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The Effects of Aging in Normal Men on Bioavailable Testosterone and Luteinizing Hormone Secretion: Response to Clomiphene Citrate*

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ABSTRACT. Serum testosterone (T) levels in men decline with age while serum LH levels, as measured by RIA, increase. To assess if the decline in serum T levels in healthy aging men is paralleled by an age-related decline in the bioavailable non-sex hormone-binding globulin (SHBG)-bound fraction of T and to determine whether there are age-related changes in LH secretion or LH control of T production, we studied 29 young (aged 22–35 yr) and 26 elderly (aged 65–84 yr) healthy men. All men had single random blood samples drawn, and 14 men in each age group underwent frequent blood sampling for 24 h, both before and after 7 days of clomiphene citrate (CC) administration. Both mean 24-h serum total T levels and non-SHBG-bound T were reduced in elderly men compared to those in young men ($P < 0.05$), while estradiol and SHBG levels were similar in the 2 age groups. Serum FSH determined by RIA and LH by RIA and bioassay were higher in the elderly men compared to those in young men ($P < 0.05$), but the ratios of LH bioactivity to immunoreactivity and the LH pulse frequency and amplitude were similar. After CC administration, mean serum total T and non-SHBG-bound levels in young men increased by 100% and 304%, respectively, while in older men these values increased by only 32% and 8%, respectively. However, CC-stimulated LH pulse characteristics and serum levels of estradiol, SHBG, FSH, and bioactive and immunoreactive LH were similar in the 2 groups.

Thus, both at baseline and after CC stimulation, elderly men had significantly lower serum total T and non-SHBG-bound (bioavailable) T levels than did young men, despite similar or increased levels of bioactive LH and similar bioactive to immunoreactive LH ratios and LH pulse characteristics. These results suggest that major age-related changes in the hypothalamic-pituitary-testicular axis occur at the level of the testes and are manifested by decreased responsiveness to bioactive LH. Administration of CC to young and elderly men resulted in similar changes in LH pulse characteristics and LH bioactivity and immunoreactivity, suggesting preserved hypothalamic-pituitary responsiveness in the elderly. (*J Clin Endocrinol Metab* **65:** 1118, 1987)

MANY aspects of testicular function decline with aging. Many (1–6), but not all (7–9), studies have shown an age-related decrease in total serum testosterone (T) and/or free T levels. Blunting or loss of the circadian rhythm in serum T levels also occurs with aging (1, 3). Other evidence of a decline in testicular function with age includes a decrease in Leydig cell mass (10), a progressive loss of other testicular cell types (11), a reduced relative or absolute serum T response to hCG (7, 8, 12), alterations in intratesticular steroid biosynthetic activity (2), and a decline in daily sperm production (10) and sperm motility (8). Recently, it has been reported that bioavailable T [the portion not bound to sex hormone-binding globulin (SHBG)] also may decrease with aging in normal men (9).

The changes in testis morphology and function with age occur in the setting of increasing serum gonadotropin levels, as measured by RIA (1, 2, 4–8, 13), suggesting that intrinsic testicular changes accompany normal aging. However, in addition to primary testicular alterations, changes in hypothalamic or pituitary control of testicular function also might contribute to testicular dysfunction in aging men (6, 13, 14). Age-associated alterations in hypothalamic or higher central nervous system control of testicular function are suggested by the loss of diurnal rhythm in serum T (3) and apparent increased sensitivity to sex steroid negative feedback (15). Some studies have examined LH pulsation in young and old men (13, 16), but these used short infrequent

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sampling periods (13) and/or only a few subjects, some of whom had gynecomastia (16). Age-related changes also may be occurring at the pituitary level. For example, some studies have shown a blunted pituitary response to LHRH in old men compared to young men (5, 8, 17). In addition, several groups have reported a moderate age-related decline in the ratio of LH bioactivity to immunoreactivity (B/I ratio) (18, 19).

This study was designed to assess LH pulse characteristics and bioactivity for a 24-h period in healthy young and elderly men and to evaluate these in relationship to their serum total T, estradiol (E_2), and non-SHBG-bound (bioavailable) T levels. These parameters were studied before and after a short course of clomiphene citrate (CC), a compound known to stimulate gonadotropin secretion (20).

Materials and Methods

Subjects

Fifty-five healthy men, 29 young (age range, 22–35 yr; mean, 27.3 yr) and 26 older men (age range, 65–84 yr; mean, 70.7 yr), were studied. The men, 53 caucasian and 2 oriental, were recruited by advertising and were within 10% of ideal body weight. All were night sleepers, nonsmokers, and nonabusers of alcohol; none was an elite athlete or taking medications. All were healthy, as determined by medical history, physical examination, complete blood count, urinalysis, and blood chemistry screen, and had normal sized testes (at least 3.6 × 2.2 cm).

Experimental protocol

All men had a single blood sample taken (uncontrolled for time of day), which was allowed to clot at room temperature. The serum was separated, and the sample was frozen and stored at −20 C.

On each of two occasions, separated by at least 8 weeks, 28 of the men (14 young and 14 elderly) also underwent more extensive blood sampling. These men were selected for more extensive study before knowledge of their random blood sample results, and selection was based solely on willingness to participate in the study protocol. The men were admitted to the Clinical Research Center of the University of Washington Hospital for acclimatization the night before each of the two study sessions. Each man received CC (Clomid, Merrill-Dow, Cincinnati, OH; 50 mg, orally, twice a day for 7 days) just preceding one of the two sessions. The last dose of CC was taken no more than 12 h before the onset of blood sampling. Half of the men in each age group received CC before their first study session, while half of the men received CC before the second session. Each age group was divided equally so as to begin blood sampling either in the morning (0800–1000 h) or in the evening (2000–2100 h). Blood was drawn every 10 min for 24 h through an indwelling 18-gauge polyethylene cannula placed in an arm vein and kept patent with heparinized lactated Ringers solution; the total amount of blood drawn in 24 h was 485 mL. During the night, the person performing the sampling was in an adjacent room so that the men were not disturbed by the blood-drawing procedure. Blood samples from the 24-h studies were handled in the same manner as the single samples.

Serum LH was measured in duplicate by RIA in all blood samples. Hourly blood samples from the 24-h studies and the random single samples were measured in duplicate for T and FSH by RIA; the hourly results from each 24-h study were averaged. In addition, equal aliquots from the hourly samples throughout each 24-h period were pooled, and these along with the single random samples were assayed for LH bioactivity. Serum E_2, SHBG, and α-subunit were also measured on the 24-h pooled samples. For each study, all samples from an individual man were analyzed at the same time.

Hormone assays

LH and FSH RIA. The RIAs for serum LH and FSH were described previously (21). The RIA for LH used a reference standard (LER 907) and first antibody (antihuman LH batch 2) supplied by the National Hormone and Pituitary Program. The tracer was purified hCG radioiodinated with ^{125}I using chloramine-T (22). The limit of detectability of the assay was 6 ng/mL [6 ng/mL = 6 μg/L in Systeme International (SI) units], and the intra- and interassay variations were 5.5% and 8.4%, respectively.

The RIA for serum FSH used reagents distributed by the National Hormone and Pituitary Program. The reference standard was LER 907, the first antibody was antihuman FSH (batch 5), and the tracer was HS-I, radioiodinated with ^{125}I using chloramine-T (22). The limit of FSH detectability was 25 ng/mL (25 μg/L), and the intra- and interassay variations were 7.3% and 9.7%, respectively. Assay results for both LH and FSH were calculated using the computer program of Burger *et al.* (23).

T and E_2 RIA. The RIAs for serum T and E_2 used reagents provided by the WHO Matched Reagent Programme. The methodologies were described previously (24). T and E_2 assays were separated from serum by ether extraction, and separation of bound from free hormone was accomplished by dextran-coated charcoal. The assay sensitivity was 0.1 ng/mL (0.35 nmol/L) for T and 8 pg/mL (29.4 pmol/L) for E_2. The intra- and interassay variabilities were 5.1% and 9.8%, respectively, for T, and 8.2% and 8.8%, respectively, for E_2.

SHBG. Serum SHBG was measured in duplicate by RIA and by [^{3}H]dihydrotestosterone (DHT) saturation analysis using methods previously described (25, 26). The SHBG antibody and standard were generous gifts of C. Y. Cheng and C. W. Bardin. The intraassay coefficients of variation were 7.4% and 7.7% for the saturation analysis and RIA, respectively. Interassay coefficients of variation of the saturation analysis and RIA were 9.6% and 12.0%, respectively. There was a strong correlation between the RIA and saturation analysis results (r = 0.93).

Non-SHBG-bound T. Serum non-SHBG-bound T was calculated from the total molar concentration of T and SHBG (measured using the RIA method) according to a modification

of the mass action equation of Pearlman (27):

$$\left(\frac{X}{(\mathrm{T}-X)}\right)\left(\frac{1}{(\mathrm{SHBG})}\right)=k\left(1-\frac{X}{(\mathrm{SHBG})}\right);\ X=\frac{b-\sqrt{b^2-4a}}{2}$$

where X is the molar concentration of SHBG-bound T, $k = 9.8 \times 10^8$ and is the association constant of T and SHBG, a = (T) + (SHBG), $b = 1/k$ + (SHBG) + (T), and non-SHBG bound T = (T) − X (28). The calculation of non-SHBG-bound T in this equation assumes that SHBG is the only binding protein for T in serum. *In vivo*, albumin is also present and may effect the distribution of T bound to SHBG; therefore, this equation may not reflect the total mass of bound T present in serum. However, since albumin-bound T readily crosses biological membranes and is available to bind to SHBG, the formula described should reflect the effects of SHBG on diffusable T (29). A formula that includes albumin in the calculation of T distribution was previously described by Södergard *et al.* (30). In our study, values for non-SHBG-bound T calculated from the Södergard *et al.* formula correlated closely with values obtained using the above formula (r = 0.96), although the absolute values differed.

LH bioactivity. Bioactive LH was measured in duplicate in the 24-h sample pools by the mouse Leydig cell *in vitro* bioassay (31), using LER 907 as a reference standard. The intra- and interassay coefficients of variation for the Leydig cell bioassay were 9.4% and 15.6%, respectively, and the assay sensitivity was 0.3 ng/mL (0.3 μg/L).

α-Subunit (RIA). Serum α-subunit levels were determined in duplicate by a double antibody RIA, using reagents provided by the National Hormone and Pituitary Program. Purified human LHα (batch I) was iodinated with chloramine-T (22), and the radioiodinated tracer was separated from free ^{125}I by Sephadex G-75 chromatography; the iodination preparation was also used as the standard. Antihuman LHα antiserum (batch I) was raised in rabbits and used in final dilution of 1:20,000. At this dilution, the antiserum bound 31–43% of [^{125}I] LHα, with a lower limit of sensitivity of 0.01 ng/tube. Goat antirabbit γ-globulin was used as the second antibody (Antibodies, Inc., Davis, CA). Pools of human serum caused the same pattern of dose-dependent inhibition in the assay as did the LHα standards (Fig. 1A). The intraassay coefficient of variation in 8 consecutive assays was 8.4%. The interassay coefficient of variation, based on 60 duplicate determinations, was 5.4%.

The maximum cross-reactivity of intact human LH (expressed relative to LER 907, the standard used in the LH RIA) in the human α-subunit assay was estimated by comparing the relative potency of a highly purified human LH preparation (LH I-3, National Hormone and Pituitary Agency) to that of human LHα in the human α-subunit assay and comparing the relative potency of LH I-3 to that of LER 907 in the LH RIA. Similarly, the maximum cross-reactivity of intact FSH in the human α-subunit assay was estimated by comparing the relative potency of a highly purified human FSH preparation (FSH I-3, National Hormone and Pituitary Agency) to that of human LH-α in the human α-subunit assay and comparing the relative potency of FSH I-3 to that of LER 907 in the FSH RIA. This method of estimating maximal cross-reactivity of intact gonadotropins, expressed in terms of LER 907 (a relatively impure human pituitary standard that contains substantial amounts of α-subunit), in human α-subunit assays has been used by other investigators (32).

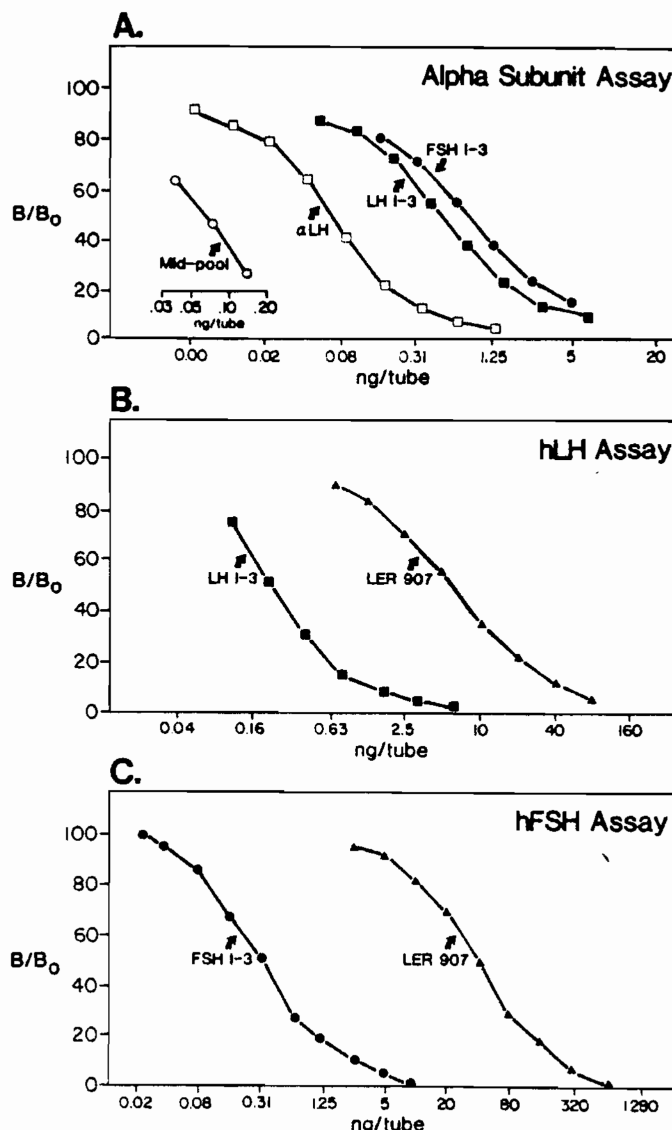

FIG. 1. A, Immunoreactivity of highly purified human LH (LH I-3), highly purified human FSH (FSH I-3), LHα, and midpool human serum (pooled human serum giving assay values near the mean for normal serum) in the LHα RIA. B, Immunoreactivity of LH I-3 and LER 907 in the LH RIA. C, Immunoreactivity of FSH I-3 and LER 907 in the FSH RIA. B/B$_0$, Bound to free ratio.

The results of these cross-reactivity studies are depicted in Fig. 1, which demonstrates the relative potencies of human LH α, LH I-3, and FSH I-3 in the human α-subunit RIA (Fig. 1A); the relative potencies of LH I-3 and LER 907 in the LH RIA (Fig. 1B); and the relative potencies of FSH I-3 and LER 907 in the FSH RIA (Fig. 1C). All curves were parallel to each other. Comparisons of relative potency and cross-reactivity were made at 50% displacement of the tracer (bound to free ratio = 50%). Based on these comparisons, intact LH, expressed

in terms of LER 907, had a maximum 0.46% cross-reactivity in the human α-subunit assay, and intact FSH, expressed in terms of LER 907, had a maximum of 0.06% cross-reactivity in the human α-subunit assay.

LH pulse analysis. Twenty-four-hour LH pulse patterns were analyzed by a modification of the method of Santen and Bardin (33). For each sampling series, measurement error was assessed on the basis of assay replicate variability, as determined by analysis of variance. A LH pulse was defined as an increase in the serum LH level from nadir to peak that was equal to or greater than 4 times the intraassay coefficient of variance of assay replicates.

Statistical analysis. The mean of all values measured, regardless of the time of onset of blood sampling, was determined for each man before and after CC administration. The data for each age group before and after CC were compared using Student's two-tailed paired t test, and the data for the young and elderly men were compared using unpaired Student's two-tailed t test. The power of the tests was determined by reference to standard tables.

Results

Basal serum T, E_2, SHBG, and non-SHBG-bound T

The healthy young men had a mean 24-h total serum T level of 4.9 ± 0.3 (±SEM) ng/mL (17.0 nmol/L; Fig. 2A). The elderly men had a significantly lower mean 24-h total serum T level of 4.1 ± 0.4 ng/mL ($P < .05$; 14.2 nmol/L; Fig. 2A). Total serum T levels obtained from single random samples were also significantly lower in older men; the single sample total T level was 4.8 ± 0.2 ng/mL (16.6 nmol/L) in the young and 4.0 ± 0.2 ng/mL (13.9 nmol/L) in the elderly. In the elderly men, the mean serum E_2 levels and SHBG by RIA and DHT saturation analysis were similar to those in young men (Table 1).

Using the total serum T levels and SHBG, as determined by RIA analysis, the calculated non-SHBG-bound T level was 0.63 ± 0.17 ng/mL (2.2 nmol/L) in the young men and 0.26 ± 0.03 ng/mL (0.90 nmol/L) in the elderly men ($P < 0.05$; Fig. 2B). As a percentage of the total serum T, the non-SHBG-bound T level in young men was 12.9%, and in elderly men it was 6.3%. For comparison, when the formula of Södergard *et al.* (30) was used to calculate non-SHBG-bound T levels, the results were 3.2 ± 0.2 ng/mL (11.1 nmol/L) or 65% of the total T for young men, and 1.9 ± 0.1 ng/mL (6.6 nmol/L) or 46% of the total T for elderly men. The large difference in non-SHBG-bound T between the two age groups despite somewhat similar SHBG levels can be explained by the binding characteristics of T to SHBG. T binds to SHBG in a nonlinear fashion, and in the physiological T range, the binding curve is steep. Therefore, relatively small increases in T or small decreases in SHBG can raise dramatically the amount of non-SHBG-bound T (34).

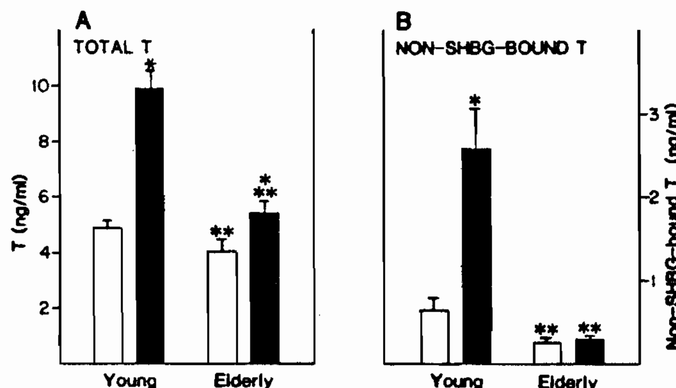

FIG. 2. Mean (±SEM) 24-h serum total T levels (A) and calculated non-SHBG-bound T levels (B) in 14 young and 14 elderly men before (□) and after CC administration (■). *, $P < 0.01$ compared to baseline; **, $P < 0.05$ compared to young men (1 ng/mL total or non-SHBG-bound T = 3.5 nmol/L).

Basal serum FSH RIA, LH RIA, and LH bioactivity

The elderly men had a significantly higher mean 24-h serum FSH level than the young men (Table 1). FSH levels in single random blood samples showed a similar relationship, with FSH levels of 117 ± 10 and 211 ± 21 ng/mL (117 and 211 μg/L) in young and elderly men, respectively ($P < 0.05$).

The mean 24-h serum LH level by RIA in young men was 24 ± 2 ng/mL (24 μg/L). By comparison, elderly men had a significantly higher ($P < 0.05$) mean 24-h LH level of 34 ± 3 ng/mL (34 μg/L; Fig. 3A). Single sample data for LH by RIA was similar to the mean 24-h data [28 ± 2 ng/mL (28 μg/L) in the young men and 41 ± 4 ng/mL (41 μg/L) in the elderly men; $P < 0.05$].

The mean 24-h serum LH bioactivity level in young men was 240 ± 26 ng/mL (240 μg/L), compared to a significantly higher value of 342 ± 40 ng/mL (342 μg/L) in elderly men (Fig. 3B; $P < 0.05$). Serum LH bioactivity in single random blood samples was also significantly higher in the elderly (353 ± 35 ng/mL; 353 μg/L) than in the young men (247 ± 17 ng/mL; 247 μg/L; $P < 0.05$).

Basal LH B/I ratio and α-subunit

The baseline LH B/I ratios for mean 24-h samples were similar in young men (9.9 ± 0.7) and elderly men (10.1 ± 0.8; Fig. 3C). LH B/I ratios also were similar in the single random blood samples (9.0 ± 0.5 in young men and 8.8 ± 0.6 in elderly men). The mean levels of α-subunit were the same in young and elderly men (Table 1).

Basal LH pulse characteristics

Examples of the LH pulse patterns in young and elderly men are depicted in Fig. 4. Young men had a

TABLE 1. Mean 24-h serum E_2, SHBG, FSH, and α-subunit levels in young and elderly men before and after CC administration

	Young (n = 14)		Elderly (n = 14)	
	Baseline[a]	+CC	Baseline[a]	+CC
Estradiol (pg/mL)[b]	37 ± 3	54 ± 4[c]	37 ± 2	47 ± 3[c]
SHBG (ng bound DHT/mL)[d]	8.0 ± 0.8	9.5 ± 1.0[c]	8.7 ± 0.8	11.3 ± 1.1[c]
SHBG (nM/L)[e]	24.5 ± 2.2	31.3 ± 3.2[c]	31.0 ± 2.83	40.0 ± 4.0[c]
FSH (ng/mL)[f]	117 ± 17	220 ± 34[c]	180 ± 26[g]	234 ± 32[c]
α-Subunit (ng/mL)[f]	0.49 ± 0.20	0.52 ± 0.08	0.45 ± 0.04	0.49 ± 0.03

Values are the mean ± SEM.
[a] Not preceded by CC administration.
[b] 1 pg/mL E_2 = 3.67 pmol/L.
[c] $P < 0.05$ compared to baseline.
[d] [^{3}H]DHT saturation analysis: 1 ng DHT/mL = 3.5 nmol/L bound DHT.
[e] Determined by RIA.
[f] 1 ng/mL FSH or α-subunit = 1 μg/L.
[g] $P < 0.05$ compared to young men.

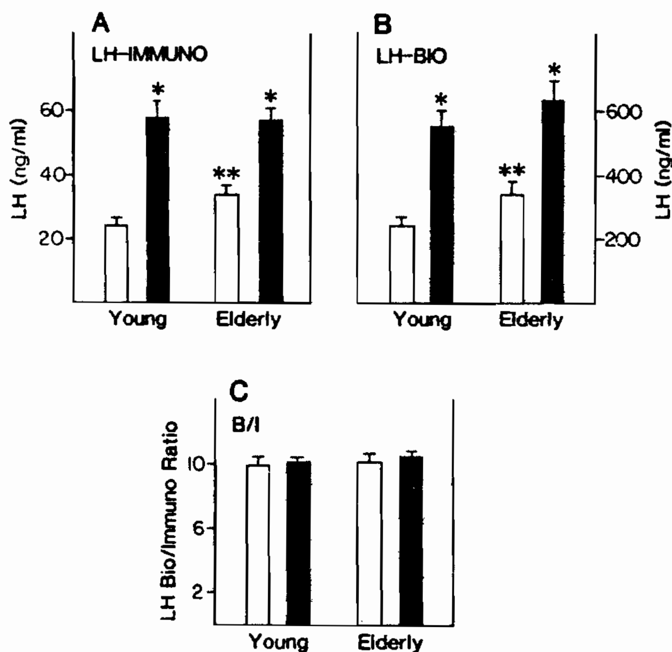

FIG. 3. Mean (±SEM) serum levels of LH determined by RIA (LH-IMMUNO) and bioassay (LH-BIO) and the mean LH B/I ratio in 14 young and 14 elderly men before (□) and after CC administration (■). *, $P < 0.01$ compared to baseline; **, $P < 0.05$ compared to young men (1 ng/mL LH by RIA or bioassay = 1 μg/L).

mean LH pulse frequency of 14.0 ± 0.6 pulses/24 h (Figs. 4A, *top*, and 5A), with a range of 11–18 LH pulses/24 h. The mean baseline LH pulse amplitude was 15 ± 1 ng/mL (15 μg/L; Fig. 5B). The elderly men had a similar baseline LH pulse frequency (Figs. 4B, *top*; and 5A) of 14.4 ± 0.7 pulses/24 h (range, 11–20 LH pulses/24 h) and a mean LH pulse amplitude of 16 ± 2 ng/mL (16 μg/L; Fig. 5B).

CC administration

After 7 days of CC stimulation, serum bioactive and immunoreactive LH levels increased to similar levels in both young and elderly men (Fig. 3, A and B). In young men, CC significantly increased bioactive and immunoreactive LH to 552 ± 50 and 58 ± 6 ng/mL (552 and 58 μg/L), respectively. In elderly men, CC significantly increased bioactive LH to 628 ± 52 ng/mL (628 μg/L) and immunoreactive LH to 56 ± 4 ng/mL (56 μg/L). The calculated LH B/I ratio did not change significantly in either age group (10.0 ± 0.6 in young men and 11.3 ± 0.4 in elderly men; Fig. 3C). The serum α-subunit levels also did not change significantly in either age group after CC administration (Table 1).

After CC administration, the mean LH pulse frequency and pulse amplitude increased significantly in both young and elderly men (Figs. 4 and 5). As a group, the 24-h mean LH pulse frequency in young men increased to 19.6 ± 1.0 pulses/24 h (range, 17–26 LH pulses/24 h), and their mean LH pulse amplitude increased to 29 ± 3 ng/mL (29 μg/L; Fig. 5). The LH pulse frequency in elderly men increased to 19.2 ± 0.7 pulses/24 h (range, 15–23 pulses/24 h), and their LH pulse amplitude increased to 21.1 ± 2.6 ng/mL (21 μg/L). The LH pulse amplitude after CC administration was slightly but not significantly lower in elderly men than in young men.

For young and elderly men, CC administration resulted in similar increases in serum FSH, E_2, and SHBG levels (Table 1). The mean 24-h total serum T level in young men increased to 9.8 ± 0.7 ng/mL (34.0 nmol/L), a significant 100% increase over baseline values (Fig. 2A). In comparison, the mean 24-h total serum T level in elderly men increased only to 5.4 ± 0.5 ng/mL (18.7 nmol/L), a 34% increase over baseline. In addition, the calculated non-SHBG-bound T level increased to 2.55 ± 0.58 ng/mL (8.8 nmol/L), a 304% increase over baseline, only in young men (Fig. 2B). The non-SHBG-bound T level in elderly men after CC treatment was 0.28 ± 0.03 ng/mL (0.98 nmol/L), the same as the baseline value. CC-stimulated values for non-SHBG-bound T levels,

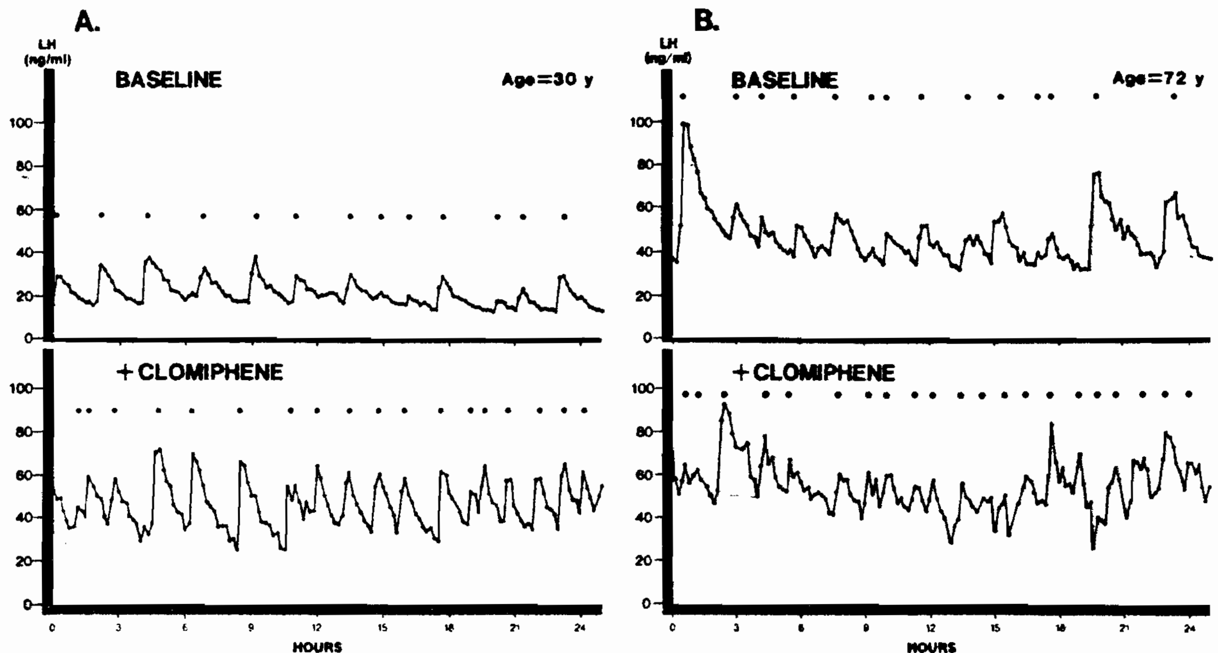

FIG. 4. Twenty-four-hour serum LH RIA levels in one young (A; 30 yr old) and one elderly (B; 72 yr old) normal man before and after 7-day administration of CC. ●, A LH pulse. Zero time is 0900 h for the elderly man and 2100 h for the young man (1 ng/mL LH = 1 μg/L).

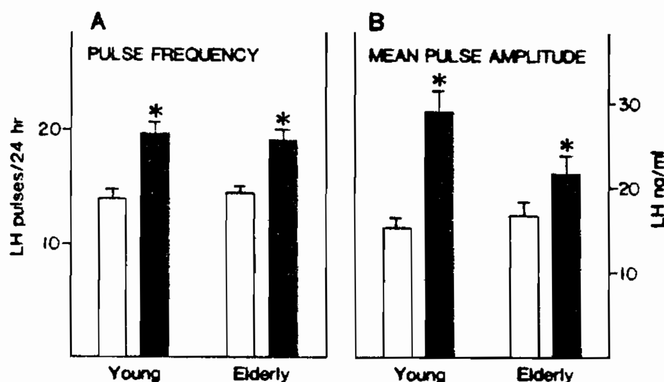

FIG. 5. Mean (±SEM) LH RIA pulse characteristics in 14 young and 14 elderly men before (□) and after CC administration (■). *, $P < 0.01$ compared to baseline (1 ng/mL LH = 1 μg/L).

calculated using the Södergard *et al.* formula, were 6.5 ± 0.9 ng/mL (22.5 nmol/L, or 66% of total T) and 2.3 ± 0.5 ng/mL (8.0 nmol/L, or 42% of total T) in the young and elderly men, respectively.

Discussion

We found that, compared to young men, healthy elderly men had lower mean serum total T levels and lower non-SHBG-bound T levels before and after CC administration. Baseline and CC-stimulated levels of serum bioactive LH and the frequency and amplitude of LH secretory episodes were similar in young and elderly men. Baseline and CC-stimulated serum E_2, SHBG, α-subunit, and LH B/I ratios also were similar in the two age groups.

The finding of lower serum total T in aged compared to young men agrees with most recent studies of healthy men (1–6). Of note, however, is the markedly lower non-SHBG-bound T levels found in the elderly men. This age-related difference in the biologically available portion of serum T is even greater than would be predicted by differences in total T alone and is consistent with other recent data obtained using different methods of determining non-SHBG-bound T (9, 35). However, the absolute values for non-SHBG-bound T using our calculated method are lower than other values in the literature, most of which were measured using direct fractional binding methods (7, 9). There is, however, no methodology to measure what portion of total serum T is truly bioavailable to tissues. Our calculated method of determining non-SHBG-bound T levels is based on the premises that non-SHBG-bound T represents both unbound T plus T that is bound to plasma proteins other than SHBG (particularly albumin) and that this portion of T closely represents the amount of hormone available to tissues (28, 36). Values for bioavailable T obtained in this manner correlate well with estimates of bioavailable T determined by measurement of the brain uptake index of T, as evaluated by the *in vivo* Oldendorf carotid artery injection technique (29), and by correlation with serum LH increases in the hyperthyroid state (37).

Our study subjects were very healthy and screened to eliminate factors, such as obesity, heavy alcohol intake, concomitant medications, and smoking, that might confound the results. None of the men was black, which may affect comparison of results with other studies that included black men, since there is some evidence that

young adult black men have higher total and free serum T levels than white men (38).

The similar serum E_2 levels in the young and elderly men agree with some (7, 8, 13), but not all (4, 6), previous reports. Our finding that baseline SHBG levels do not increase significantly with age is consistent with the finding of similar E_2 levels in young and elderly men, since E_2 is a major stimulator of SHBG production (39). However, most (6, 13), but not all (35), investigators have reported a rise in SHBG levels with age. The good health and normal body weight of our study subjects as well as differences in study methodology may account for some of the differences.

Our finding that basal serum immunoreactive FSH and LH levels were elevated in elderly compared to young men agrees with the results of other studies (1, 4–8, 13) and suggests, in the setting of lower serum T levels, primary gonadal failure. The significantly higher mean bioactive LH levels in the aged men are consistent with some (18), but not other (19), study findings. The study that did not find elevated bioactive LH levels in aged men (19) included men in the aged group who were much younger (>40 yr) than the men studied by us and others (18).

Because both serum bioactive and immunoreactive LH levels were elevated in our elderly men, the baseline LH B/I ratios were similar, in contrast to other studies (18, 19) which showed a moderate decline (22–39%) in LH B/I as men aged. The difference seems to be largely that our elderly men, compared to young men, had lower immunoreactive LH levels than those found in some other studies (19). Differences in the LH RIA, including antibody specificity and purity of standards used, may account for this difference in LH immunoreactivity. From the number of men in our study, we calculate that using our assay systems we should have been able to detect, with 95% certainty, a difference of 20% or greater in the LH B/I ratio between the two age groups, if such a difference had been present.

The finding that serum α-subunit levels were similar in young and elderly men is consistent with the finding of comparable B/I ratios in the two age groups. Since free α-subunits are devoid of bioactivity and have some cross-reactivity in the LH RIA, if they were secreted in large amounts, serum could contain high LH immunoreactivity and low LH bioactivity. However, serum LH bioactivity can decrease without a change in α-subunit secretion (40).

LH secretory episodes, as measured by RIA, were similar in frequency and amplitude in the young and elderly men, despite higher mean immunoreactive LH levels in the elderly men. These results extend the findings of a previous study in normal men, in which blood was sampled every 20 min for 8 h (13). One other group (16) reported a near-total loss of LH pulsatile secretion in four aged men, but these men were not totally healthy. The reasons for elevated mean immunoreactive LH levels in elderly men despite LH pulse characteristics similar to those in young men are not known. Possible explanations include an age-related decrease in LH clearance or an age-related increase in nonpulsatile basal release of LH from the pituitary.

CC was used in this study because it is known to stimulate gonadotropin secretion (20), although its exact mechanism of action is uncertain (41). Most evidence points to a hypothalamic target site (42), where it appears to displace endogenous estrogens from hypothalamic receptors, thereby blocking steroid negative feedback and augmenting LHRH release. The use of CC allows for age group comparisons of stimulated hypothalamic-pituitary activity (as reflected in the peripheral blood pulsatile LH response) and gonadal responses to the increased gonadotropin levels.

After CC administration, immunoreactive FSH and bioactive and immunoreactive LH levels increased, while α-subunit levels did not change in both young and elderly men. The CC-stimulated increases in LH pulse frequency and amplitude were similar in the two groups. These data suggest that the pituitaries of young and elderly men are capable of similar responses when stimulated. Both E_2 and SHBG increased similarly in the young and elderly men after CC administration. The increase in E_2 after CC administration has been reported in young men (43), and most likely is due to peripheral conversion of the CC-stimulated elevated serum T level and increased testicular estrogen production. The elevation in SHBG levels found after CC administration also is consistent with previous studies in young men (43). The stimulus for SHBG production could be the increased E_2 levels (39) or the direct estrogenic effects of CC on hepatic cells (43). Despite high serum bioactive LH levels and increased LH pulse frequency with CC stimulation in elderly men, both absolute and relative increases in serum total T were much lower than those in young men, and the CC-stimulated non-SHBG-bound T levels were even more different in the two age groups. These results indicate that the changes in testicular function that occur with age, especially the lower T secretion, are most likely the result of primary testicular insufficiency. Whether elderly men are capable of the same maximal testicular T production as that found in young men, but are slower in responding to a stimulus, cannot be determined from this study. More than 7 days may be required to achieve the maximal testicular response to CC stimulation (44).

Finally, although our data are consistent with primary testicular decline with aging, these studies do not rule out some age-related alterations in testicular control at the hypothalamic-pituitary level. Although elderly men

have increased serum T levels in response to CC administration, they do not have serum total T or non-SHBG-bound T values as high as those in young men; yet, despite this diminished T negative feedback, their LH pulse characteristics are unaltered compared to those of young men. Since in young men decreased T levels lead to increased LH pulse frequency (45), there may be an age-related increased sensitivity to steroid negative feedback, a possibility suggested by other studies (4, 15).

In conclusion, despite higher serum LH bioactivity and similar LH B/I ratio and LH pulse characteristics, elderly men have significantly lower serum total T and dramatically lower non-SHBG-bound T levels than young men. These findings suggest that major age-related changes in the hypothalamic-pituitary-testicular axis occur at the level of the testes and are manifested by decreased responsiveness to bioactive LH. In addition, since CC administration in young and elderly men resulted in similar changes in LH pulse characteristics and LH bioactivity and immunoreactivity, hypothalamic-pituitary responsiveness is preserved in the elderly.

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