Older men exhibit reduced efficacy of and heightened potency downregulation by intravenous pulses of recombinant human LH: a study in 92 healthy men

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Veldhuis JD, Liu PY, Keenan DM, Takahashi PY. Older men exhibit reduced efficacy of and heightened potency downregulation by intravenous pulses of recombinant human LH: a study in 92 healthy men. Am J Physiol Endocrinol Metab 302: E117–E122, 2012. First published October 4, 2011; doi:10.1152/ajpendo.00450.2011.—Direct sampling of the human spermatic veins has disclosed concomitant LH and testosterone (T) pulses, suggesting pulsatile LH concentration-dependent stimulation of T secretion. However, studies to date have examined this hypothesis using only pharmacological stimulation with hCG. The present study tests the hypothesis that age is marked by decreased T secretory responses to repeated near-physiological iv pulses of recombinant human LH administered in a Clinical Translational Science Center. Participants included 92 healthy men aged 18–75 yr with BMI 18–34 kg/m2. The contribution of endogenous LH pulses was minimized by combined injection of a selective GnRH receptor antagonist sc and successive pulses of biosynthetic LH iv. A new analytical dose response model was applied to estimate the properties of exogenous LH’s drive of T secretion. Regression of LH-T dose response potency estimates on age showed that the efficacy of pulses of biosynthetic LH progressively decreased with age (P = 0.014, r = 0.26). Testis sensitivity to exogenous LH pulses also declined with age (P = 0.011, r = 0.27). Moreover, estimated Leydig cell downregulation by LH pulses rose significantly with age (P = 0.039, r = 0.22). These outcomes were selective, since the recovery potency of infused LH was not affected by age but was reduced by increasing BMI (P = 0.011, r = 0.27). Assuming stable bioactivity of infused recombinant human LH, these novel data indicate that factors associated with age and BMI attenuate LH efficacy and testis sensitivity and augment Leydig cell downregulation in healthy men.

METHODS

Human subjects. Ninety-two healthy men were studied after they provided voluntary, witnessed, informed consent that was approved by the Mayo Clinic Institutional Review Board. The protocol was reviewed by the FDA under an investigator-initiated off-label use of ganirelix (Organon, West Orange, NJ) and recombinant human LH (Serono, Rockland, MA).

Protocol. Each subject underwent an overnight study in the Mayo Clinic Translational Science Center. The protocol comprised 2200 sc injection of 1 mg/m2 ganirelix, a selective GnRH receptor antagonist (2). Age does not affect ganirelix absorption or blood levels (33). Intravenous (6- or 8-min square-wave) pulses of 6.25–50 IU Serono LH were given every 1–3 h for 8 h to 2 days, beginning 2 h after the ganirelix injection. Blood was sampled every 10 min concurrently for LH and T measurements.

Hormone assays. LH was measured by robotics-automated immunochemiluminescence assay in duplicate in each subject exactly as described (33). No samples were undetectable (all >0.1 U/L, Second International Reference Preparation). In this assay, 75 IU Serono LH represents 30 IU Second International Reference Preparation LH by direct assay. T was measured by the same platform, which correlates at r2 = 0.98, slope = 1.07 (P < 0.001), with tandem mass spectrometry (20).

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Table 1. Table of rhLH subjects according to study design

<table>
<thead>
<tr>
<th>Study (Infusion Paradigm)</th>
<th>Means ± SE (Ranges)</th>
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</thead>
<tbody>
<tr>
<td>6.25, 12.5, 25, and 37.5 IU rhLH pulses, each twice iv in random order every 3 h for 20 h (n = 15)</td>
<td>Age, yr: 48 ± 4.6 (21–75) BMI, kg/m²: 27 ± 0.82 (21–32)</td>
</tr>
<tr>
<td>12.5 (every hour) or 25 IU (every 2 h) rhLH over 22 h (n = 15)</td>
<td>40 ± 5.4 (19–73) 26 ± 1.1 (19–34)</td>
</tr>
<tr>
<td>12.5 IU rhLH every 2 h over 22 h (n = 23)</td>
<td>41 ± 3.0 (19–72) 26 ± 0.71 (20–32)</td>
</tr>
<tr>
<td>37.5 IU rhLH boluses administered 2 h apart over 8 h (n = 20)</td>
<td>35 ± 2.8 (18–70) 26 ± 0.73 (18–31)</td>
</tr>
<tr>
<td>50 IU rhLH iv every 2 h for 2 days (n = 19)</td>
<td>41 ± 4.9 (19–73) 28 ± 0.75 (22–32)</td>
</tr>
<tr>
<td>6.25–50 IU rhLH iv every 1–3 h for 8 h to 2 days (all 5 studies; n = 92)</td>
<td>40 ± 1.8 (18–75) 27 ± 0.37 (18–34)</td>
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</tbody>
</table>

Values are means ± SE; n = 92 men. rhLH, recombinant human LH; iv, intravenous; BMI, body mass index. Pulses were infused as 6- or 8-min square-wave boluses.

Results

Age, BMI, and recombinant human LH-infusion schedules are given in Table 1. All subjects completed the full sampling protocol. Mild injection site tenderness was noted after gannarelaxation injection, but not after treatment. There were no other adverse events. Age ranged from 18 to 75 yr and BMI from 18 to 34 kg/m². By linear regression, age was associated with increases in BMI (P < 0.001), sex hormone-binding globulin (P = 0.004), and FSH (P < 0.001) and decreases in both prolactin (P < 0.001) and bioavailable T concentrations (P < 0.001) at screening. LH, E₂, and total T did not differ with age (P > 0.07).

Estimated potency of initial (onset) LH stimulation averaged 2.5 ± 0.47 IU/l (median 2.3) in the 92 volunteers (Table 2). Downregulated (offset) LH potency averaged 6.6 ± 0.79 IU/l (median 5.2). The latter values denote significant pulse-by-pulse downregulation [P < 0.001 by paired 2-tailed t-test compared with initial (onset) EC₅₀]. Regression analysis revealed no significant effect of age on the initial (onset) LH EC₅₀ (P = 0.64) or on the delayed (recovery) LH EC₅₀ (P = 0.16). In contrast with age, BMI predicted onset (initial) LH EC₅₀ (P = 0.054, r = 0.20; Fig. 1, top) and offset (downregulated) LH EC₅₀ (P = 0.011, r = 0.27; Fig. 1, bottom). The positive slopes of BMI on EC₅₀ define loss of LH potency. The hypothesis of heightened downregulation of LH potency}

The sensitivity of pulsatile T secretion to pulsatile exogenous LH drive (slope term) averaged 1.4 ± 0.21 (median 1.7) (ng·dl⁻¹·min⁻¹·IU/l) in the 92 men studied (Table 2). There were significantly negative regressions of testis sensitivity on age (P = 0.011, r = −0.27), BMI (P = 0.029, r = −0.23), and age × BMI product (P = 0.005, r = −0.29; Fig. 3).

Table 2. LH-T 2-potency dose response data in men: rhLH

<table>
<thead>
<tr>
<th>rhLH</th>
<th>Means ± SE</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC₅₀ onset, IU/l</td>
<td>2.5 ± 0.47</td>
<td>2.3 (0.003–19)</td>
</tr>
<tr>
<td>EC₅₀ recovery, IU/l</td>
<td>6.6 ± 0.79</td>
<td>5.2 (0.81–38)</td>
</tr>
<tr>
<td>Potency onset (exponent)</td>
<td>−3.5 ± 0.76</td>
<td>−4.9 (−40 to −0.003)</td>
</tr>
<tr>
<td>Potency recovery (exponent)</td>
<td>−9.3 ± 1.5</td>
<td>−9.4 (−40 to −0.18)</td>
</tr>
<tr>
<td>Sensitivity (slope)</td>
<td>1.4 ± 0.21</td>
<td>1.7 (0.001–8.7)</td>
</tr>
<tr>
<td>Efficacy, ng·dl⁻¹·min⁻¹</td>
<td>9.5 ± 0.58</td>
<td>9.9 (1.7–29)</td>
</tr>
<tr>
<td>Basal, ng·dl⁻¹·min⁻¹</td>
<td>4.1 ± 0.76</td>
<td>5.4 (0.001–26)</td>
</tr>
<tr>
<td>Downregulation delay, min*</td>
<td>30 ± 1.5</td>
<td>40 (10–50)</td>
</tr>
<tr>
<td>Model SD, ng·dl⁻¹·min⁻¹</td>
<td>3.1 ± 0.13</td>
<td>3.0 (0.68–6.7)</td>
</tr>
<tr>
<td>Random effects, ng·dl⁻¹·min⁻¹</td>
<td>0.77 ± 0.21</td>
<td>2.1 (0.001–9.1)</td>
</tr>
</tbody>
</table>

Data are the geometric means ± SE and median (range); n = 92. T, testosterone. *Time lag from potency onset to recovery (see METHODS).
Efficacy of infused LH pulses averaged 9.5 ± 0.58 (median 9.9) in the full cohort (Table 2). Regression analysis showed a strongly negative influence of age on pulsatile LH efficacy (P = 0.014, r = −0.26; Fig. 4, top). This indicates that maximal pulsatile exogenous LH drive of pulsatile T secretion decreases approximately linearly as age increases. In addition, there was a significant interaction between age and BMI in jointly predicting decreased LH efficacy (P = 0.047, r = −0.21; Fig. 4, bottom). There was no effect of BMI alone.

Basal (nonpulsatile) T secretion averaged 4.1 ± 0.76 ng·dl⁻¹·min⁻¹ (median 5.4), with no change with age (Table 2). The time delay to downregulation averaged 30 ± 1.5 min (median 40 min; n = 92 men). Neither age nor BMI affected this value. Model fit, defined statistically by the model residual SD, was 3.1 ± 0.13 (3.0) ng·dl⁻¹·min⁻¹. Model SD declined with age (P = 0.004, r = −0.30). Random effects on T secretory burst mass were invariant of age (P = 0.46) and BMI (P = 0.27) and BMI (P = 0.46).

**DISCUSSION**

In 92 healthy men aged 18–75 yr, pulsatile intravenous (iv) infusion of biosynthetic human LH at 1- to 3-h intervals for 8–48 h unveils a strong age-associated loss of stimulatory efficacy. Confounding by endogenous LH was minimized by prior subcutaneous administration of a potent selective GnRH receptor antagonist, ganirelix, which lowers LH concentrations by ≥85% (21, 34, 37, 38). Earlier studies showed that age per se does not affect ganirelix blood levels in healthy men (21). Accordingly, assuming validity of the pulsatile recombinant human LH clamp paradigm, the present outcomes establish an age-associated reduction of maximal Leydig cell steroidogenic responses to nearly physiological LH pulses. This inference is strengthened by evidence that GnRH antagonists exert no significant direct gonadal effects (9) and that deconvolution estimates of the mass and half-life of injected LH pulses were well within the range of normal endogenous LH pulses (15).

Deconvolution analysis of T concentration series obtained during the exogenous pulsatile recombinant human LH clamp provided an independent verification and a mechanistic explanation for diminished testis responsiveness in older individuals. In particular, the mass (amount of) T secreted per burst declined significantly with age (P = 0.003, r = −0.31). The age-related finding was selective, since nonpulsatile (basal) T secretion did not change with age (P = 0.634, r = 0.051). Data are from n = 92 men given repeated iv pulses of rhLH after ganirelix (GnRH receptor blocker) injection. *Linear regression of sensitivity on BMI was also significant (P = 0.029, r = −0.23). †Only potency difference (P = 0.008, r = 0.28) and sensitivity (P = 0.005, r = −0.29) were determined by an age × BMI interaction.
secretion was independent of age across the five-decade span evaluated here.

Calculated testis sensitivity to recombinant human LH pulses decreased markedly with age, BMI, and their interaction. The largest absolute effect was for the interaction between age and BMI, where $r = -0.29$ ($P = 0.005$). Sensitivity corresponds to a slope term defined by the degree of augmentation of T secretion rate induced by each unit rise in LH concentrations. Lower sensitivity to individual LH pulses could be due to reductions in either LH receptor activation or postreceptor signaling. In this regard, data in the aged male rat demonstrate decreases in testicular LH-driven AMP accumulation and in cholesterol’s delivery to inner mitochondrial membranes (18, 32). Postulated pathophysiological mechanisms in aging include formation of reactive oxygen species and impairment of antioxidant defenses in aging steroidogenic cells (5). In contrast, we speculate that an absolute reduction in the number of LH receptors or Leydig cells could better explain reduced LH efficacy (lower maximal T secretion) (27). Accumulation of systemic inflammatory cytokines in aging and obesity may also confer a mechanism for reduced Leydig cell function (39). The pulsatile LH clamp model provides a means to test such hypotheses prospectively.

The finding of age-invariant LH potency is consistent with infusion of recombinant human LH of fixed biological potency. The earlier inferred reduction in endogenous LH potency in aging men (15) might be due to reduction in circulating LH bioactivity (6, 11, 25, 36). Whereas detailed analysis of LH isoforms will be required to clarify this issue, the pulsatile recombinant human LH clamp paradigm used here bypasses endogenous LH due to overnight hypogonadotropism induced by ganirelix pretreatment. Under these experimental circumstances, LH potency estimates were comparable in young and older men whether assessed during the rising (onset) or falling (offset) phases of LH pulses.

Intraindividual EC_{50} differences (recovery EC_{50}—initial EC_{50}) provide a measure of intrapulse downregulation of LH-stimulated T secretion. This difference (downregulation) term increased significantly with age in the cohort of 92 men ($P = 0.023$, $r = 0.24$). Heightened downregulation of Leydig cell T responses to distinct LH pulses in older men suggests a novel mechanism for reduced testis responsiveness in aging individuals. If verified, this mechanism could also contribute to decreased hCG effects in older men, since pharmacological hCG is itself a strong downregulating signal to T secretion in men and animals (see INTRODUCTION) (8, 35).

Caveats include the issue of parameter correlation in all models, including dose response models. Here, the concern is mitigated by deconvolving the otherwise strongly autocorrelated T concentration time series into a T secretion profile and

Fig. 3. Inverse relationships between age, BMI, and age × BMI and the natural logarithm of T secretory sensitivity to infused pulses of rhLH in 92 healthy men. The data point in the box is an outlier at $P < 0.001$ by $F$ ratio testing.

Fig. 4. Negative association between the natural logarithm of the efficacy of pulsatile intravenous rhLH infusions (y-axis) and age (x-axis) (top) as well as age × BMI (bottom) in 92 healthy men.
by parameter estimation using a maximum-likelihood-based convergence method. The asymptotic (tending to a plateau) nature of the logistic dose response function also provides an implicit upper and lower boundary. Additional caveats include the need to replicate these outcomes in other cohorts of healthy men, the restriction that exogenous LH pulses may not be identical to endogenous LH pulses in shape (viz., the former were ~2 min shorter) or in biochemical isoform distribution, the recognition that the present experimental infusions test mainly short-term testis responses, and the need for longitudinal data to test cause and effect.

In summary, by using a paradigm of pulsatile iv infusion of recombinant human LH along with analytical LH-T dose response estimation, the present studies identify three distinguishable age-related defects in testis responsiveness to LH action: decreased LH efficacy, diminished Leydig cell sensitivity, and increased LH-T downregulation. Together, these mechanisms could provide a powerful basis for reduced effec-tual LH drive in older men.

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GRANTS

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DISCLOSURES

The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS

J.D.V. and P.Y.L. did the conception and design of the research; J.D.V., P.Y.L., and P.Y.T. performed the experiments; J.D.V., D.M.K., and P.Y.T. interpreted the results of the experiments; J.D.V. and D.M.K. prepared the figures; J.D.V. drafted the manuscript; J.D.V., P.Y.L., and P.Y.T. edited and revised the manuscript; J.D.V. approved the final version of the manuscript; D.M.K. analyzed the data.

REFERENCES


