# Testosterone Supplementation in Older Men Restrains Insulin-Like Growth Factor's Dose-Dependent Feedback Inhibition of Pulsatile Growth Hormone Secretion

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**Background:** Pulsatile GH secretion declines in older men. The causal mechanisms are unknown. Candidates include deficient feedforward (stimulation) by endogenous secretagogues and excessive feedback (inhibition) by GH or IGF-I due to age and/or relative hypoandrogenemia.

**Hypothesis:** Testosterone (T) supplementation in healthy older men will restrain negative feedback by systemic concentrations of IGF-I.

**Subjects:** Twenty-four healthy men (ages, 50 to 75 yr; body mass index, 24 to 30 kg/m$^2$) participated in the study.

**Methods:** We performed a prospectively randomized, double-blind, placebo-controlled assessment of the impact of pharmacological T supplementation on GH responses to randomly ordered separate-day injections of recombinant human IGF-I doses of 0, 1.0, 1.5, and 2.0 mg/m$^2$.

**Analysis:** Deconvolution and approximate entropy analyses of pulsatile, basal, and entropic (pattern-sensitive) modes of GH secretion were conducted.

**Results:** Recombinant human IGF-I injections 1) elevated mean and peak serum IGF-I concentrations dose-dependently (both $P < 0.001$); 2) suppressed pulsatile GH secretion ($P = 0.003$), burst mass ($P = 0.025$), burst number ($P = 0.005$), interpulse variability ($P = 0.032$), and basal GH secretion ($P = 0.009$); and 3) increased secretory pattern regularity ($P = 0.020$). T administration did not alter experimentally controlled IGF-I concentrations, but it elevated mean GH concentrations ($P = 0.015$) and stimulated pulsatile GH secretion (frequency $P = 0.037$, mass per burst $P = 0.038$). Compared with placebo, T attenuated exogenous IGF-I's inhibition of GH secretory-burst mass ($P < 0.038$) without restoring pulse number, basal secretion, or pattern regularity.

**Conclusion:** The capability of systemic T to mute IGF-I feedback on pulsatile GH secretion suggests a novel mechanism for augmenting GH production. **(*J Clin Endocrinol Metab* 94: 246–254, 2009)**

GH concentrations decline exponentially with age, beginning at the end of puberty (1–3). One analysis estimated that GH secretion rates decrease by 50% every 7 yr after age 18 in men (4). Interventional studies indicate that GH production in older men can be stimulated by 90-min iv pulses of GHRH, twice-daily sc doses of GHRH, and continuous infusion of the GH-releasing peptide, GHRP-2 (5–7). Testosterone (T) replacement in hypogonadal boys, T-deficient men, and healthy older men also stimulates GH secretion (8–15). T administration selectively augments pulsatile GH secretion (15), which is medi-

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Abbreviations: ANCOVA, Analysis of covariance; ApEn, approximate entropy; CV, coefficient of variation; $E_2$, estradiol; ER, estrogen receptor; rhIGF-I, recombinant human IGF-I; T, testosterone.

ated in part by amplification of GH secretory-burst size and attenuation of autonegative feedback by GH (16). However, T supplementation in men, unlike in prepubertal boys (17), does not potentiate maximal stimulation by the principal peptidyl secretagogues, GHRH and/or GH-releasing peptide (18–20). In the only study of its kind, iv infusion of a single dose of recombinant human IGF-I (rhIGF-I) suppressed the stimulatory effect of GHRH by 27% in older men, and T supplementation overcame suppression (21). Whether T can reverse IGF-I's concentration-dependent inhibition of GH secretion is unknown. Because pharmacological T administration doubles pulsatile GH secretion in older but not young men (15), the present investigation uses an analogous paradigm to examine T's regulation of IGF-I feedback.

## Subjects and Methods

The postulate is that pharmacological T supplementation can relieve the negative-feedback effect of physiological IGF-I concentrations on GH secretion in healthy older men. To this end, the experimental design, as depicted in Fig. 1, comprised: 1) overnight fasting after a standardized evening meal; 2) graded elevation of systemic IGF-I concentrations by sc injection of saline or three doses of rhIGF-I (1.0, 1.5, and 2.0 mg/m$^2$) in randomly assigned order on separate days fasting, wherein the highest dose approaches the normal young male IGF-I production rate (19); and 3) prospectively randomized, double-blind administration of placebo (1 ml saline) *vs.* T (175 mg T enanthate every 6 d for three injections) to create a hyperandrogenemic milieu, based upon the efficacy of 200 mg T enanthate given weekly in an earlier study (15).

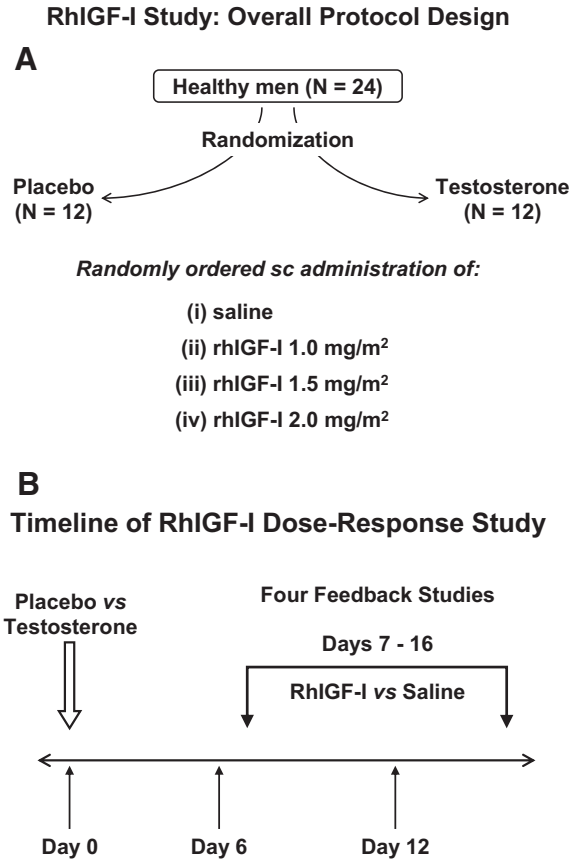


**FIG. 1.** Outline of randomization plan (A) and timeline of studies (B).

### Subjects

Participants (n = 24) provided written informed consent approved by the Mayo Institutional Review Board (IRB). Subjects were compensated financially for time spent in the study at an IRB-approved rate. The protocol was reviewed by the National Institutes of Health and U.S. Food and Drug Administration under an investigator-initiated new drug assignment for rhIGF-I. Inclusion criteria comprised age 50–75 yr, body mass index 20–30 kg/m$^2$, a normal physical examination, and normal screening laboratory tests of hepatic, renal, endocrine, metabolic, and hematological function. Exclusion criteria included known or suspected cardiac, cerebrovascular, or peripheral arterial disease; prostate abnormalities, including benign prostatic hypertrophy, prostate-specific antigen greater than 2 $\mu$g/liter, prostatic nodules, or adenocarcinoma; concomitant or recent use of neuroactive medications; anemia; and, failure to provide written informed consent. Also disallowed were recent transmeridian travel (exceeding three time zones within 10 d), night-shift work, significant weight change ($\geq$2 kg in 3 wk), acute or chronic systemic disease, psychiatric illness requiring treatment, and alcohol or drug abuse.

### Time-line of interventions

Men were each studied four times, as summarized in Fig. 1. Admissions to the Clinical Translational Science Center were scheduled during the administration of either placebo or T. Visits were randomly ordered and at least 48 h apart within the 10-d time window comprising d 7–16 inclusive.

### Blood sampling and injection protocol

To obviate nutritional confounds, participants received a standardized meal the night before at 1800 h (10 kcal/kg of an American Diabetes Association diet) and remained fasting thereafter until the end of sampling at 1600 h the next day. Blood was withdrawn repetitively (1.0 ml every 10 min) for 8 h beginning at 0800 h. Caffeinated beverages, sleep, and exercise were disallowed during the sampling session. Saline or rhIGF-I (1.0, 1.5, and 2.0 mg/m$^2$) [maximal single dose, 4 mg] was injected sc at 0800 h on separate randomly ordered days after the first blood sample was obtained. The doses reflect an estimated daily blood production rate of 3–3.5 mg IGF-I in young adults (19).

### Hormone assays

Serum GH concentrations were determined in duplicate by automated ultrasensitive two-site immunoenzymatic chemiluminescence assay performed on the DxI automated system (Beckman Instruments, Chaska, MN). Interassay coefficients of variation (CVs) were 6.1% at 0.46 $\mu$g/liter, 4.3% at 3.0 $\mu$g/liter, 5.0% at 7.2 $\mu$g/liter, and 4.8% at 13.6 $\mu$g/liter. Intraassay CV's were 4.7% at 0.37 $\mu$g/liter, 3.5% at 2.5 $\mu$g/liter, and 3.2% at 14.8 $\mu$g/liter. The lowest detectable GH concentration at 95% confidence is 0.008 $\mu$g/liter, determined by processing a six-point calibration curve, five quality controls, and 10 replicates of zero calibrator in multiple assays.

T and estradiol concentrations were quantified by tandem liquid chromatography ion-spray mass spectrometry (ThermoFisher Scientific, Franklin, MA, and Applied Biosystems-MDS Sciex, Foster City, CA). For T, the analytic range is 7–2000 ng/dl (multiply by 0.0347 for nmol/liter). Intraassay CV's were 3.3, 2.8, 2.2, and 2.0% at T concentrations of 16, 64, 184, and 927 ng/dl, respectively. Corresponding interassay CV's were 5.1, 3.8, 3.7, and 2.8%. For estradiol, the analytic range is 3–500 pg/ml. Intraassay CV's were 3.1, 5.0, and 3.5% at 29, 109, and 325 pg/ml, respectively [multiply by 3.68 to convert to pmol/liter]. Interassay CV's were 8.6, 9.0, 6.6, and 4.8% at 24, 61, 125, and 360 pg/ml, respectively. SHBG and albumin were assayed, and bioavailable and free T concentrations were calculated, as described (22).

Total IGF-I concentrations were measured by immunoradiometric assay (Diagnostic Systems Laboratories, Webster, TX), as described

**TABLE 1.** Subject characteristics and hormonal measurements

| | Placebo (n = 12) | T (n = 12) | *P* value[a] |
|---|---|---|---|
| Subject characteristics | | | |
| Age (yr) | 62 (50–74) | 56 (50–75) | NS |
| BMI (kg/m$^2$) | 27 (25–30) | 27 (24–30) | NS |
| After intervention | | | |
| IGF-I ($\mu$g/liter) | 143 ± 16 | 170 ± 17 | NS |
| IGF binding protein-3 (mg/liter) | 3.5 ± 0.17 | 3.4 ± 0.23 | NS |
| LH (IU/liter) | 4.9 ± 0.96 | 0.92 ± 0.30 | <0.001 |
| FSH (IU/liter) | 5.9 ± 0.67 | 2.5 ± 0.81 | 0.001 |
| Prolactin ($\mu$g/liter) | 5.4 ± 0.62 | 6.3 ± 0.63 | NS |
| SHBG (nmol/liter) | 32 ± 3.1 | 32 ± 4.7 | NS |
| T (ng/dl)[b] | 431 ± 43 | 1403 ± 76 | <0.001 |
| $E_2$ (pg/ml)[c] | 22 ± 4.4 | 51 ± 5.8 | 0.013 |

Data are the mean ± SEM. NS denotes $P > 0.05$. Measurements were made at 0800 h fasting on the day of saline injection.

[a] Unpaired two-tailed Student's *t* test.

[b] Multiply by 0.0347 for units of nmol/liter.

[c] Multiply by 3.68 to obtain units of pmol/liter.

(23). Intraassay CVs were 3.4% at 9.4, 3% at 55, and 1.5% at 264 $\mu$g/liter. Interassay CVs for IGF-I were 9% at 64 $\mu$g/liter and 6.2% at 157 $\mu$g/liter.

LH and FSH were assayed using the DxI automated two-site immunoenzymatic system (Beckman Instruments). For LH, intraassay CVs were 4.3 and 4.0% at 1.2 and 38.5 IU/liter, and interassay CVs were 9.3, 6.0, and 6.0% at 1.4, 15.6, and 48.8 IU/liter, respectively. For FSH, intraassay CVs were 3.2 and 2.8% at 8.6 and 47.1 mIU/ml, and interassay CVs were 3.6, 3.2, and 4.7% at 6.5, 16.7, and 58.0 mIU/ml, respectively.

## Deconvolution analysis

Deconvolution analysis was employed to estimate underlying basal and pulsatile GH secretion, as well as the shape and size of secretory events (24). Each 8-h GH concentration time series was analyzed using a recently validated deconvolution method (24). The automated Matlab program first detrends the data and normalizes concentrations to the unit interval (0, 1) (25). Second, successive potential pulse-time sets, each containing one fewer burst, are created by a smoothing process (a nonlinear adaptation of the heat-diffusion equation). Third, a maximum-likelihood estimation method (Matlab Direct-Search Algorithm) calculates all secretion and elimination rates simultaneously for each candidate pulse-time set. The deconvolution model specifies basal secretion ($\beta_0$), two half-lives ($\alpha_1$, $\alpha_2$), an accumulation process and weak interpulse-length dependency for secretory-burst mass ($\eta_0$, $\eta_1$), random effects on burst mass ($\sigma_A$), procedural and measurement error ($\sigma_\varepsilon$), and a three-parameter secretory-burst waveform ($\beta_1$, $\beta_2$, $\beta_3$). In the present analysis, the rapid half-life was assumed to be 3.5 min and to contribute 37% of total decay, with the second-phase (slow) half-life estimated analytically from the data (26). Model selection is performed to distinguish among the candidate pulse-time sets using the Akaike information criterion (27). Observed interpulse intervals are described by a two-parameter Weibull process (more general form of a Poisson process). The parameters (and units) are frequency (number of bursts per unit time, $\lambda$ of Weibull distribution), regularity of interpulse intervals (unitless $\gamma$ of Weibull), slow half-life (minutes), basal and pulsatile secretion rates (concentration per unit time), mass secreted per burst (concentration), and waveform mode (time delay to maximal secretion after burst onset in minutes) (24, 25). Model precision is determined technically by the analytical SD of the likelihood function (24, 25). The CV of parameter estimates is 2–14%. Sensitivity, specificity, and discriminative accuracy are 92, 90, and 91%, respectively, for GH data sampled every 10 min.

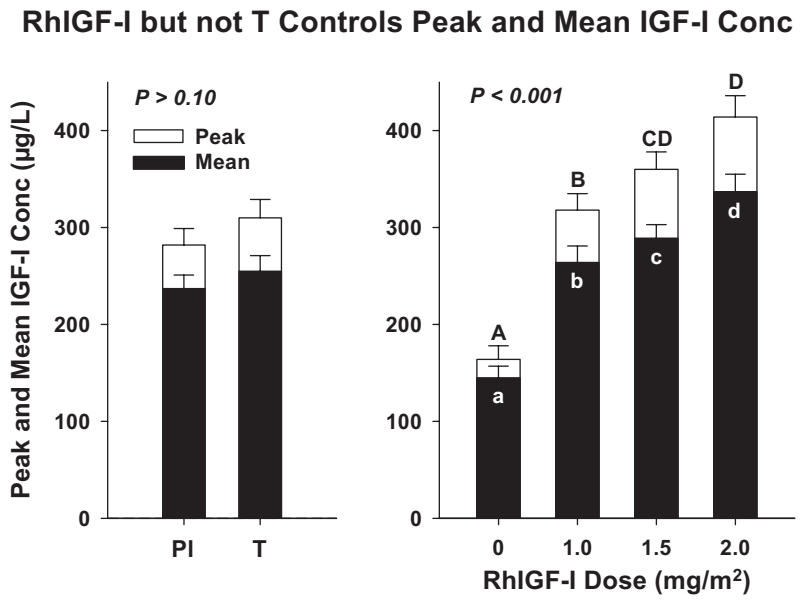


**FIG. 2.** Mean (8-h) and peak total IGF-I concentrations observed after the indicated doses of rhIGF-I in 12 men administered placebo and 12 administered T parenterally. Data are the mean ± SEM. *Different* (unshared) *alphabetic superscripts* denote significantly different means by *post hoc* Fisher's least-significantly different test.

## Statistical analysis

The primary outcome was the mean GH concentration enforced after injection of saline and each dose of rhIGF-I (n = 12 placebo, n = 12 T). To adjust for within-subject correlations, the statistical model comprised hierarchical mixed-effect two-way analysis of covariance (ANCOVA), wherein the baseline (saline) outcome served as the covariate (28). The model-specification parameters were: 1) placebo and T; and 2) three nonsaline doses of rhIGF-I (29). Logarithmic transformation was used to limit heterogeneity of variance. The equal-slopes assumption of the ANCOVA structure was verified by a generalized F-ratio test, followed by restricted maximum-likelihood estimation of parameters. Rejection of

prespecified hypotheses was based on a multiple-comparison experiment-wise type I error rate of less than 0.05 using Tukey's honestly, significantly different test (28). Computations were performed using PROC MIXED in SAS version 8.0 (SAS Institute Inc., Cary, NC). When the covariate was not significant ($P > 0.05$), ANOVA was used. All $P$ values are two-tailed.

In pilot analysis, a single dose (1.0 mg/m$^2$) of rhIGF-I reduced mean GH concentrations by 45% ± 19% (SD). Statistical power to detect 50% attenuation of this response by T *vs.* placebo exceeds 95% at $P < 0.05$ by one-tailed unpaired $t$ test if 19 individuals complete the study. Hourly mean values were defined as the mean of six consecutive 10-min samples (0800–0850 h, 0900–0950 h, *etc.*). An unpaired two-tailed Student's $t$ test with unknown variance was applied to contrast mean hormone concentrations. Three-way ANOVA was used to assess the effect of hourly or 10-min sampling times, T or placebo, and four IGF-I doses on GH concentrations. Regressions of GH secretion on T concentrations assume that 1) the men were assigned randomly to receive T or placebo injections; 2) a wide range of T values was thereby created; and 3) endogenous and exogenous T acts similarly.

### Safety monitoring

Electrocardiographic activity was monitored continuously, and plasma glucose was measured every hour during saline and IGF-I infusion.

## Results

Screening endocrine data were comparable in the two cohorts (data not shown). Age and body mass index did not differ by group (Table 1). T compared with placebo supplementation elevated T and estradiol ($E_2$) concentrations and reduced LH and FSH concentrations measured at 0800 h fasting on the saline day.

Administration of rhIGF-I (0, 1.0, 1.5, and 2.0 mg/m$^2$) increased mean and peak IGF-I concentrations significantly (dose effect $P < 0.001$ for both measures by two-way ANCOVA). Mean and peak IGF-I concentrations were independent of T exposure ($P = 0.19$ and $P = 0.14$, respectively) (Fig. 2).

Exogenous IGF-I suppressed 8-h mean GH concentrations ($P < 0.001$ overall effect), beginning at a dose of 1.0 mg/m$^2$ ($P = 0.001$ *vs.* saline) and at both higher doses as well ($P < 0.001$ *vs.* saline) (Table 2). Suppression by the 1.0-mg dose was significantly relieved by T *vs.* placebo ($P = 0.015$). In particular, administration of T elevated 10-min GH concentrations after saline injection (from 0.38 ± 0.16 to 0.83 ± 0.11 μg/liter) and analogously by 2-fold after rhIGF-I doses of 1.0 and 2.0 mg/m$^2$ ($P < 0.001$ by three-way ANOVA for time, IGF-I dose, and T status) (Fig. 3A). These effects were confirmed by T-dependent increases in hourly mean GH concentrations on the saline day ($P < 0.001$) and after injection of rhIGF-I doses (mg/m$^2$) of 1.0 ($P < 0.001$), 1.5 ($P = 0.029$), and 2.0 ($P < 0.001$) rhIGF-I. In particular, rhIGF-I reduced hourly mean GH concentrations by a mean of 70 ± 3% ($P < 0.001$) in a dose-dependent manner ($P < 0.001$). The decrease was opposed by T compared with placebo supplementation ($P < 0.001$ by two-way ANCOVA) (Fig. 3B). Peak and nadir GH concentrations (μg/liter) changed analogously, *viz.* GH peaks declined from 1.8 to 0.75 (placebo) and from 3.3 to 1.3 (T) and GH nadirs from 0.037 to 0.016 (placebo) and from 0.100 to 0.021 (T) ($P < 0.01$ for each IGF-I and T effect). Table 2 summarizes these statistical inferences.

**TABLE 2.** Summary data for 8-h GH time series after T or placebo injection and four doses of rhIGF-I

| | Placebo (n = 12) | | | | T (n = 12) | | | | *P* values | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Saline | 1.0 dose | 1.5 dose | 2.0 dose | Saline | 1.0 dose | 1.5 dose | 2.0 dose | Dose | Treatment |
| Mean GH conc (μg/liter) | 0.38 ± 0.16$^{A}$ | 0.13 ± 0.054$^{B}$ | 0.14 ± 0.096$^{B}$ | 0.15 ± 0.10$^{B}$ | 0.83 ± 0.11$^{A}$ | 0.48 ± 0.085$^{AC}$ | 0.24 ± 0.10$^{BC}$ | 0.31 ± 0.084$^{BC}$ | <0.001 | 0.015 |
| Peak GH conc (μg/liter) | 1.8 ± 0.70$^{AB}$ | 0.87 ± 0.42$^{C}$ | 0.75 ± 0.47$^{C}$ | 0.92 ± 0.70$^{ACD}$ | 3.3 ± 0.45$^{B}$ | 2.4 ± 0.53$^{BDE}$ | 1.3 ± 0.56$^{ACE}$ | 1.5 ± 0.51$^{ACE}$ | 0.006 | 0.076 |
| Nadir GH conc (μg/liter) | 0.037 ± 0.016$^{A}$ | 0.017 ± 0.007$^{B}$ | 0.017 ± 0.007$^{B}$ | 0.016 ± 0.007$^{B}$ | 0.10 ± 0.019$^{A}$ | 0.029 ± 0.007$^{BC}$ | 0.021 ± 0.008$^{C}$ | 0.027 ± 0.008$^{BC}$ | <0.001 | 0.102 |
| Peak IGF-I conc (μg/liter) | 142 ± 16$^{A}$ | 309 ± 20$^{BF}$ | 359 ± 20$^{CE}$ | 407 ± 22$^{DE}$ | 189 ± 20$^{A}$ | 328 ± 29$^{B}$ | 362 ± 31$^{BC}$ | 420 ± 39$^{CDF}$ | <0.001 | 0.142 |
| Mean IGF-I conc (μg/liter) | 130 ± 16$^{A}$ | 253 ± 20$^{BF}$ | 292 ± 19$^{CD}$ | 331 ± 20$^{D}$ | 160 ± 17$^{A}$ | 276 ± 28$^{B}$ | 286 ± 23$^{BCE}$ | 342 ± 32$^{DE}$ | <0.001 | 0.194 |
| No pulses (per 8 h) | 3.4 ± 0.43$^{AD}$ | 2.9 ± 0.32$^{ABC}$ | 3.0 ± 0.43$^{ABC}$ | 2.2 ± 0.26$^{BC}$ | 4.4 ± 0.34$^{A}$ | 4.0 ± 0.45$^{AE}$ | 2.5 ± 0.43$^{CD}$ | 3.0 ± 0.47$^{CDE}$ | 0.005 | 0.283 |
| Slow half-life (min) | 17 ± 0.87$^{A}$ | 16 ± 0.91$^{AB}$ | 17 ± 1.1$^{AB}$ | 15 ± 0.78$^{B}$ | 15 ± 0.86$^{AB}$ | 16 ± 0.76$^{AB}$ | 15 ± 0.82$^{AB}$ | 16 ± 0.83$^{AB}$ | 0.477 | 0.636 |
| Secretory-burst mode (min) | 23 ± 2.0 | 18 ± 4.2 | 25 ± 7.3 | 21 ± 4.9 | 19 ± 1.7 | 20 ± 2.1 | 16 ± 2.1 | 19 ± 2.5 | 0.962 | 0.312 |
| Basal secretion (μg/liter/8 h) | 1.1 ± 0.62$^{A}$ | 0.47 ± 0.21$^{B}$ | 0.50 ± 0.24$^{AB}$ | 0.49 ± 0.31$^{BC}$ | 1.3 ± 0.84$^{AC}$ | 0.78 ± 0.21$^{AB}$ | 0.44 ± 0.28$^{B}$ | 0.70 ± 0.23$^{AB}$ | 0.009 | 0.945 |
| Pulsatile secretion (μg/liter/8 h) | 6.0 ± 2.8$^{AC}$ | 2.4 ± 0.85$^{B}$ | 1.2 ± 2.1$^{B}$ | 2.2 ± 2.1$^{BC}$ | 16 ± 2.6$^{A}$ | 9.5 ± 2.0$^{AC}$ | 4.0 ± 1.8$^{BC}$ | 5.1 ± 1.6$^{BC}$ | 0.003 | 0.025 |
| Mass per burst (μg/liter) | 1.8 ± 0.69$^{A}$ | 0.83 ± 0.32$^{B}$ | 0.64 ± 0.55$^{B}$ | 0.79 ± 0.48$^{AB}$ | 3.6 ± 0.6$^{A}$ | 2.4 ± 0.51$^{A}$ | 1.7 ± 0.63$^{AB}$ | 1.7 ± 1.1$^{AB}$ | 0.025 | 0.038 |
| γ (regularity) | 10 ± 55$^{AB}$ | 5.7 ± 31$^{AB}$ | 5.8 ± 45$^{AB}$ | 37 ± 62$^{A}$ | 2.4 ± 1.0$^{B}$ | 4.7 ± 42$^{BC}$ | 8.0 ± 64$^{AB}$ | 22 ± 48$^{AC}$ | 0.032 | 0.349 |

Data are the geometric mean ± SEM, n = 24. *Unshared alphabetic superscripts* denote significantly different means due to dose effects within rows. Dose values are mg/m$^2$ rhIGF-I. Comparisons were made *post hoc* by the Tukey test. Conc, Concentration.

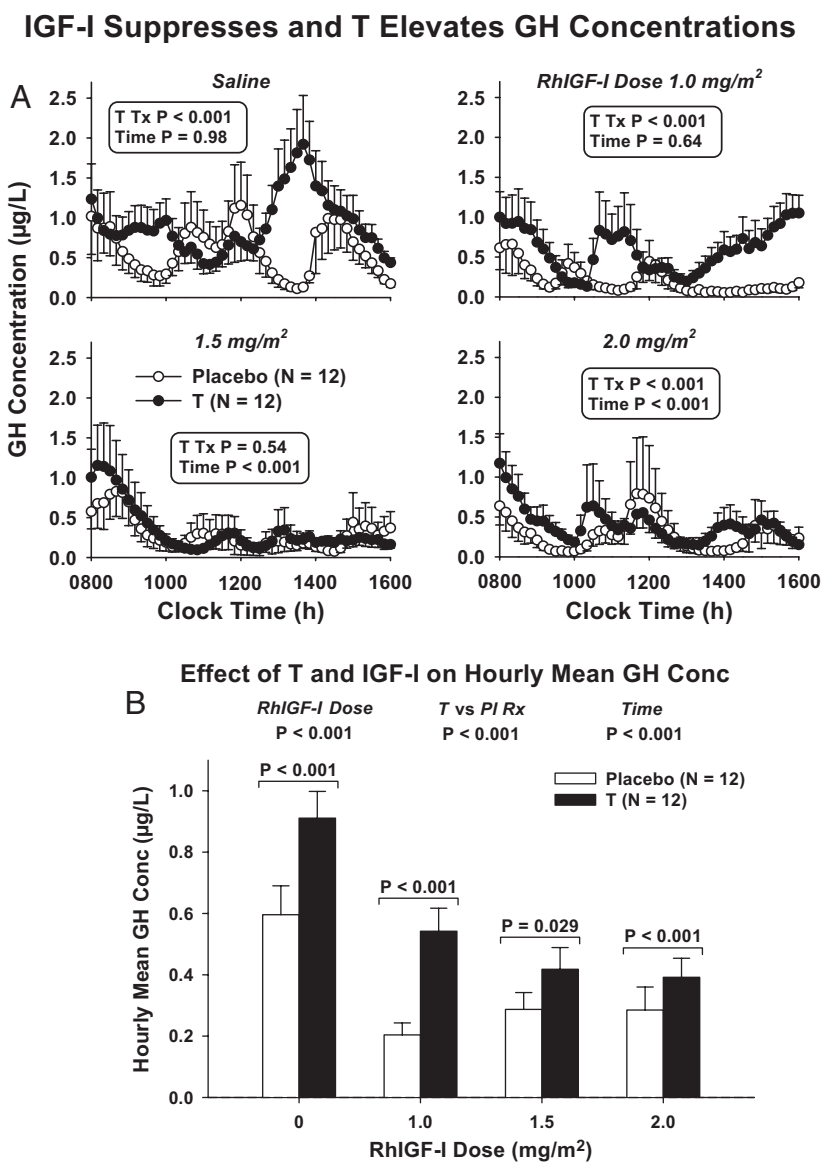


**FIG. 3.** A, GH concentration profiles sampled every 10 min for 8 h in 12 men given placebo (*open circles*) and 12 others given T (*closed circles*), followed by administration of randomly ordered sc doses of rhIGF-I on 4 separate days. B, Mean hourly GH concentrations evaluated by two-way analysis of covariance. Data are presented as described in Fig. 2.

Deconvolution analysis disclosed that IGF-I repressed pulsatile GH secretion dose-dependently ($P = 0.003$) and that T supplementation significantly relieved inhibition ($P = 0.025$, overall two-way ANCOVA $P < 0.001$) (Fig. 4A). T opposed IGF-I's submaximal (1.0 mg/m$^2$ dose; $P = 0.03$; overall ANCOVA, $P < 0.001$), but not maximal, inhibition of pulsatile GH secretion. The mechanisms of IGF-I's inhibition entailed: 1) a reduction in GH secretory-burst mass ($P = 0.025$), which effect was attenuated by T administration ($P = 0.038$, overall ANCOVA $P < 0.001$); and 2) a decrease in GH pulse frequency ($P = 0.005$), which was not relieved by T administration ($P = 0.28$, overall ANCOVA $P < 0.001$) (Fig. 4B). IGF-I injections also significantly suppressed basal GH secretion ($P = 0.009$, overall $P < 0.001$), and T did not reverse this effect ($P = 0.95$) (Fig. 4C).

The combined effects of rhIGF-I and T on pulsatile and basal secretion are captured in total GH secretion (µg/liter·8 h), which IGF-I suppressed ($P < 0.001$) and T stimulated ($P < 0.022$ by two-way ANCOVA). T specifically muted inhibition by the 1.0 mg/m$^2$ dose of rhIGF-I ($P = 0.011$). Figure 5 depicts the positive curvilinear (exponential) relationships in this cohort of men between total GH secretion and each of total, bioavailable, and free T concentrations after injection of saline and 1.0 mg/m$^2$ rhIGF-I. Salient findings were that: 1) 50% of maximal stimulation of total GH secretion on the saline day was attained by physiological T concentrations, *viz.*, 433 ng/dl (total), 98 ng/dl (bioavailable), and 9.5 ng/dl (free T); and 2) higher concentrations of each T fraction were required to achieve the same percentage effect after administration of 1.0 mg/m$^2$ IGF-I, *viz.*, 693 ng/dl (total T), 173 ng/dl (bioavailable T), and 15.4 ng/dl (free T) [each $P < 0.001$]. The T effect was not significant at the higher two IGF-I doses (1.5 and 2.0 mg/m$^2$). Exploratory regressions of the same GH-secretion parameters on $E_2$ concentrations were not significant.

The highest dose of IGF-I increased the regularity of GH secretory-burst intervals ($\gamma$ of Weibull probability distribution) from 2.4 ± 0.6 to 15 ± 2.7 ($P = 0.032$) independently of T administration ($P = 0.68$). GH secretory-burst duration (mode, 20 ± 1.1 min) and GH half-life (mean, 16 ± 0.82 min) were invariant of IGF-I or T administration (Table 2).

As a model-free estimate of IGF-I's feedback effects, approximate entropy (ApEn; a pattern-sensitive statistic) was computed for each 8-h GH profile. Two-way ANOVA indicated that exogenous IGF-I decreases ApEn irregularity values dose-dependently, signifying enhanced orderliness (regularity) of GH secretion patterns ($P = 0.02$, overall $P < 0.001$). Exposure to T did not alter pattern orderliness ($P = 0.11$).

Unifocal ventricular premature beats were observed in five men without attendant symptoms or signs during rhIGF-I (three subjects) or saline (two subjects) administration. No treatment was required. Plasma glucose concentrations (absolute range) were 64–87 mg/dl after saline and 61–81 mg/dl after rhIGF-I injection.

## Discussion

The present investigation demonstrates that experimental elevation of systemic IGF-I concentrations into the young-adult range in older men: 1) reduces mean GH concentrations and pulsatile GH secretion by suppressing secretory-burst mass and

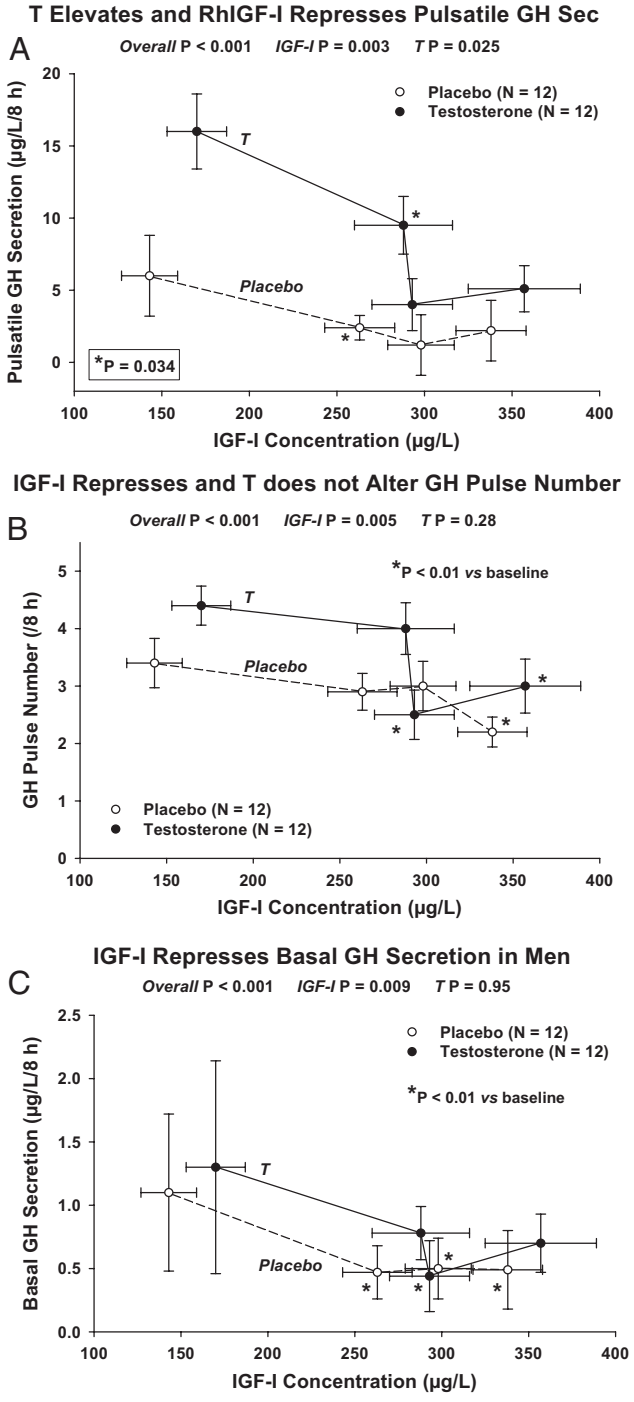


**FIG. 4.** Distinctive impact of T and rhIGF-I injections on pulsatile GH secretion (A), GH secretory-burst mass number (B), and basal GH secretion (C) in 24 older men. See Fig. 2 for data presentation.

frequency; 2) inhibits basal (nonpulsatile) GH secretion; 3) augments regularity of the GH pulse-renewal process; 4) increases the orderliness of GH secretion patterns; and 5) has no effect on secretory-burst duration or GH half-life. Concomitant pharmacological T supplementation in an amount that stimulates GH secretion in older but not young men (15) attenuates IGF-I's suppression of pulsatile GH secretion and secretory-burst mass, without altering IGF-I's repression of pulse frequency and basal GH secretion or its stimulation of pulse-renewal regularity and pattern orderliness. The selectivity of IGF-I's inhibitory mechanisms and the specificity of T's opposition require a new model of GH feedback control in healthy men.

Under physiological conditions, GH secretory-burst mass is determined via tripartite mechanisms involving stimulation by GHRH, potentiation by ghrelin, and inhibition by somatostatin (19). In contradistinction, GH pulse frequency putatively reflects primarily the episodic release of GHRH, which is subject to hypothalamic restraint by GH feedback-induced somatostatin release (30–32). Because both the mass and the frequency of GH secretory bursts were inhibited by the highest dose of rhIGF-I, a simple unifying postulate would be that IGF-I, like GH, evokes hypothalamic somatostatin outflow (18), which in turn decreases both (hypothalamic) GHRH pulsatility and (pituitary) GH release.

In experimental animals, IGF-I represses GH secretion *in vitro* and inhibits expression of genes encoding pituitary GHRH and ghrelin receptors (18, 33–35). In clinical studies, infusion of a putative inhibitor of somatostatin secretion, L-arginine, overcomes IGF-I's suppression of fasting and GHRH-stimulated GH secretion in young adults (36, 37), arguing against direct pituitary inhibition. Whether L-arginine is also able to counteract IGF-I's suppression of ghrelin-stimulated GH secretion is not known. If this were the case, then available data would be consistent with our hypothesis that IGF-I stimulates periventricular-nucleus somatostatin secretion, which quenches arcuate-nucleus GHRH pulses and inhibits hypothalamo-pituitary actions of secretagogues.

The further question emerges how T or its metabolites mute feedback actions of IGF-I. A testable model is that, whereas IGF-I evokes periventricular-nucleus somatostatin release (30, 31), T or its metabolic products counter the intrahypothalamic effect of somatostatin and stimulate arcuate-nucleus GHRH secretion (Fig. 6). In this construction, IGF-I-induced somatostatin outflow would reduce the number and size of GHRH pulses, basal GH release, and pituitary responses to GHRH and ghrelin. Conversely, T would selectively oppose suppression of GHRH pulse size and GHRH action, thereby augmenting GH secretory-burst mass. T fails to relieve IGF-I's inhibition of basal GH secretion or GHRH/GH pulse number. In relation to stimulation by GHRH, T enhances postsomatostatin rebound-like release of GH, which is a GHRH-dependent event (16, 19, 38, 39). The aromatized product of T, $E_2$, augments the potencies of GHRH and ghrelin and blunts negative feedback by somatostatin and GH in women (23, 40–42). Because estrogen receptor $\alpha$ (ER$\alpha$) is expressed highly in GHRH neurons and the androgen receptor in somatostatin neurons (18, 19, 43–45), T's postulated stimulation of GHRH secretion may be transduced by ER$\alpha$-mediated stimulation on GHRH neurons or androgen receptor-mediated repression of somatostatin neurons. In addition, ER$\beta$ is present in the hypothalamus and pituitary gland, which could transduce some of T's actions via its estrogenic product, 5$\alpha$-androstane-3$\beta$,17$\beta$-diol (46). Whether T or its products also regulate IGF-binding proteins in the human brain is not known.

The orderliness of GH secretory patterns is strongly determined by negative feedback (13, 47). ApEn provides a model-

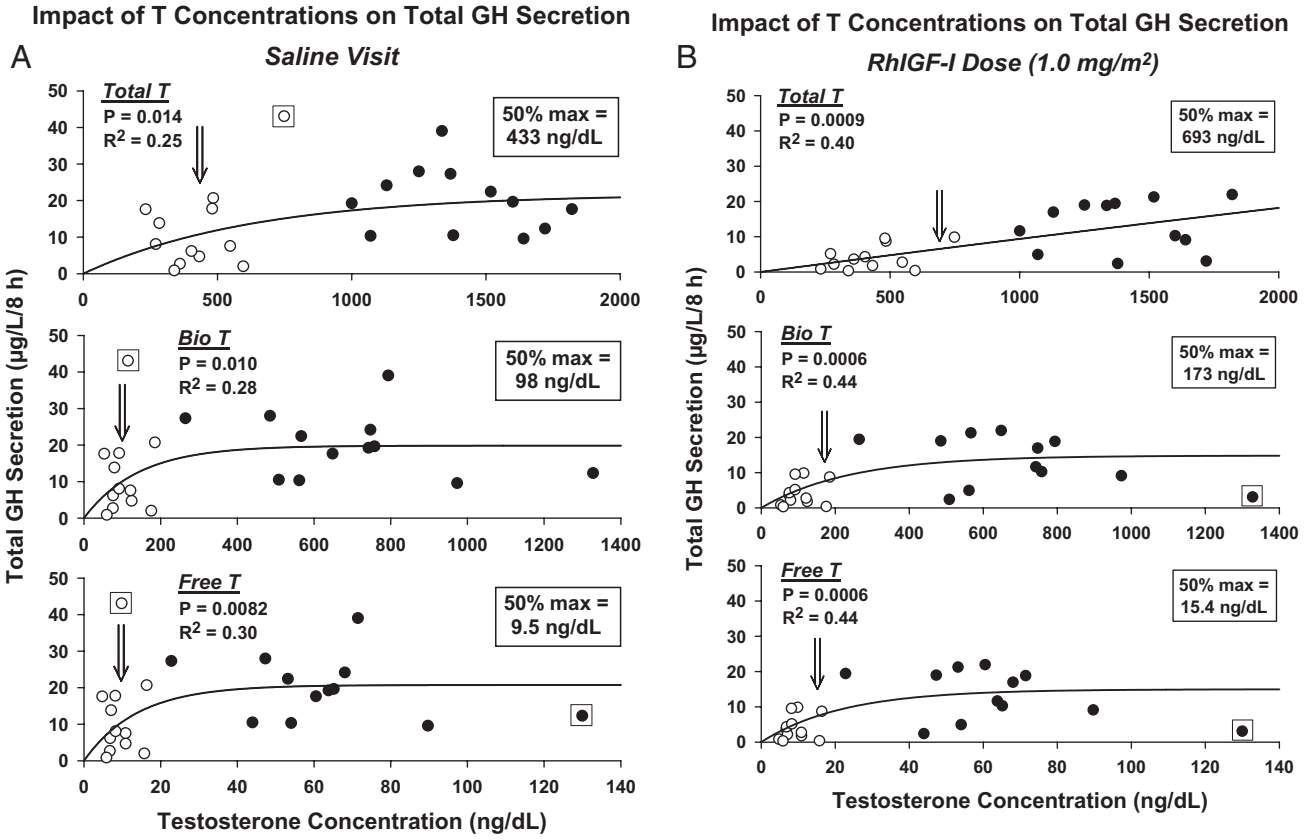


**FIG. 5.** Regression of total (sum of pulsatile and basal) GH secretion estimated after saline injection (A) and after 1.0 mg/m$^2$ rhIGF-I injection (B) on total (*top*), bioavailable (*middle*), and free (*bottom*) T concentrations in 24 men pretreated with placebo (*open circles*) or T (*closed circles*). An inverse-exponential function was used in the regression, assuming randomized T *vs.* placebo injection; a *circle enclosed by a box* denotes a statistical outlier (defined by leverage $P < 0.01$). Apparent T potencies (50% maximal stimulation) are noted by *double arrows*.

and scale-independent measure of such pattern regularity (48, 49). IGF-I administration reduced ApEn, thus quantifying greater pattern reproducibility. T was not able to restore baseline irregularity, suggesting a noncompetitive action of IGF-I.

Nonlinear regression analyses disclosed that T concentrations predict 25–44% of the intersubject variability in total GH secretion. T correlated positively with pulsatile, but not basal, GH secretion. Fifty percent maximal GH secretion occurred at systemic T concentrations within the low-normal (saline) and mid-normal (IGF-I) range of young-adult values. In contradistinction, although $E_2$ is thought to mediate important actions of T on the GH axis (18, 19), systemic $E_2$ concentrations did not correlate with basal, pulsatile, or total GH secretion. However, estrogen can stimulate GH secretion by pituitary cells *in vitro* and by ectopically transplanted pituitary tissue *in vivo* (50, 51). In addition, transgenic inactivation of the aromatase gene in mice diminishes pituitary expression of GH, *pit-1*, GHRH-receptor, and ghrelin-receptor genes, while augmenting expression of somatostatin receptors (52). Estrogen administration to such animals restores wild-type mRNA levels except for ghrelin-receptor transcripts (53). If T acted via local, rather than exclusively systemic, aromatization, one would expect that supplementation with a high dose of T could rescue IGF-I-inhibited GH secretory-burst mass, as observed here. Because nonpulsatile (basal) GH release was not affected by T, other mechanisms may play a role in regulating this mode of GH secretion. Accordingly, dissecting the precise network of signals involved in the opposing actions of T and IGF-I will require further basic and clinical studies.

Caveats include the pharmacological T milieu used and potential variability of T levels during the 6 d after im injection. The

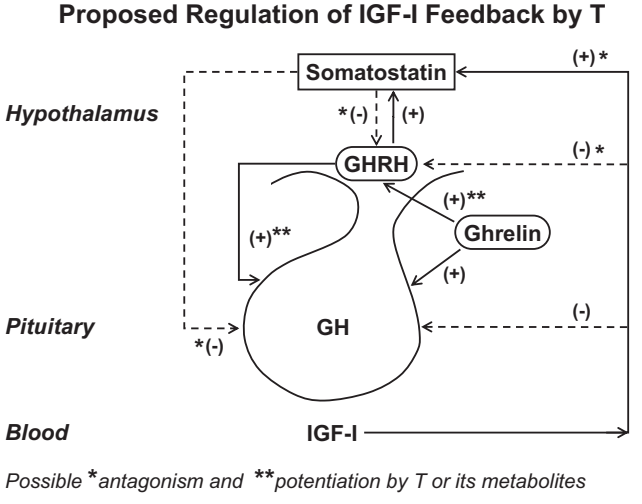


**FIG. 6.** Postulated model in which IGF-I elicits periventricular-nucleus somatostatin release and T evokes arcuate-nucleus GHRH secretion. The noncompetitive nature of somatostatin's inhibition of GHRH action would explain why high concentrations of T fail to overcome the maximal inhibitory effect of IGF-I.

exact dose dependency of T's action on the GH/IGF-I axis has not been established. How food intake alters IGF-I negative feedback in low and high androgenic milieus is not known. Although elevated T concentrations double GH secretion in older but not young men, suggesting possible differences in sex-steroid effects on the GH axis by age (15), whether this age-related difference is due to change in T's capability to mute IGF-I negative feedback is not known.

In summary, experimental elevation of systemic IGF-I concentrations into the young-adult range in older men suppresses mean GH concentrations dose-dependently by inhibiting both basal and pulsatile GH secretion. Reduced pulsatility is due to decreases in both the mass and number of GH secretory bursts with no change in waveform or GH half-life. Pharmacological T supplementation is sufficient to oppose IGF-I's suppression of pulsatile GH secretion but is not able to relieve IGF-I's inhibition of basal GH secretion, GH secretory-burst frequency, or pattern irregularity. The data collectively indicate that systemic T can selectively amplify GH secretion and oppose IGF-I feedback in aging men, thus framing a basis for reciprocal feedforward and feedback control of GH production.

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