Unequal Impact of Short-Term Testosterone Repletion on the Somatotropic Axis of Young and Older Men

A. GENTILI, T. MULLIGAN, M. GODSCHALK, J. CLORE, J. PATRIE, A. IRANMANESH, AND J. D. VELDHUIS

Department of Internal Medicine (J.C.), General Clinical Research Center, Virginia Commonwealth University, Medical College of Virginia, Geriatrics and Extended Care Service Line, Richmond, Virginia 23298; Geriatrics and Extended Care Service (A.G., T.M., M.G.), McGuire Veterans Affairs Medical Center, Richmond, Virginia 23249; Division of Endocrinology (J.P., J.D.V.), Department of Internal Medicine, General Clinical Research Center, Center for Biomathematical Technology, University of Virginia School of Medicine, Charlottesville, Virginia 22908-0202; and Endocrine Section (A.I.), Medical Service, Salem Veterans Affairs Medical Center, Salem, Virginia 24153

The present clinical study compares the impact of low- and high-dose parenteral testosterone (T) supplementation on daily GH secretory patterns and serum IGF-I, IGFBP-1, and IGFBP-3 concentrations in healthy older (60–82 yr) and young (20–40 yr) men. To this end, we administered three consecutive weekly injections of randomly ordered saline and either a low (100 mg) or a high (200 mg) dose of testosterone enanthate im; namely, saline (n = 17, young; n = 16, older), a low dose (n = 8 young; n = 8 older) and a high dose (n = 8 young; and n = 8 older) of androgen. To monitor somatotropic-axis responses, blood was sampled every 10 min for 24 h for later chemiluminescence-based assay of serum GH, RIA of serum IGF-I, and immunoradiometric assay of serum IGFBP-1 and IGFBP-3 concentrations. Data were analyzed via a nested analysis of covariance statistical design. At baseline (saline injection), older, compared with young, men maintained: 1) similar serum total T, (1, 2, 3, 16, 19), and age-related increased serum LH concentrations (15); 2) a 2.03-fold rise in the mean (24-h) serum GH concentration (P = 0.0053, compared with the response to saline); 2) a 1.20-fold increase in basal (nonpulsatile) GH production (P = 0.039); 3) a 2.17-fold elevation of the Mesor of nyclothemeral GH output (P = 0.031); 4) a 1.79-fold enhancement in GH approximate entropy (P = 0.0002); and 6) a 40% increase in the fasting serum IGF-I concentration (P = 0.000005). Multivariate statistical analysis indicated that following high-dose T administration, the E2 increment significantly predicted the IGF-I increment in both age groups combined (P = 0.003); T dose positively forecast the serum total IGF-I concentration (P = 0.0031); and age and T dose jointly determined serum LH concentrations (P = 0.031). In summary, neither a physiological nor a pharmacological dose of T administered parenterally for 3 wk augments daily GH secretion in eugonadal young men. In contrast, a high dose of aromatizable androgen significantly amplifies 24-h basal, pulsatile, entropic, and nyclothemically rhythmic GH production and elevates the serum IGF-I concentration in older men. The mechanistic basis for the foregoing age-related distinction in GH/IGF-I axis responsivity to T is not known. (J Clin Endocrinol Metab 87: 825–834, 2002)
three time zones within the preceding 10 d) and/or any acute weight change (>2 kg in 3 wk). Each subject provided written informed consent and was paid for participation in the study.

The design was a prospectively randomized, double-blind, placebo-(saline) controlled, nested intervention, wherein each volunteer received saline and either a low or high dose of T. Eight young and eight older subjects received 0.5 cc saline and testosterone enanthate 100 mg im weekly for 3 consecutive wk. This dose was intended to produce young-adult serum total T concentrations (23). Nine other young and eight older subjects received 0.5 cc saline and testosterone enanthate 200 mg im weekly for 3 wk to impose a supraphysiological androgen stimulus. Sampling for GH/IGF-I was conducted 3–5 d after the third injection of saline or T.

Volunteers were admitted to the General Clinical Research Center (GCRC) on the evening before blood sampling. Subjects received a standardized weight-maintaining diet of 55% carbohydrate, 30% fat, and 15% protein in meals served at 0800, 1200, and 1700 h. Lights were put out at 2300 h. After overnight adaptation to the GCRC, an indwelling catheter was placed in a forearm vein to collect blood samples every 10 min for 24 h (0800 to 0800 h). Volunteers were allowed to ambulate to the lavatory but were not permitted to sleep during the daytime or exercise vigorously. A minimum washout interval of 4 wk was imposed between the saline and T interventions.

**Assays**

Blood samples were allowed to clot at room temperature. Sera were separated and stored at −20 C. Serum GH concentrations were assayed in duplicate in each sample using a chemiluminescence-based assay with a sensitivity of 0.005 μg/liter (3 sd above the zero-dose tube) and an intraassay precision (coefficient of variation) of 4.6–8.5% for the concentration range measured here (4–6). All sera in a given subject (n = 289 samples) were assayed together. Serum total T, E2, SHBG, IGF-I, IGFBP-1, IGFBP-3, FSH, and LH concentrations were measured by RIA (T), chemiluminescence (E2), or immunoradiometric assay. The remainder in a single pool of serum prepared from all 145 samples collected on a given admission, as described earlier (2–7, 15, 24–25).

**Deconvolution analysis**

Multiparameter deconvolution analysis was used to quantify the basal (nonpulsatile) GH secretion rate; the number and mass of significant GH secretory bursts; the endogenous GH half-life; and thereby total daily GH secretion (4–6). Ninety-five percent statistical confidence intervals (CIs) for GH pulse mass were determined by the Monte Carlo support-plane procedure (26–29).

**Nyctohemeral (24-h) rhythmicity of GH release**

Twenty-four-hour rhythms of serum GH concentrations were evaluated by regression of a simple cosine function of 1440-min periodicity on each time series (30, 31). We calculated the amplitude (half the difference between the zenith and nadir), acrophase (time of maximal value within the 24-h rhythm), and mesor (mean value about which the cosine rhythm varied).

**Approximate entropy (ApEn)**

ApEn is a model-independent regularity statistic designed to quantify the regularity or orderliness of a time series (32–35). ApEn is a single
nonnegative number that monitors relative pattern consistency in serial data (32, 34, 36, 37). To compute ApEn, two input parameters are specified, $m$ (pattern length) and $r$ (de facto tolerance). In this study, we used $m = 1$ and $r = 20\%$ of the sd of the individual subject’s time series, as validated earlier (33–35, 38).

Statistical analyses

Baseline (placebo) data in young and older men were compared by the two-tailed Welch $t$ test, which extends the $t$ test to accommodate unequal variance. To assess within-subject interventional effects, we calculated as the ratio of the GH/IGF-I response to supplementation with T vs. saline. The logarithms were analyzed to equalize within-group residual variance. A primary analysis of covariance model was specified to include two classification variables: age (young, older) and T dose (low, higher) and a term to identify any age-by-dose interaction. Analyses were based on restricted maximum likelihood with a multiple comparison type I error rate of 0.05 using the least significant difference criterion. Data are presented as the geometric mean (±sem) ratios of the response to T vs. saline. Linear regression analysis was used to relate the logarithm of incremental (treatment minus placebo) changes in serum concentrations T or E2 to the primary response variables. Statistical computations were carried out in SAS version 6.12 with the mixed model software of Proc Mixed (SAS version 6.12 SAS/STAT Software Changes and Enhancements, 1996; SAS Institute, Cary, NC).

Results

Twenty-four hour serum GH concentration profiles and corresponding deconvolution-calculated GH secretory rates are illustrated graphically for several subjects in Fig. 1. Serum total IGF-I concentrations (and responses to T) are summarized in Fig. 2A. Statistical analyses of saline-pretreated subjects (baseline data) revealed that older, compared with young, men exhibited: 1) a significantly lower mean (±sem) 24-h serum GH concentration of 0.27 plus or minus 0.04 vs. 0.41 plus or minus 0.06 µg/liter ($P = 0.024$) (Fig. 2B); 2) a comparable mean (24-h pooled) serum total T concentration of 468 plus or minus 43 vs. 516 plus or minus 34 ng/dl ($P = 0.001$) (Fig. 3); 3) a reduced global mean basal (saline-exposed subjects) serum total IGF-1 concentration of 160 plus or minus 15 vs. 280 plus or minus 18 µg/liter, ($P = 0.001$) (Fig. 2); 4) a lower mass of GH secreted per burst (0.68 ± 0.09 vs. 1.2 ± 0.20 µg/liter, $P = 0.031$) (Fig. 4); 5) higher GH ApEn, denoting more disorderly GH release (0.501 ± 0.058 vs. 0.288 ± 0.021, $P < 0.001$) (Fig. 5); and 6) a blunted mean 24-h rhythmic GH amplitude and mesor (Table 1).

In young men, administration of a low dose of T did not alter the mean serum total T concentration. A higher dose increased the latter value by 2.7-fold [95% CIs (2.14, 3.34), $P <
In older men, the serum total T concentration increased by (geometric mean) 1.3-fold \([1.03, 1.65], P = 0.027\) after the low-dose and by 3.5-fold \([3.4, 4.47], P < 0.001\) after the high-dose intervention. T repletion was biologically effectual in older men because serum SHBG concentrations declined in the elderly cohort (Fig. 3). Serum T/SHBG ratios were thus lower in older subjects at all time points examined. Serum LH and FSH concentrations fell by several-fold in response to both T doses in all subjects \((P < 0.001, 10^{-3}; \text{Fig. 6 and Table 1})\). By multivariate analysis, age and T dose jointly determined the degree of decline in serum LH (but not FSH) concentrations \((P = 0.031)\). In particular, older men exhibited greater LH suppression during low-dose, and lesser LH suppression during high-dose, androgen repletion than young individuals. E2 concentrations did not change significantly in either age cohort administered the low dose of T (Table 1) but rose following the high dose by 1.5-fold \([1.15, 1.98], P = 0.004\) in young men and by 2.8-fold \([2.08, 3.75], P < 0.0001\) in older men (Fig. 3).

Nested analysis of covariance revealed that T dose influenced mean serum concentrations of IGF-I \((P = 0.0031)\) and IGFBP-3 \((P = 0.049)\) but not IGFBP-1 \((P = NS)\) (Table 1 and Fig. 2). In addition, age and T dose jointly predicted the serum total IGF-I concentration \((P = 0.007)\). In young men, IGF-I increased by 1.21-fold \([1.05–1.34], P = 0.0098\) and 1.22-fold \([1.07, 1.35], P = 0.0027\), respectively, during low- and high-dose T supplementation. In older men, IGF-I did not change after the low dose but increased by 1.4-fold \([1.22, 1.54], P < 0.0001\) following the high dose of androgen. Deconvolution analysis was applied to quantitate specific measures of GH secretion after saline vs. T injection. Neither dose of androgen altered any measure of daily GH production in young subjects. The low dose of T was also ineffectual in older individuals. In contrast, in aging men, compared with...
TABLE 1. Selected measures of GH dynamics and reproductive hormone concentrations in young and older men administered saline and either a low or high dose of T im for 3 wk

<table>
<thead>
<tr>
<th>Measure</th>
<th>Placebo (n = 17)</th>
<th>Low dose (n = 8)</th>
<th>High dose (n = 9)</th>
<th>Placebo (n = 16)</th>
<th>Low dose (n = 8)</th>
<th>High dose (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH mesor (µg/liter)</td>
<td>0.41 ± 0.06a</td>
<td>0.40 ± 0.04a</td>
<td>0.56 ± 0.18a</td>
<td>0.28 ± 0.04b</td>
<td>0.19 ± 0.04c</td>
<td>0.59 ± 0.09b</td>
</tr>
<tr>
<td>GH cosine amplitude (µg/liter)</td>
<td>0.47 ± 0.08a</td>
<td>0.49 ± 0.06a</td>
<td>0.44 ± 0.13a</td>
<td>0.25 ± 0.05b</td>
<td>0.16 ± 0.04c</td>
<td>0.26 ± 0.06b</td>
</tr>
<tr>
<td>GH cosine acrophase (min before 0800 h)</td>
<td>364 ± 51</td>
<td>386 ± 17</td>
<td>555 ± 119</td>
<td>483 ± 83</td>
<td>544 ± 128</td>
<td>535 ± 178</td>
</tr>
<tr>
<td>GH half-life (min)</td>
<td>17 ± 0.61</td>
<td>18 ± 0.71</td>
<td>15 ± 0.89</td>
<td>16 ± 0.81</td>
<td>16 ± 1.2</td>
<td>15 ± 1.4</td>
</tr>
<tr>
<td>FSH (IU/liter)</td>
<td>3.0 ± 0.3a</td>
<td>0.58 ± 0.3a</td>
<td>0.24 ± 0.02b</td>
<td>9.3 ± 2.9a</td>
<td>0.60 ± 0.3b</td>
<td>1.3 ± 0.5b</td>
</tr>
<tr>
<td>LH (IU/liter)</td>
<td>3.7 ± 0.3a</td>
<td>0.74 ± 0.4a</td>
<td>0.24 ± 0.01</td>
<td>5.2 ± 1.1a</td>
<td>0.26 ± 0.1b</td>
<td>0.78 ± 0.4b</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>41 ± 4.8a</td>
<td>54 ± 8.6a</td>
<td>45 ± 6.3a</td>
<td>30 ± 3.1a</td>
<td>36 ± 3.0a</td>
<td>99 ± 26b</td>
</tr>
<tr>
<td>IGFBP-3 (µg/liter)</td>
<td>3659 ± 131a</td>
<td>3753 ± 206a</td>
<td>3489 ± 162a</td>
<td>2912 ± 165a</td>
<td>2706 ± 220b</td>
<td>3000 ± 281c</td>
</tr>
<tr>
<td>IGFBP-1 (µg/liter)</td>
<td>20 ± 3.5</td>
<td>20 ± 3.5</td>
<td>23 ± 9.4</td>
<td>29 ± 6.2</td>
<td>37 ± 9.5</td>
<td>26 ± 8.2</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM. Low-dose (100 mg) and high-dose (200 mg) T enanthate administered im weekly.

* P < 0.05, b P < 0.005; means with shared alphabetic superscripts do not differ significantly. To convert E2 (pg/ml) to pmol/liter, multiply by 3.67.

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saline, injection of the high dose of T relative to placebo injections stimulated a 2.03-fold [(1.26, 3.27), P = 0.005] increase in the mean serum GH concentration (Fig. 2B); a 2.15-fold augmentation [(1.36, 3.41), P = 0.002] of GH secretory burst mass (Fig. 4); and a 1.10-fold increase [95% CI (1.02, 2.30), P = 0.039] in daily basal/nonpulsatile, with a corresponding elevation in total daily GH secretion (Fig. 7). On the other hand, the GH interpulse interval and GH half-life were not altered by either intervention (Fig. 4 and Table 1). Linear regression analysis revealed a significantly positive correla-
tion between the (logarithmic) incremental changes in serum total E2 and IGF-I concentrations only following the high dose of T supplementation (Fig. 8).

The ApEn statistic was used to quantitate the regularity of GH release patterns (33–35, 39). Low-dose T administration had no effect on GH ApEn in either age group (Fig. 5). Injections of a high dose of T elevated GH ApEn only in older men, namely, by 1.79-fold [(1.34, 2.38), \( P = 0.0003 \)], which signifies more irregular GH secretion.

Nyctohemeral GH release was appraised by cosinor analysis. A low dose of T paradoxically lowered the mesor and amplitude of 24-h rhythmic serum GH concentrations in older men (Table 1). However, the high dose of androgen elevated the GH mesor in older men by 2.17-fold [(1.10, 3.83), \( P = 0.025 \)]. Neither intervention affected the acrophase of 24-h rhythmic GH release.

**Discussion**

The present clinical study demonstrates that administration of a pharmacological dose of T administered parenterally for 3 wk significantly stimulates basal (nonpulsatile) GH release, augments GH secretory burst mass, increases pulsatile and total 24-h GH production, heightens the disorderliness of GH profiles, amplifies nyctohemeral GH output, and elevates serum IGF-I concentrations significantly in healthy older (but not young) men. Serum IGFBP-1 and IGFBP-3 concentrations showed minimal alterations over this interval, whereas LH and FSH release was suppressed markedly. An identical T supplementation regimen failed to stimulate any measured end points of GH/IGF-I secretion in young men, except for increasing the serum IGF-I concentration slightly. The foregoing multiple responses of the GH-IGF-I axis in older men to a high dose of T resemble the consistent facilitation of basal, pulsatile, entropic, and 24-h rhythmic GH secretion and concomitant elevation of serum IGF-I concentrations observed in normal puberty and in hypogonadal boys replaced with T (7, 39–42).

Supraphysiological T supplementation increased serum IGF-I concentrations in older men by 40% and in young men by 20%. Serum E2 concentrations rose concomitantly in both age groups and predicted the incremental changes in IGF-I (Fig. 8). T also stimulates combined GH and IGF-I production in prepubertal children, hypogonadal middle-aged men, and transsexual women (8, 13, 15, 39, 41, 43–56). However, estrogen and nonaromatizable androgens typically fail to elevate systemic IGF-I concentrations in the human (13, 15, 39, 56–58) (Fig. 9). Thus, why incremental serum E2 concentrations in young and older men given T injections correlate positively with incremental IGF-I production is not clear. One consideration is that other selected in situ estrogenic or androgenic metabolites of T mediate augmented GH and IGF-I output in this setting (14, 48, 59–62). Although only a single type of AR is known, certain androgenic metabolites appear to exert tissue-preferential effects (23, 63). In addition, recent studies in transgenic mice harboring homologous disruption of the \( \alpha \) or \( \beta \) estrogen receptor gene point to a role for the former in mediating GH IGF-I generation (64). Moreover, in the adult male rat, the \( \alpha \) (rather than \( \beta \)) E2 receptor subtype is expressed in 70% of hypothalamic GHRH neurons (65). These observations allow for, but do not prove, possible selective control of the GH-IGF-I axis via E2 receptor subtype-specific mechanisms.
T supplementation in a high dose stimulated both basal and pulsatile (and, hence, total daily) GH secretion in older men. The clinical mechanisms that control basal GH release are not well established. Limited human investigations indicate that, whereas estrogen does not alter, somatostatin and octreotide can repress and GHRH and GHRP-2 can stimulate basal GH production (5–6, 66–68). Such findings suggest that a high dose of T may suppress hypothalamic somatostatin secretion and/or enhance GHRH/GHRP release in older men (Fig. 8). Why young men do not respond analogously is not known.

Short-term administration of a high dose of T increased GH ApEn in older men only. This outcome denotes the induction of less orderly GH secretory patterns (35). Comparable changes in GH secretory regularity occur following supplementation with an aromatizable androgen or estrogen in children and/or postmenopausal women (39, 39, 41) and emerge transiently in normal midpuberty in boys (34, 38–41, 55, 69). From a mathematical perspective, more disorderly GH output indicates altered within-axis feed-forward and/or feedback control (32–35). In this regard, fixed infusions of GHRP-2 or GHRH likewise drive greater irregularity (elevated GH ApEn) of 24-h serum GH concentration profiles (5, 41, 66, 67, 70). Conversely, injections of somatostatin or IGF-I enforce more orderly patterns of GH secretion (lower GH ApEn) (70, 71). By inference, therefore, T may raise GH ApEn in older men by facilitating endogenous secretagogue action and/or by muting negative-feedback signaling within the GH-IGF-I axis.

The low (midphysiological) dose of T used here increased the mean serum total T concentration by 30% and tended to lower SHBG concentrations in older (but not young) men. These age distinctions may reflect the elevated baseline serum SHBG concentration in older subjects, which can retard the metabolic clearance of T (23). The low dose of androgen also doubled the mesor of 24-h rhythmic GH release in elderly, but not young, adults. Nyctohemeral GH secretion is governed conjointly by nutrient intake, the sleep-wake activity cycle, circadian inputs and hypothalamic GHRH, GHRP, and/or somatostatinergic signals (8, 72–74). Non-GHRH and non-GHRP signals may be relevant because ectopic tumoral secretion of GHRH or sustained iv infusion of GHRH and/or GHRP-2 increases, whereas loss-of-function mutation of the human GHRH receptor markedly diminishes 24-h rhythmic GH secretion (5, 66, 67, 75).

Maintenance of serum total T concentrations within the young-adult male range for 3 wk in older men elevated 24-h rhythmic GH release but did not stimulate basal, pulsatile, or entropic measures of daily GH secretion or normalize serum...
IGF-I concentrations. Such observations contradict the a priori hypothesis that relative hyposomatropism in older men is owing solely to an age-related decline in systemic T availability, at least over the short term. Whether more prolonged and/or more physiological androgen supplementation would be more effectual in older men is not known. Indeed, the precise threshold and/or dose dependency of T’s stimulation of GH and IGF-I output in older men remains to be established.

Short-term replacement of T in physiological amounts stimulates the GH-IGF-I axis in prepubertal boys and middle-aged hypogonadal men (8, 13, 39, 41, 58). In contrast, eugonadal young men fail to respond analogously. Thus, we speculate that responsiveness of the GH-IGF-I axis to androgen repletion may be conditional on androgen dose, age, and/or degree of T deficiency (23, 76).

From a multivariate statistical perspective, age and T dose jointly determined the degree of suppression of mean (24-h) serum LH but not FSH concentrations. Whereas both doses of T inhibited LH release significantly in young and older men, the low does was more and the high dose less effectual than the low dose in older individuals. The basis for this age-related contrast is not known. Indeed, the precise threshold and/or dose dependency of T’s stimulatory action on selected target tissues will be required to clarify whether and how aging alters the tissue-specific effects of T and/or its principal metabolites.

In summary, parenteral administration of a high dose of T for 3 wk in healthy older men stimulates basal, pulsatile, and total daily GH secretion; heightens the irregularity (ApEn) of GH release patterns; enhances 24-h rhythmic GH production; normalizes serum IGF-I concentrations; and reduces serum LH, FSH, and SHBG concentrations. Serum IGFBP-1 and IGFBP-3 levels do not change remarkably. Young men treated in the same manner show suppression of LH and FSH release but only a small rise in serum IGF-I concentrations and no detectable amplification of GH secretion. The precise neuroendocrine mechanism(s) subserving these age-related contrasts and the threshold amount of T required to elicit such distinctions are not known.

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References


58. Giustina A, Cappelli C 1997 Interaction between testosterone and growth


