Aging alters feed-forward and feedback linkages between LH and testosterone in healthy men

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Mulligan, Thomas, Ali Iranmanesh, Michael L. Johnson, Martin Straume, and Johannes D. Veldhuis. Aging alters feed-forward and feedback linkages between LH and testosterone in healthy men. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1407–R1413, 1997.—To discern the effect of aging on coordinate luteinizing hormone (LH) and testosterone secretion, we sampled healthy older men (age 62–74 yr, n = 11) and young controls (age 21–34 yr, n = 13) every 2.5 min overnight. Deconvolution analysis and cross-correlation were used to relate serum LH concentrations to calculated testosterone secretion rates (feed-forward stimulation), as well as serum testosterone concentrations to computed LH secretion rates (feedback inhibition). Despite statistically similar mean LH and testosterone concentrations in the young and older men, older individuals had diminished feed-forward stimulation of LH concentrations on calculated testosterone secretion rates, as well as delayed feedback inhibition of testosterone concentrations on computed LH secretion rates.

biological timing; luteinizing hormone; hormone secretion; age; male

MULTIPLE FACTORS (e.g., polysynaptic neuronal inputs) coordinate hypothalamic secretion of bursts of gonadotropin-releasing hormone (GnRH) in women and men. Pulsatile release of GnRH into the hypothalamic-pituitary portal venous system stimulates pituitary secretion of luteinizing hormone (LH). Episodic secretion of LH into the systemic circulation in men in turn drives pulsatile testicular production of testosterone (feed-forward effect of LH on testosterone). Biologically active testosterone in the plasma completes the loop, resulting in feedback inhibition of GnRH and LH secretion (1, 17, 22, 25).

The impact of aging on the hypothalamic-pituitary-testicular axis is important because aging is associated with diminished testosterone secretion (13). In turn, limited testosterone availability can be accompanied by decreased muscle mass and strength (12), reduced bone mineral density (18), greater risk of hip fracture (9), loss of sexual interest (6), coronary artery disease (14), and impaired spatial cognition (10). Importantly, alterations in testosterone secretion could be caused by reduced feed-forward (blood LH concentrations stimulating Leydig cell testosterone secretion) or feedback (testosterone concentrations inhibiting GnRH-LH secretion) interactions between the hypothalamic-gonadotroph unit and Leydig cells. We postulated here that healthy older men even in the absence of significant alterations in mean serum LH and/or testosterone concentrations might exhibit decreased coordinate LH and testosterone release via disruption of either feed-forward or feedback signaling.

To test the hypothesis of altered LH and testosterone interactions in aging men, we used frequent venous sampling (every 2.5 min) overnight and cross-correlation analysis of the LH-testosterone time series in healthy young versus older individuals. We studied nocturnal release of LH and testosterone, because earlier 24-h studies in young men showed that LH and testosterone secretion are maximal overnight at approximately 0300–0800 (28). In addition, because serum LH concentrations presumably drive testosterone secretion, cross-correlation analysis was carried out between serum LH concentration values and deconvolution-calculated testosterone secretion rates (rather than between the serum concentrations of the two hormones under investigation). Conversely, because the feedback actions of testosterone on secretory output of the hypothalamic-pituitary unit are presumptively mediated via serum testosterone concentrations acting ultimately on LH secretion, cross-correlation analysis was also applied to serum testosterone concentrations versus calculated LH secretion rates. These new strategies unmasked alterations of LH-testosterone feed-forward and feedback relationships in healthy older men compared with younger individuals, who had statistically similar mean serum LH and testosterone concentrations. Such findings suggest that more subtle dynamic disruption of the GnRH-LH-testosterone axis may occur in older men, reflecting deterioration of coordinate within-axis regulation.

METHODS

This study was approved by the University of Virginia Human Investigation Committee. We studied healthy young (age 21–34 yr, n = 13) and older (age 62–74 yr, n = 11) men who had no acute or chronic illness, ingested no drugs or medications, were nonsmokers, were within 20% of ideal body weight, and had not undergone any transmeridian travel in the past 6 wk.

After giving written informed consent, subjects spent two consecutive nights in the sleep laboratory of the General Clinical Research Center. The first night was to habituate the volunteer to the experimental setting, including polysomnography. During the second night, at 2200, a catheter was inserted into the forearm vein in each subject and connected via long tubing to a slow infusion of heparinized saline. While the subject slept, electroencephalogram (EEG) monitoring was carried out, and a 2.5-ml serum sample was obtained every 2.5 min. Sampling was terminated when the volunteer awakened, allowing an average of 7 h of study. Blood samples
were allowed to clot at room temperature, and the sera were frozen at −20°C for later assay. EEG records were analyzed according to the criteria of Rechtschaffen and Kales (16a). This provides a standardized assessment of sleep latency (time from lights out to sleep onset), sleep efficiency (total sleep time/total time in bed), percentage of sleep time spent in the various sleep stages (I-IV), and time spent in rapid eye movement (REM) versus non-REM sleep.

We performed serum LH assays in duplicate via a fully automated robotics system, using a two-site monoclonal immunoradiometric assay (IRMA, Nichols Laboratory, San Juan Capistrano, CA). The sensitivity of the LH IRMA was 0.20 IU/l. The interassay coefficient of variation was <10%. Serum testosterone measurements were also performed in duplicate for each sample, using a radioimmunoassay (Diagnostic Products, Los Angeles, CA) with an assay sensitivity of 2.8 ng/dl and an interassay coefficient of variation of <10%. For analysis of the LH and testosterone time series, dose-dependent within-assay sample standard deviations and hence coefficient of variations were calculated via a power function fit of the relationship “hormone concentration (dose) versus within-sample standard deviation squared (variance)” for all overnight assay replicates in each subject (26). Pooled overnight sera were used to assay (mean) per subject cortisol, thyroid-stimulating hormone, thyroxine (T4), follicle-stimulating hormone, luteinizing hormone, LH, prolactin, androstenedione, dehydroepiandrosterone sulfate (DHEA-sulfate), sex hormone-binding globulin (SHBG), insulin-like growth factor (IGF) I, and IGF-binding protein (BP)-III concentrations.

To test the hypothesis that elevations of the serum LH concentrations are closely associated with increasing testosterone secretory rates (rather than with the temporally delayed and more autocorrelated fluctuations in serum testosterone concentrations), we first applied deconvolution analysis (PULSE) to the serum testosterone concentration time series to calculate sample testosterone secretory rates (19). PULSE is a waveform-independent method, in which we assumed a two-component testosterone disappearance model with corresponding rapid and slow phase half-lives reported by others (8). Similarly, in a second phase of the analysis, serum testosterone concentrations were correlated with deconvolution-calculated (PULSE) LH secretion rates assuming a two-component LH disappearance model of first and second phase half-lives of 18 and 90 min, respectively, with 37% of the decay amplitude contributed by the slow phase, as measured earlier via infusions of human LH (24). This strategy also helps to reduce spurious cross-correlation introduced otherwise by sustained autocorrelations within each individual hormone concentration time series.

The serum LH concentrations and testosterone secretion rates, and the serum testosterone concentrations and LH secretion rates, were then submitted to cross-correlation analysis. Cross-correlation is a form of lagged linear correlation, in which appropriately paired serial data (e.g., serum LH concentrations and calculated sample testosterone secretion rates) are correlated at each of various time lags of interest (e.g., zero lag correlates all the simultaneous LH concentrations and testosterone secretion rates, and as such is a simple linear correlation). Cross-correlation r values are calculated in each subject at each of multiple time lags. In particular, here LH was allowed to lead or lag testosterone by 0–150 min (27). We next determined whether and where there were statistically significant cross-correlations within the groups of 13 young and 11 older men between 1) the serum LH concentrations and the testosterone secretion rates to assess feed-forward stimulation and 2) the serum testosterone concentrations and LH secretion rates to assess feedback inhibition. To this end, individual r values were converted to standard deviate scores (z scores) by dividing each original r value by its corresponding standard deviation. The latter was determined by Monte Carlo simulations, in which the order of values in each paired LH and testosterone time series was shuffled 300 times, and the corresponding r value at each lag was recalculated from the new (randomly ordered) pair. The standard deviation so observed for the r values at any particular lag in any given (randomly shuffled) paired LH-testosterone series in any given subject was used to compute a corresponding z score. These empirically determined standard deviations yielded inferences that agreed well with independently calculated values, assuming that standard deviation of r equals [1/(n − k)]1/2, where n is the number of sample pairs and k is the number of lag units (e.g., one or more sampling intervals, etc.).

We used Student’s t-test to determine the significance of differences if any between mean serum LH or testosterone concentrations in young and older men and time spent in given sleep stages. Correlations between LH and testosterone in the young (n = 13) and older men (n = 11) were evaluated statistically by the Kolmogorov-Smirnov test under the null hypothesis that z scores matching the individual r values at any given lag are randomly and normally distributed about a zero mean with unit standard deviation. Additionally, we used the Student’s t-test to compare the z score distributions in young and older men at any given lag. In all cases we used two-tailed statistics and accepted as significant P < 0.05, except for cross-correlations where a protected P value of 0.01 was required to limit false positive associations to ≤1 per 100 lags.

RESULTS

As outlined in Table 1, older subjects had less efficient sleep and spent a greater proportion of their sleep time in stage 1 sleep. However, there were no significant differences between young and older men in the percentage of time they spent in deep sleep (slow-wave sleep, stages 3–4) or REM sleep. Pooled hormone measurements from overnight sera revealed group (young vs. older) differences for cortisol, T4, FSH, prolactin, DHEA-sulfate, IGF-I, and IGFBP-III but not for the other hormones measured here (Table 2). Means ± SE overnight serum LH concentrations in the young and older men were virtually identical at 2.5 ± 0.2 vs. 2.5 ± 0.3 IU/l, respectively (P = 1.0). Serum testosterone concentrations in the two age groups were not significantly different (461.5 ± 40.4 vs. 403.8 ± 37.5 ng/dl, P = 0.36). However, visual inspection of overnight serum LH and testosterone concentration profiles

<table>
<thead>
<tr>
<th>Table 1. Sleep parameters in young and older subjects</th>
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<tr>
<td>Young</td>
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<tr>
<td>Sleep latency, min</td>
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<tr>
<td>Sleep efficiency, %</td>
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<td>Stage 1, %</td>
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<td>Stage 2, %</td>
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<td>Stages 3 and 4, %</td>
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<td>REM, %</td>
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<td>REM latency, min</td>
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Values are means ± SE; n = 13 young and 11 older subjects. REM, rapid eye movement.
in young men suggested an apparent coupling of serum LH concentration elevations to later serum testosterone elevations. This relationship was less visually apparent in the older men (Fig. 1) and hence was quantified by cross-correlation analysis.

Cross-correlation analysis showed sustained positive [or presumptively feed-forward (stimulatory)] correlations between serum LH concentrations and lagged testosterone secretion rates in young men; see Fig. 2A, where median r values at different lags are plotted for the group of 13 young volunteers. Specifically, a rise in the calculated testosterone secretion rate tended to follow an increase in the serum LH concentration by 15–120 min in young men. Statistical evaluation also suggested negative cross-correlations between testosterone secretion rates and LH concentrations at a lag of 80–90 min; i.e., when testosterone secretion increased, LH concentrations fell 80–90 min later. This relationship was tested more appropriately by cross-correlating serum testosterone concentrations and calculated pituitary LH secretion rates.

In contrast to young individuals, older men (n = 11) showed an attenuation of the positive cross-correlation between serum LH concentrations and calculated testosterone secretion rates, indicating a putatively diminished LH-testosterone feed-forward effect (Fig. 2B). Specifically, a rise in testosterone secretion rates followed an increase in serum LH concentrations over a narrower range of lags, namely by 40–50 min. This apparent difference between young and older individuals was affirmed further statistically by Student's t-testing of the group cross-correlation coefficients (or Kolmogorov-Smirnov testing of their corresponding z scores) in young and older men at different lags (not shown).

Cross-correlation also demonstrated significant negative correlations (presumptive feedback inhibition) between serum testosterone concentrations and calculated LH secretion rates in young men (Fig. 3A).

Table 2. General endocrine measures in young and older subjects

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Older</th>
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<tbody>
<tr>
<td>Cortisol, µg/dl</td>
<td>5.1 ± 0.7</td>
<td>7.2 ± 0.6</td>
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<td>TSH, µU/ml</td>
<td>1.3 ± 0.2</td>
<td>1.6 ± 0.2</td>
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<tr>
<td>T4, µg/dl</td>
<td>5.1 ± 0.4</td>
<td>4.1 ± 0.1</td>
<td>0.04</td>
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<tr>
<td>FSH, IU/l</td>
<td>2.7 ± 0.4</td>
<td>4.8 ± 0.6</td>
<td>0.006</td>
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<tr>
<td>Prolactin, µg/l</td>
<td>9.5 ± 1.2</td>
<td>4.3 ± 0.8</td>
<td>0.005</td>
</tr>
<tr>
<td>Estradiol, pg/ml</td>
<td>39 ± 8</td>
<td>21 ± 7</td>
<td>0.12</td>
</tr>
<tr>
<td>DHEA-S, µg/dl</td>
<td>272 ± 30</td>
<td>113 ± 29</td>
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<tr>
<td>GH, µg/l</td>
<td>1.7 ± 0.5</td>
<td>0.7 ± 0.2</td>
<td>0.11</td>
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<tr>
<td>IGF-I, µg/l</td>
<td>160 ± 33</td>
<td>39 ± 16</td>
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</tr>
<tr>
<td>IGFBP-3, µg/l</td>
<td>1,767 ± 61</td>
<td>1,378 ± 131</td>
<td>0.009</td>
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</tbody>
</table>

Values are means ± SE; n = 13 young and 11 older subjects. TSH, thyroid-stimulating hormone; T4, thyroxine; FSH, follicle-stimulating hormone; DHEA, dehydroepiandrosterone; GH, growth hormone; IGF, insulin-like growth factor; BP, binding protein.

Fig. 1. Illustrative plots of serum luteinizing hormone (LH; A and B) and testosterone (T; C and D) concentrations from representative young (A and C) and older (B and D) men sampled at 2.5-min intervals overnight. Note that for young men (A and C) there is a prominent increase in serum LH concentrations at ~50 min, followed about 50 min later by a sustained increase in serum testosterone concentrations. This apparent time-lagged coupling of elevations of serum LH and testosterone concentrations is attenuated in older men (B and D).
Specifically, LH secretion declined within $-10$ to $+25$ min of the testosterone concentration rise. In contrast to young subjects, older men demonstrated a longer lag in the feedback inhibition of testosterone concentrations on LH secretion rates (Fig. 3B). Rather than rapid feedback (within 25 min), older men exhibited negative cross-correlations of testosterone concentrations on LH secretion rates after a delay of 32–37.5 min and again

Fig. 2. Median cross-correlation coefficients ($r$ values) between serum LH concentrations and calculated testosterone secretion rates for 13 young men (A) and 11 older men (B). For young men there is sustained positive cross-correlation (feed-forward stimulation), which is statistically significant from 15 to 120 min (noted by plus signs above series of $r$ values). In contrast, there is restriction of this feed-forward linkage in older men to the interval between 40 and 50 min only.

Fig. 3. Median cross-correlation coefficients ($r$ values) between serum testosterone concentrations and calculated LH secretion rates for 13 young men (A) and 11 older men (B). In young men there is rapid and sustained negative cross-correlation (feedback inhibition) when androgen levels precede LH secretory rates by $-10$ to $+25$ min (noted by plus signs above series of $r$ values). In contrast there is delay of this negative feedback effect in older men to shorter lag windows of 32–37.5 and 55–62.5 min.
after 55–62.5 min. Thus older men's statistically inferred suppression of LH secretion by an increase in testosterone concentration occurs after a more substantial time lag.

**DISCUSSION**

We confirm a significant time-lagged cross-correlation relationship between LH and testosterone in young men (3). By way of deconvolution analysis of serum LH and testosterone time series obtained by intensively sampling (every 2.5 min) blood overnight in healthy young men, we have extended this finding to LH concentrations positively correlated with (putatively acting on) calculated testosterone secretion rates. Conversely, we observed that young men also show a statistically significant negative cross-correlation (apparent feedback inhibition) of testosterone concentrations on calculated LH secretion rates, as inferred in more direct studies in experimental animals (16). Because hormone concentrations in blood lag their underlying glandular secretion rates and thus obscure the feedback relationship, e.g., between serum androgen concentrations and actual LH release, here we used deconvolution analysis to calculate underlying hormone secretion rates. We could then cross-correlate, e.g., serum testosterone concentrations and calculated LH secretion rates to evaluate negative feedback. This analysis in the young men studied here showed rapid (within -10 to +25 min) apparent negative feedback of blood androgen concentrations on calculated LH secretion rates in healthy young adults. Results in older men were then contrasted with these new findings, because the impact of aging on either of these two major (feed-forward and feedback) endogenous interactions has not been previously delineated in the human or experimental animal to our knowledge.

This clinical investigation disclosed two evident age-related disturbances in the dynamics of the male hypothalamic-pituitary-gonadal axis. First, whereas we observed a highly significant and sustained positive cross-correlation between the serum LH concentration and calculated testosterone secretion rate after a biologically plausible lag time of 15–120 min in young men, this feed-forward relationship was attenuated in the aged men to only a 40- to 50-min lag. Of interest, this inferred erosion of positive LH-testosterone (feed-forward) coupling in older men occurred despite statistically similar mean serum LH and testosterone concentrations in the two age groups. Such data are consistent with a postulate of attenuated effectiveness of the in vivo LH-Leydig cell testosterone drive in healthy aged men. Postulated impairment of functional LH-testosterone feed-forward coupling could be due to the decline in high-amplitude bioactive LH secretory pulses in older individuals (5, 30) and/or the concomitantly increased frequency of low-amplitude pulsations (5). Other possible explanations include a primary decrease in Leydig cell steroidogenic responsiveness to minute-to-minute changes in circulating blood LH concentrations, reduced vascular delivery of LH to and/or within the testes, and/or altered kinetics of Leydig cell testosterone secretion into or distribution within plasma. In relation to these considerations, both diminished endogenous (bioactive) LH secretory pulses (20) and reduced Leydig cell responsiveness to exogenous LH-human chorionic gonadotropin injections have been inferred in older men (7). Available data do not distinguish further among these possibilities.

Second, whereas we found evidence of rapid (within -10 to +25 min) feedback inhibition by (negative cross-correlation between) serum testosterone concentrations on LH secretion rates in the young men, this inferred physiological autofeedback mechanism was altered in the aged men. Older individuals lacked this short-lagged negative cross-correlation between serum testosterone concentrations and LH secretion rates (the latter estimated by deconvolution analysis) and rather showed delayed (32- to 37.5- and 52- to 62.5-min lagged) negative feedback. This inferred loss of rapid feedback inhibition and the apparent operation of time-delayed feedback suppression, within the LH-testosterone axis could be due to possible age-associated changes in testosterone secretory patterns (i.e., loss of either 24-h nyctohemeral and/or ultradian pulsatile rhythms) as recognized recently (2, 13) and/or postulated alterations in hypothalamic-pituitary responsiveness to negative feedback actions of testosterone. Although not proven to our knowledge, disrupted autonegative feedback within the aging testosterone-LH axis could be due to age-dependent changes in testosterone metabolism (e.g., aromatization or 5-α reduction) and/or in androgen-receptor activity. These postulated considerations would arise from indirect observations in the rodent and human (23). Whether the amount and frequency of pulsatile GnRH and LH release in older men is inappropriately regulated by endogenous androgen negative feedback is not known, but older men do show normal increases in pulsatile bioactive LH secretion when androgen negative feedback is partially blocked by administration of the antiandrogen flutamide (29). In contrast, increased sensitivity to the inhibitory actions of exogenously (constantly) delivered androgen has been inferred in older men after intravenous infusion of testosterone (32) or transdermal delivery of 5-α-dihydrotestosterone (31). The identification of occasional negative lags in the testosterone-LH negative-feedback relationship suggests stochastic elements within the feedback control system and/or the influence of experimental uncertainty within the sampling and assay components of this in vivo analysis. We cannot distinguish among these possibilities with the currently available data. Further clinical investigation will be required to characterize postulated alterations in androgen autonegative feedback regulation of the GnRH-LH-testosterone axis in healthy aging men.

As discussed previously, the present work suggests that endogenous androgen negative feedback signaling of LH release is disrupted in healthy older men, even when mean overnight serum LH and testosterone
concentrations do not differ from those in younger individuals. Presumptive loss of normal minute-to-minute LH-testosterone synchrony in aging men has also been inferred recently using a novel independent statistic designed to measure conditional (pair-wise) disorderliness of bihormonal release, namely, cross-approximate entropy (15). This conceptually and mathematically distinct statistic is calculated independently of lag, unlike cross-correlation or cross-spectral analysis, and hence may imply a more general deterioration of LH-testosterone coordinated release with aging. The relevance of these new insights in the reproductive axis to aging-related disturbances in other neuroendocrine axes that are also subject to feedback and feed-forward control will require further study, as will their potential application (if any) to female reproductive aging. However, ACTH, GH, and insulin release all exhibit reduced synchrony in the same individual during healthy aging, for example, DHEA-S, the relatively higher serum cortisol and some of these age-related differences are well known, i.e., DHEA-S, the relatively higher serum cortisol and lower serum T₄ concentrations have not been previously reported. These age-related alterations may be a function of changes in the serum concentration of the respective hormone-binding-globulin or hormone clearance. Nevertheless, these preliminary observations warrant further investigation.

Perspectives

The relative hypogonadism of older age in men is associated with such adverse outcomes as hip fracture. However, the underlying biological mechanisms responsible for the lower testosterone production rate have not been adequately delineated. In our investigation, we found significant alterations in the LH-testosterone feed-forward and feedback systems, despite statistically similar mean serum LH and testosterone concentrations. To our knowledge, this is the first in vivo assessment of the joint relationships between human endogenous serum LH concentrations and calculated testosterone secretion rates (feed-forward stimulation), and testosterone concentrations and computed LH secretion rates (feedback inhibition). Importantly, this perspective could not have been accomplished without the technique of frequent venous sampling performed over at least four ultradian rhythms coupled with deconvolution analysis of the endocrine time series. These newly described age-related disruptions could be due to alterations in signal efficacy or strength (amplitude or waveform of the LH secretory event) or in the ability of the target cell to respond to one or more signals (e.g., impaired Leydig cell signal transduction). Furthermore, this intriguing observation of age-associated alterations in feed-forward and feedback reproductive-hormone signaling needs to be investigated in other axes (e.g., ACTH and cortisol).

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