Volunteers were treated for 48 h with placebo (P) versus pyridostigmine (Mestinon, M) 60 mg orally four times daily, the latter in order to presumptively reduce hypothalamic somatostatin's inhibition of the GH axis, and possibly to heighten rebound endogenous GHRH release *(53)*. The ability of pyridostigmine to increase GH secretion over placebo (M-P) was negatively correlated with the BMI in these normal men. In contrast, age did not have any significant impact on GH responsiveness to pyridostigmine (*see* text pages 5 and 6 for discussion). (Adapted with permission from ref *53*.)

effect *(53)*. This experiment suggests that the relative obesity component of aging's suppression of GH secretion is not fully overcome by pyridostigmine's presumptive reduction of somatostatin release (with possible rebound GHRH release concomitantly), whereas that of aging *per se* may be more nearly overcome. However, pharmacological agents are not yet available that offer proven complete or specific somatostatin withdrawal. For example, some studies suggest that pyridostigmine may also release endogenous GHRH, which could occur secondarily following somatostatin withdrawal in view of somatostatin's reciprocal (inhibitory) connection to GHRH secretion *(76–78)*. Thus, any procedure

that reduces somatostatin release could in turn limit the ability of somatostatin to inhibit GHRH secretion, resulting in relatively increased GHRH release. Thus, somatostatin-withdrawing agents are unlikely to test a single component of the GH axis.

The GH axis can also be driven by hexapeptide agonists of the GHRP family, and nonpeptide analogs of such agonists *(32,79–81)*. In general, these GHRP agonists strongly facilitate GHRH action, and in combination with GHRH or L-arginine will evoke massive GH secretion even in obesity *(82,83)*. Recent studies using an oral non-peptidyl analog of GHRP show that older individuals can mount significant increases in 24-h GH secretion as well as in plasma total IGF-I concentrations following short-term administration of such agents *(32)*. Moreover, the release of GH in this pharmacologically stimulated context remains pulsatile, thus presumptively emulating physiological GH secretion. Further studies will be required to ultimately identify potential endogenous ligands of GHRP receptors, and to clarify more precisely the mechanisms of GHRP action both in the young and older individual. Similarly, the influence of sex-steroids, body composition, aerobic capacity, and such, on the stimulatory efficacy of GHRP and its analogs will require further evaluation.

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References

1. De Gennaro Colonna, V., Zoli, M., Cocchi, D., Maggi, A., Marrama, P., Agnati, L. F., and Muller, E. E. (1989). *Peptides* **62,** 705–708.
2. Cella, S. G., Moiraghi, V., Minuto, F., Barreca, A., Cocchi, D., De Gennaro Colona, V., Reina, G., and Muller, E. E. (1989). *Acta. Endocrinologica.* **121,** 177–184.
3. Nicklas, B. J., Ryan, A. J., Treuth, M. M., Harman, S. M., Blackman, M. R., Hurley, B. F., and Rogers, M. A. (1995). *Int. J. Sports Med.* **16,** 445–450.
4. Pavlou, E. P., Harman, S. M., Merriam, G. R., Gelato, M. C., and Blackman, M. R. (1986). *J. Clin. Endocrinol. Metab.* **62,** 595–600.
5. Penalva, A., Pombo, M., Carballo, A., Barreiro, J., Casanueva, F. F., and Dieguez, C. (1993). *Clin. Endocrinol.* **38,** 87–91.
6. Rudman, D. and Shetty, K. R. (1994). *J. Am. Geriatr. Soc.* **42,** 522–527.
7. De Gennaro Colonna, V., Cocchi, D., Lima, L., Maggi, A., and Muller, E. E. (1991). *Peptides* **12,** 309–312.
8. Laron, Z., Doron, M., Amikam, B. (1970). *Med. Sport.* **4,** 126–131.
9. Iovino, M., Monteleone, P., and Steardo, L. (1989). *J. Clin. Endocrinol. Metab.* **68,** 910–913.
10. Hoffman, D. M., O'Sullivan, A. J., Baxter, R. C., and Ho, K. Y. (1994). *Lancet* **343,** 1064–1068.
11. Hartman, M. L., Pezzoli, S. S., Hellmann, P. J., Suratt, P. M., and Thorner, M. O. (1996). *J. Clin. Endocrinol. Metab.* **81,** 2694–2701.
12. Ghigo, E., Goffi, S., Arvat, E., Nicolosi, M., Procopio, M., Bellone, J., Imperiale, E., Mazza, E., Baracchi, G., and Camanni, F. (1990). *Acta. Endocrinol.* **122,** 169–174.
13. Ghigo, E., Aimartti, G., Gianotti, L., Bellone, J., Arvat, E., and Camanni, F. (1996). *Eur. J. Endocrinol.* **134,** 352–356.
14. Elahi, D., Muller, D. C., Tzankoff, S. P., Andres, R., and Tobin, J. D. (1982). *J. Gerontol.* **37,** 385–391.
15. Dudl, R. J., Ensinck, J. W., Palmer, H. E., and Williams, R. H. (1973). *J. Clin. Endocrinol. Metab.* **37,** 11–16.
16. Zadik, Z., Chalew, S. A., McCarter, Jr. R. J., Meistas, M., and Kowarski, A. A. (1985). *J. Clin. Endocrinol. Metab.* **60,** 513–516.
17. Rudman, D., Kutner, M. H., Rogers, M., Lubin, M. F., Fleming, G. A., and Bain, R. P. (1981). *J. Clin. Invest.* **67,** 1361–1369.
18. Arvat, E., Gianotti, L., Grottoli, S., Imbimbo, B. P., Lenaerts, V., Deghenghi, R., Camanni, F., and Ghigo, E. (1994). *J. Clin. Endocrinol. Metab.* **79,** 1440–1443.
19. Corpas, E., Harman, S. M., Pineyro, M. A., Roberson, R., and Blackman, M. R. (1993). *J. Clin. Endocrinol. Metab.* **76,** 134–138.
20. De Boer, H., Blok, G.-J., Popp-Snijders, C., and van der Veen, E. (1994). *Lancet* **343,** 1645,1646.
21. Finkelstein, J., Roffwarg, H., Boyar, P., Kream, J., and Hellman, L. (1972). *J. Clin. Endocrinol. Metab.* **35,** 665–670.
22. Florini, J. R., Prinz, P. N., Vitiello, M. V., and Hintz, R. L. (1985). *J. Gerontol.* **40,** 2–7.
23. Sonntag, W. E., Steger, R. W., Forman, L. J., and Meites, J. (1980). *Endocrinol.* **107,** 1875–1879.
24. Martha, Jr., P. M., Goorman, K. M., Blizzard, R. M., Rogol, A. D., and Veldhuis, J. D. (1992). *J. Clin. Endocrinol. Metab.* **74,** 336–344.
25. Hartman, M. L., Faria, A. C., Vance, M. L., Johnson, M. L., Thorner, M. O., and Veldhuis, J. D. (1991). *Am. J. Physiol.* **260,** E101–E110.
26. Iranmanesh, A., Lizarralde, G, and Veldhuis, J. D. (1991). *J. Clin. Endocrinol. Metab.* **73,** 1081–1088.
27. Veldhuis, J. D., Iranmanesh, A., Ho, K. K., Lizarralde, G., Waters, M. J., and Johnson, M. L. (1991). *J. Clin. Endocrinol. Metab.* **72,** 51–59.
28. Weltman, A., Weltman, J. Y., Hartman, M. L., Abbott, R. A., Rogol, A. D., Evans, W. S., and Veldhuis, J. D. (1994). *J. Clin. Endocrinol. Metab.* **78,** 543–548.
29. Veldhuis, J. D., Liem, A. Y., South, S., Weltman, A., Weltman, J., Clemmons, D. A., Abbott, R., Mulligan, T., Johnson, M. L., Pincus, S., Straume, M., and Iranmanesh, A. (1995). *J. Clin. Endocrinol. Metab.* **80,** 3209–3222.
30. Pincus, S. M., Gevers, E., Robinson, I. C. A., Roelfsema, F., Hartman, M. L., and Veldhuis, J. D. (1996). *Am. J. Physiol.* **270,** E107–E115.
31. Van den Berg, G., Veldhuis, J. D., Frolich, M., and Roelfsema, F. (1996). *J. Clin. Endocrinol. Metab.* **81,** 2460–2466.
32. Chapman, I. M., Bach, M. A., Cauter, E. V., Farmer, M., Krupa, D., Taylor, A. M., Schilling, L. M., Cole, K. Y., Skiles, E. H., Pezzoli, S. S., Hartman, M. L., Veldhuis, J. D., Gormley, G. J., and Thorner, M. O. (1996). *J. Clin. Endocrinol. Metab.* **81,** 4249–4257.

33. Iranmanesh, A., Liem, A. Y., South, S., Clemmons, D., Weltman, A., Thorner, M. O., and Veldhuis, J. D. (1995). A 3-day exogenous pulsatile GHRH clamp unmasks strongly negative determination of GH secretory burst mass by age and percentage body fat and positive control by testosterone in healthy men. Presented at the Clinical Research Meeting, San Diego, California, May 5–8.
34. Schaefer, F., Baumann, G., Faunt, L. M., Haffner, D., Johnson, M. L., Mercado, M., Ritz, E., Mehls, O., and Veldhuis, J. D. (1996). *J. Clin. Endocrinol. Metab.* **81,** 22–31.
35. Iranmanesh, A., Grisso, B., and Veldhuis, J. D. (1994). *J. Clin. Endocrinol. Metab.* **78,** 526–535.
36. Veldhuis, J. D. (1996). *J. Pediatr. Endocrinol.* **9,** 237–253.
37. Iranmanesh, A. and Veldhuis, J. D. (1992). Veldhuis, J. D. (ed) Endocrinology and Metabolism Clinics of North America. W. B. Saunders, Philadelphia, PA, 783-816.
38. Veldhuis, J. D. (1995). *Curr. Med. Lit.-Growth Horm. Growth Factors* **10,** 59–68.
39. Vahl, N., Jorgensen, J. O. L., Skjaerback, C., Veldhuis, J. D., Orskov, H., and Christiansen, J. (1997). *Am. J. Physiol.* (in press).
40. Ho, K. Y., Evans, W. S., Blizzard, R. M., Veldhuis, J. D., Merriam, G. R., Samojlik, E., Furlanetto, R., Rogol, A. D., Kaiser, D. L., and Thorner, M. O. (1987). *J. Clin. Endocrinol. Metab.* **64,** 51–58.
41. Krieg, Jr. R. J., Latta, K., Niimi, K., Veldhuis, J. D., and Chan, J. C. M. (1995). *J. Endocrinol.* **146,** 509–517.
42. Holl, R. W., Hartman, M. L., Veldhuis, J. D., Taylor, W. M., and Thorner, M. O. (1991). *J. Clin. Endocrinol. Metab.* **72,** 854–861.
43. Hartman, M. L., Veldhuis, J. D., Johnson, M. L., Lee, M. M., Alberti, K. G., Samojlik, E., and Thorner, M. O. (1992). *J. Clin. Endocrinol. Metabol.* **74,** 757–765.
44. Borkan, G. A., Hults, D. E., Gerzof, S. G., Robbins, A. H., and Silbert, C. K. (1983). *J. Gerontol.* **38,** 673–677.
45. Hartman, M. L., Pincus, S. M., Johnson, M. L., Matthews, D. H., Faunt, L. M., Vance, M. L., Thorner, M. O., and Veldhuis J. D. (1994). *J. Clin. Invest.* **94,** 1277–1288.
46. Pincus, S. M. (1991). *Proc. Natl. Acad. Sci. USA* **88,** 2297–2301.
47. Pincus, S. M. and Keefe, D. L. (1992). *Am. J. Physiol.* **262,** E741–E754.
48. Veldhuis, J. D., Matzger, D. L., Martha, Jr., P. M., Mauras, N., Keenan, B., Rogol, A. D., and Pincus, S. M. (1997). *Endocrinol. Metab.* (in press).
49. Meneilly, G. S., Ryan, A. S., Veldhuis, J. D., and Elahi, D. (1997). *J. Clin. Endocrinol. Metab.* (in press).
50. Pincus, S. M., Mulligan, T., Iranmanesh, A., Gheorghiu, S., Godschalk, M., and Veldhuis, J. D. (1996). *Proc. Natl. Acad. Sci. USA* **93,** 14,100–14,105.
51. Pincus, S. M., Veldhuis, J. D., Mulligan, T., Iranmanesh, A., and Evans W. S. (1997). *Am. J. Physiol.* (in press).
52. Van den Berg, G., Pincus, S., Veldhuis, J. D., Frolich, M., and Roelfsema, F. (1997). *Eur. J. Endocrinol.* (in press).
53. Friend, K., Iranmanesh, A., Login, I. S., and Veldhuis, J. D. (1997) *Eur. J. Endocrinol.* (in press).
54. Giustina. A., Scalvini, T., Tassi. C., Desenzani, P., Poiesi, C., Wehenberg, W., Rogol, A., and Veldhuis, J. D. (1997). *J. Clin. Endocrinol. Metab.* **82,** 1210–1219.
55. Friend, K. E., Hartman, M. L., Pezzoli, S. S., Clasey, J. L., and Thorner, M. O. (1996). *J. Clin. Endocrinol. Metab.* **81,** 2250–2256.
56. Campagnoli, C., Biglia, N., Altare, F., Lanza, M. G., Lesca, L., Cantamessa, C., Peris, C., Fiorucci, G. C., and Sismondi, P. (1993). *Gynecol. Endocrinol.* **7,** 251–258.
57. Gravholt, C. H., Veldhuis, J. D., and Christiansen, J. S. (1997). *J. Clin. Endocrinol. Metab.* (in press).
58. Fryburg, D. A., Weltman, A., Jahn, L. A., Weltman, J. Y., Samolijik, E., and Veldhuis, J. D. (1997). *J. Clin. Endocrinol. Metab.*, in press.
59. Weissberger, A. J. and Ho, K. K. (1993). *J. Clin. Endo. Metab.* **76,** 1407–1412.
60. Deversa, J., Lois, N., Arce, V., Diaz, M. J., Lima, L., Tresguerres, J. A. (1991). *J. Steroid Biochem. Mol. Biol.* **40,** 165–173.
61. Weltman, A., Weltman, J. Y., Schurrer, R., Evans, W. S., Veldhuis, J. D., and Rogol, A. D. (1992). *J. App. Physiol.* **76,** 2188–2196.
62. Rogol, A. D., Weltman, A., Weltman, J. Y., Seip, R. L., Snead, D. B., Levine, S., Haskvitz, E. M., Thompson, D. L., Schurer, R., Dowling, E., Walberg-Rankin, J., Mueller, B., Evans, W. S., and Veldhuis, J. D. (1992). *J. Appl. Physiol.* **72,** 1571–1580.
63. Borsst, S. E., Millard, W. J., and Lawenthal, D. T. (1994). *J. Am. Geriatr. Soc.* **42,** 528–535.
64. Craig, B. W., Brown, R., and Everhart, J. (1989). *Mech. Aging Dev.* **49,** 159-169.
65. Pyka, G., Wiswell, R. A., and Marcus, R. (1992). *J. Clin. Endocrinol. Metab.* **75,** 404–407.
66. Veldhuis, J. D., Iranmanesh, A. (1993). *Recent Advances in the Neuroendocrinology of Aging.* Scherbaum, W., Rossmanith, W. G. (eds.), Walter De Gruyter & Co, Berlin, 71–93.
67. Corpas, E., Harman, S. M., Pineyro, M. A., Roberson, R., and Blackman, M. R. (1992). *J. Clin. Endocrinol. Metab.* 75, 530–535.
68. Holloway, L., Butterfield, G., Hintz, R., Gesundheit, N., and Marcus, R. (1994). *J. Clin. Endocrinol. Metab.* **79,** 470–494.
69. Cordido, F., Penalva, A., Dieguez, C., and Casanueva, F. F. (1993). *J. Clin. Endocrinol. Metab.* **76,** 819–823.
70. Ghigo, E., Arvat, E., and Valente, F. (1991). *Neuroendocrinology* **54,** 291–294.
71. Ghigo, E., Goffi, S., Nicolois, M., Arvat, E., Procopio, M., Bellone, J., Mazza, E., and Camanni, F. (1990). *J. Clin. Endocrinol. Metab.* **71,** 1481–1485.
72. Ghigo, E., Goffi, S., Nicolosi, M., Arvat, E., Valente, F., Mazza, E., Ghico, M. C., and Camanni, F. (1990). *J. Clin. Endocrinol. Metab.* **71,** 1481–1485.
73. Kalk, W. J., Winik, A. I., Pimstone, B. L., and Jackson, W. P. (1973). *J. Gerontol.* **28,** 431–433.
74. Ghigo, E., Goffi, S., Arvat, E., Nicolosi, M., Prpcopio, M., Bellone, J., Imperiale, E., Mazza, E., Baracchi, G., and Camanni, F. (1990). *Acta Endocrinol.* **123,** 169–174.
75. Sohmiya, M., and Kato, Y. (1992). *J. Clin. Endocrinol. Metab.* **75,** 1487–1490.
76. Tannenbaum, G. S. (1991). *Acta Paediatr Scand (Suppl)* **372,** 5–16.
77. Tannenbaum, G. S. (1994). *Clin. Pediat. Endocrinol.* **3,** 97–110.
78. Liposits, Z. S., Merchenthaler, I., Paull, W. K., and Flerko, S. (1988). *Histochemistry* **89,** 247–252.
79. Ghigo, E., Arvat, E., Gianotti, L., Grottoli, S., Rizzi, G., Ceda, G. P., Bolghen, M. F., Deghenghi, R., and Camanni, F. (1996). *Eur. J. Endocrinol.* 135, 407–412.
80. Bowers, C. Y., Reynolds, G. A., Durham, D., Barrera, C. M., Pezzoli, S. S., and Thorner, M. O. (1990). *J. Clin. Endocrinol. Metab.* 975–982.
81. Ghigo, E., Arvat, E., and Gianotti, L. (1994). *J. Clin. Endocrinol. Metab.* **78,** 693–698.
82. Ghigo, E., Mazza, E., Imperiale, E., Goffi, S., Arvat, E., Bellone, J., Procopio, M., Ullio, E., Boghen, M., and Camanni, F. (1988). *Acta Paed. Scand.* **S343,** 182,183.
83. Arvat, E., Gianotti, L., Divito, L., Imbimbo, B. P., Lenaerts, V., Deghenghi, R., Camanni, F., and Ghigo, E. (1995). *Neuroendocrinology* 61, 51–56.