

Decreased Serum Inhibin Levels in Normal Elderly Men: Evidence for a Decline in Sertoli Cell Function with Aging*

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ABSTRACT. Compared to young men, normal elderly men have decreased sperm production despite elevated serum gonadotropin levels. To determine whether the seminiferous tubule defect in elderly men includes decreased Sertoli cell function, we measured serum immunoreactive inhibin concentrations in young and elderly men before and after clomiphene citrate (CC) administration. Thirty-eight healthy men, 19 young (aged 22–35 yr) and 19 elderly (aged 65–85 yr), were studied before CC administration. The mean baseline serum inhibin level was significantly lower ($P < 0.001$) in the elderly men than in the young men [416 ± 22 (\pm SE) vs. 588 ± 30 U/L], while serum

immunoreactive FSH and LH levels were higher in the older men, and bioactive FSH levels were similar in the two age groups. Eleven young men and 13 elderly men were studied after 1 week of CC administration. The mean serum inhibin level increased by 71%, from 566 ± 36 to 970 ± 82 U/L, in the young men, but it increased by only 24%, from 421 ± 26 to 520 ± 38 U/L, in the elderly men. Serum immunoreactive LH and bioactive and immunoreactive FSH concentrations increased to similar levels in both groups after CC administration. We conclude that the seminiferous tubule defect of elderly men includes decreased Sertoli cell function. (*J Clin Endocrinol Metab* 67: 455, 1988)

DAILY sperm production and serum testosterone (T) levels are decreased in normal elderly men compared to those in young men (1–8). These decreases in testicular function occur despite increased serum immunoreactive gonadotropin levels in the elderly (4–9), suggesting that there is an intrinsic decrease in both seminiferous tubule and Leydig cell function with normal aging.

Inhibin is a gonadal glycoprotein thought to be important in the regulation of FSH secretion (10). It is produced by Sertoli cells in response to FSH (11, 12). Until recently, no assay was available to measure inhibin in serum. With the isolation of purified bovine inhibin from follicular fluid (13, 14), sensitive and specific RIAs for measuring serum inhibin have been developed (15, 16).

In this work we measured serum inhibin levels by RIA in healthy young and elderly men, both before and after

1 week of clomiphene citrate (CC) administration. CC was given to stimulate gonadotropin secretion and thus allow determination of the Sertoli cell response to the increased endogenous gonadotropin secretion (17).

Materials and Methods

Subjects

We studied 38 healthy men, 19 young (age range, 22–35 yr; mean, 29.2 yr) and 19 elderly (age range, 65–85 yr; mean, 71.0 yr). All were community dwellers and were within 15% of ideal body weight. None was a smoker, abused alcohol, was an elite athlete, or took any medications. All were healthy, as determined by medical history, physical examination, complete blood count, urinalysis, and blood chemistry screen. Average testis volumes, determined by comparison to ellipsoids of known volume, were similar in the 2 age groups [mean, 18.8 ± 0.5 (\pm SE) mL in the young men and 17.8 ± 0.4 mL in the elderly men; $P > 0.1$]. The studies were approved by the University of Washington Human Subjects Review Committee, and informed consent was obtained from each man.

Experimental protocol

Ten men (5 in each age group) had single morning blood samples drawn; 28 men (14 in each age group) had blood

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sampling performed every 10 min for 24 h through an indwelling arm venous cannula as part of a previously described protocol (4). Subsequently, 11 of the young men and 13 of the elderly men received CC (Clomid, Merrill-Dow, Cincinnati, OH; 50 mg, orally, twice a day for 1 week). Starting within 12 h after their last CC dose, these men again underwent 10-min blood sampling for a 24-h period. All blood samples were allowed to clot at room temperature; the serum was separated, frozen, and stored at -20°C .

Hormone analyses

Three blood samples drawn at hourly intervals (0800–1000 h) during the 24-h studies and the single morning blood samples were assayed for T and FSH by RIA; the three values from the 24-h studies were averaged. All samples drawn between 0800 and 1000 h were analyzed for LH by RIA, and the results were averaged. In addition, equal aliquots of each sample collected between 0800–1000 h were pooled, and these, along with the single samples, were assayed for inhibin by RIA. An equal volume from each of the 24-h samples were pooled, and FSH was measured by bioassay in these serum pools. For each hormone, all samples from an individual man were analyzed in duplicate in the same assay (triplicate in the FSH bioassay). Hormone values from the group of men sampled singly were not significantly different from those obtained from men with multiple sampling.

Inhibin RIA

Serum inhibin was measured by a previously described heterologous RIA (16), except that a new inhibin antiserum (As 1989), raised in a New Zealand White rabbit to 31K bovine follicular fluid inhibin, was used. Serum from castrated men did not react in this assay, and its sensitivity was enhanced compared to that of the previous method (16). Transforming growth factor- β , bovine activin-A, and free inhibin subunits obtained after reduction and alkylation of 31K bovine inhibin had less than 1% cross-reactivity in the assay. A partially purified human follicular fluid inhibin preparation was used as the RIA standard (16). This material was calibrated in terms of its *in vitro* inhibin bioactivity using a bovine follicular fluid inhibin standard preparation, itself previously calibrated against an ovine testicular lymph preparation with a defined unitage of 1 U/mg (18). As previously described (19), serum samples from men gave dose-response lines parallel to those of the RIA standard and a serum pool obtained from women undergoing ovulation induction. In the RIA, 200- μL samples were analyzed in duplicate. The sensitivity of the assay (ED_{10}) was 100 U/L serum, and the ED_{50} was 550 U/L. The interassay variability was 11% ($n = 5$), and the intraassay variability in the upper, mid-, and lower portions of the standard curve were 12.0%, 3.3%, and 4.8%, respectively ($n = 5$).

LH and FSH RIAs

The RIAs for serum LH and FSH were described previously (20). The LH RIA 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 the chloramine-T method (21). The limit of detectability was 6 $\mu\text{g/L}$, and the intra- and interassay variabilities were 5.5% and 8.4%, respectively.

The RIA for serum FSH also 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-1, radioiodinated with ^{125}I using the chloramine-T method (21). The limit of detectability was 25 $\mu\text{g/L}$, and the intra- and interassay variabilities were 7.3% and 9.7%, respectively. Assay results for both LH and FSH were calculated using the computer program of Burger *et al.* (22).

T RIA

The RIA for serum T used reagents provided by the WHO Matched Reagent Programme. The methodology has been described previously (23). T was removed from serum by ether extraction, and the separation of bound from free hormone was accomplished by dextran-coated charcoal. The assay sensitivity was 0.35 nmol/L (0.1 ng/mL), and the intra- and interassay variabilities were 5.1% and 9.8%, respectively.

FSH bioassay

Serum bioactive FSH was measured using the *in vitro* granulosa cell bioassay (24, 25). The assay measures FSH-stimulated estrogen production by rat granulosa cells obtained from animals primed with diethylstilbestrol. The standard was LER-907. The estrogen antiserum, raised in rabbits against 17 β -estradiol, was a gift from Dr. Y. X. Liu, Academia Sinica (Beijing, China). The inter- and intraassay variabilities for the estrogen assay were 13% and 10%, respectively. The sensitivity of the FSH bioassay was 3 ng/culture well for LER-907, with inter- and intraassay variabilities of 16% and 11%, respectively.

Statistical analysis

The data for each age group before and after CC administration were compared using Student's paired *t* test, and the data for the young and elderly men were compared using Student's unpaired *t* test.

Results

Basal measurements

Basal serum inhibin levels were significantly lower ($P < 0.001$) in the 19 elderly men [mean, 416 ± 22 ($\pm\text{SE}$) U/L] than in the 19 young men (588 ± 30 U/L; Table 1). In contrast, baseline serum bioactive FSH levels were similar in the young and elderly men, as previously described (Table 1). Immunoreactive FSH and LH baseline measurements were significantly higher ($P < 0.05$) and basal serum T levels were significantly lower ($P < 0.05$) in the elderly men than in the young men (Table 1).

TABLE 1. Basal serum immunoreactive inhibin, FSH, LH, T, and bioactive FSH concentrations in young (Y) and elderly (E) men

	Y (n = 19)	E (n = 19)
Inhibin (U/L)	588 ± 30	416 ± 22 ^a
FSH, bioassay (μg/L) ^b	511 ± 118	536 ± 168
FSH, RIA (μg/L)	121 ± 15	172 ± 17 ^a
LH (μg/L)	26 ± 2	35 ± 3 ^a
T (nmol/L)	18.6 ± 0.7	15.2 ± 0.8 ^a

Values are the mean ± SE.

^a *P* < 0.05 compared to Y.

^b *n* = 12 Y and 13 E.

TABLE 2. Serum immunoreactive inhibin, FSH, LH, T, and bioactive FSH concentrations in 11 young (Y) and 13 elderly (E) men before and after 1 week of CC administration

	Baseline		1 week of CC	
	Y	E	Y	E
Inhibin (U/L)	566 ± 36	421 ± 26 ^a	970 ± 82 ^b	520 ± 38 ^{a,b}
FSH, bioassay (μg/L)	487 ± 179	536 ± 168	1117 ± 426 ^b	992 ± 227 ^b
FSH, RIA (μg/L)	116 ± 20	175 ± 23 ^a	218 ± 38 ^b	244 ± 33 ^b
LH (μg/L)	26 ± 2	35 ± 3 ^a	58 ± 6 ^b	60 ± 4 ^b
T (nmol/L)	18.6 ± 0.7	15.2 ± 0.8 ^a	35.7 ± 2.4 ^b	19.6 ± 1.8 ^{a,b}

Values are the mean ± SE.

^a *P* < 0.05 compared to Y.

^b *P* < 0.05 compared to baseline.

CC administration

After 1 week of CC administration, serum inhibin levels increased by 71% (*P* < 0.001) in the 11 young men, from a mean of 566 ± 36 to 970 ± 82 U/L (Table 2 and Fig. 1). In the 13 elderly men, although inhibin levels increased significantly (*P* < 0.01) after 1 week of CC treatment, levels rose only 24%, from 421 ± 26 to 520 ± 38 U/L (Table 2 and Fig. 1), and were markedly lower than those in the young men after CC administration.

After 1 week of CC, serum bioactive FSH rose to similar levels in the two age groups, increasing by 129% to 1117 ± 426 μg/L in the young men and by 85% to 992 ± 227 μg/L in the elderly men (Table 2 and Fig. 1). Immunoreactive FSH and LH rose to similar levels in the two age groups after CC administration. T levels also increased significantly in both age groups, although the T increases after 1 week of CC treatment were much smaller in the elderly men (Table 2).

Discussion

We found that serum inhibin levels were significantly lower in elderly men than in young men. Furthermore, inhibin levels rose to a lesser extent in elderly men after CC-induced gonadotropin stimulation. These lower inhibin levels in elderly men occurred in the presence of serum bioactive FSH levels that were similar to those in young men and serum levels of immunoreactive FSH and LH that were higher than those in young men. Many

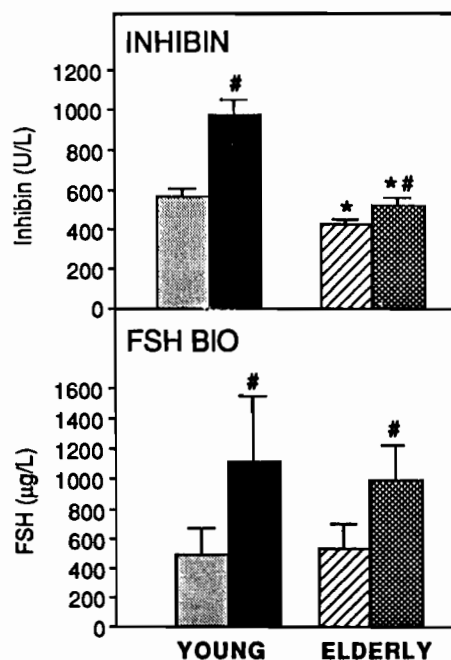


FIG. 1. Mean (±SE) serum inhibin and bioactive FSH levels in 11 young and 13 elderly men before (□ and ▨) and after (■ and ■) 1 week of CC administration. ★, *P* < 0.01 compared to young; #, *P* < 0.01 compared to baseline.

previous studies have demonstrated that Sertoli cells are the source of testicular inhibin and that FSH stimulates Sertoli cell secretion of inhibin (10–12). Therefore, inhibin may represent a circulating protein marker of Sertoli cell function. The demonstration of lower serum inhibin levels in the presence of similar bioactive FSH levels in elderly men compared to those in young men implies that Sertoli cell inhibin production decreases with aging.

The lower serum inhibin levels in the elderly men could be due to a smaller number of Sertoli cells producing inhibin and/or a decline in the maximum ability of each Sertoli cell to produce inhibin. The number of Sertoli cells in the testes is reduced in older men (26), and our results suggest that the capacity of Sertoli cells to produce inhibin in response to stimulation is decreased. Alternatively, accelerated inhibin clearance may occur with aging. However, this seems unlikely, since the clearance of many hormones decreases, rather than increases, with age (27).

Some evidence suggests that in normal male serum stored frozen for 6 yr there may be up to a 25% loss of inhibin immunoreactivity compared to that in recently collected control male serum (our unpublished data). Serum used in this study was stored frozen for 1–3 yr before inhibin assay. Furthermore, because sera from both young and elderly men were obtained at similar times, any changes with storage should be equally distributed in both age groups and, therefore, should not

alter group comparisons.

After 1 week of CC administration, serum FSH and LH levels increased in both young and elderly men. Concomitantly, serum inhibin levels increased in both age groups, consistent with the trophic action of FSH on Sertoli cell production of inhibin. Similarly, in women, exposure to exogenous FSH (28) or a combined rise in endogenous gonadotropins (29) led to an increase in serum inhibin levels.

Although serum bioactive FSH levels increased similarly after CC stimulation in the young and elderly men, the increase in inhibin levels was significantly lower in the elderly men. If the relative increase in bioactive FSH after CC treatment were considered, the inhibin levels in the elderly men increased less than half as much as those in young men for a given increase in bioactive FSH. These data imply decreased Sertoli cell reserve in the aging testis.

We recently demonstrated that either LH or FSH treatment can partially restore inhibin secretion in normal men whose endogenous gonadotropin levels were suppressed with testosterone (30). In this study we are unable to distinguish the relative roles of the two gonadotropins in stimulating inhibin secretion.

According to the inhibin hypothesis, FSH stimulates inhibin production, and inhibin decreases FSH secretion (10). Since FSH levels were higher while inhibin levels were lower in the elderly men, our data are consistent with this concept. Furthermore, the lower serum T and inhibin levels in elderly men compared to those in young men may reflect the same overall phenomenon, namely the loss of function of the testis (Leydig and Sertoli cells) with aging.

In conclusion, healthy elderly men have lower serum inhibin levels than young men, both basally and after CC stimulation of gonadotropin secretion. We conclude that normal aging results in a decline in the capability of the Sertoli cell to secrete inhibin.

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