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Serum Bioactive and Immunoreactive Follicle-Stimulating Hormone Levels and the Response to Clomiphene in Healthy Young and Elderly Men*

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ABSTRACT. Testicular function declines with normal aging, while serum immunoreactive LH and FSH levels increase. Since there are reports of an age-related decrease in the ratio of bioactivity to immunoreactivity (B/I ratio) for LH, we used a newly available bioassay for FSH to assess age-associated changes in the bioactivity and B/I ratio of FSH in man. Thirty-nine healthy men (23 young and 16 elderly) had single blood samples drawn. In addition, a subset of these men (12 young and 13 elderly) underwent frequent blood sampling for 24 h, both before and after 7 days of clomiphene citrate (CC) administration. Hourly blood samples from the 24-h sampling were pooled, and these, along with the single samples, were assayed for FSH by an *in vitro* bioassay system, using estrogen production by immature rat granulosa cells as the end point, and by RIA.

Baseline single sample mean FSH, as measured by bioassay, was similar in young and elderly men [386 ± 98 (±SEM) and 342 ± 77 ng/mL, respectively]. Baseline mean FSH, measured by RIA, was significantly higher ($P < 0.001$) in elderly men (234 ± 31 ng/mL) than in young men (122 ± 12 ng/mL). The baseline FSH B/I ratio based on single sampling was significantly lower ($P < 0.01$) in elderly men (1.4 ± 0.2) than in young men (2.7 ± 0.3). In the men given CC and sampled for 24 h, mean bioactive FSH levels increased significantly in both the young (1180 ± 282 ng/mL) and the elderly (992 ± 227 ng/mL; $P < 0.01$ for both values compared to baseline). Mean FSH by RIA also increased to similar levels in these young (217 ± 34 ng/mL) and elderly (258 ± 45 ng/mL) men. The FSH B/I ratio was 4.8 ± 0.8 in young and 4.7 ± 1.1 in elderly men after CC administration.

We conclude that 1) serum bioactive FSH levels are similar in elderly and young men, suggesting that the age-related decline in testicular function in man cannot be explained by a chronic deficiency in FSH stimulation; 2) elderly men have a lower serum FSH B/I ratio than young men, which may reflect changes in the circulating form of FSH with aging; and 3) administration of CC to young and elderly men increases both bioactive and immunoreactive serum FSH, implying preserved hypothalamic-pituitary responsiveness in the elderly. (*J Clin Endocrinol Metab* **64:** 1103, 1987)

TESTICULAR function declines with aging in healthy men. Age-related changes include a progressive loss of testicular cell types (1–3), a decrease in daily sperm production (1), and a lowering of mean serum testosterone (T) levels (4–9). FSH has been demonstrated to be important in stimulating quantitatively normal spermatogenesis (10). Since there is a quantitative decrease in spermatogenesis with aging in normal men (1–3), it is important to assess whether this decrease could be due to a primary decrease in FSH levels. Previous studies of serum FSH changes with aging have all used immunoassays and have demonstrated an age-related increase in FSH (4, 5, 8, 9, 11–14).

There is also evidence to suggest that changes in gonadotropin bioactivity may be involved in age-related alterations in testicular function. For example, pituitary extracts from aging men have been reported to contain FSH molecules with different charges from those contained in pituitaries from young men (15). Since charge changes often reflect changes in biological activity (16), the implication is that aging men may produce FSH with altered bioactivity. In addition, for LH the ratio of bioactivity to immunoreactivity (B/I ratio) is lower in older men than in young men (17, 18). However, a bioassay with sufficient sensitivity to measure FSH in human serum has not been available until very recently (19). With the development of a sensitive *in vitro* granulosa

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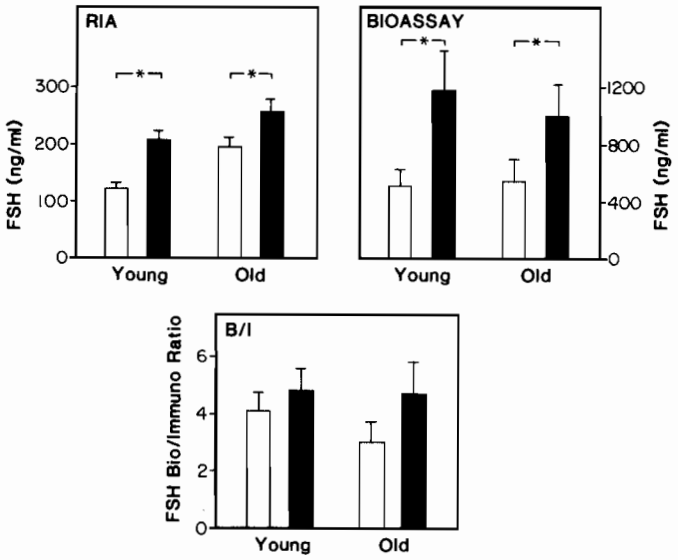

FIG. 3. Mean (±SEM) immunoreactive and bioactive FSH levels in 24-h serum pools and the calculated FSH B/I ratio in 12 normal young and 13 normal old men before (□) and after (■) 7 days of CC administration (*, $P < 0.01$ compared to before CC).

Discussion

With the development of a sensitive *in vitro* FSH bioassay (19, 20, 25), it was possible to measure serum bioactive FSH levels in healthy young and elderly men and compare these to serum immunoreactive FSH levels. Comparing either single samples or the values in a pool of serum samples obtained hourly for 24 h, healthy older men had basal serum FSH bioactivity levels similar to those in younger men. Basal immunoreactive FSH levels, however, were significantly higher in the elderly compared to the young men, a finding in agreement with previous studies (4, 8, 9, 11–14). As a consequence of their higher immunoreactive FSH levels and unchanged bioactive FSH levels, elderly men had significantly lower B/I ratios than young men. When given CC for 7 days, both young and elderly men showed similar significantly increased serum bioactive and immunoreactive FSH levels. These increases resulted in similar CC-stimulated FSH B/I ratios in the two age groups.

The men studied were physically and mentally active, had no major coexisting disease, and were taking no medications. Therefore, our results were not confounded by the problems of interacting illnesses or drugs. Although very healthy, the elderly men did have some evidence of testicular failure, as evidenced by their significantly lower mean serum T level compared to that in the younger men.

The lower basal FSH B/I ratio in elderly men compared to that in young men is interesting, especially in light of previous animal studies which have shown that the molecular forms and bioactivity of gonadotropins can vary with hormonal state, such as during puberty or after ovariectomy (27–29). Other studies have shown that aging pituitaries contain FSH forms with different charges than FSH contained in young pituitaries (15) and that charge changes often reflect alterations in biological activity (30). Additionally, some (17, 18), but not all (31), studies have indicated that the B/I ratio of LH is lower in old men than in young men. In addition, a decline in the B/I ratio of serum FSH was found in normal men treated with a potent GnRH antagonist using the same *in vitro* FSH bioassay reported here (32).

The lower single sample FSH B/I ratio found in elderly men in this study might be due to several factors, including pituitary secretion of a FSH with decreased bioactivity, alteration of the FSH molecule while circulating in serum, changes in the relative clearance of the FSH glycoprotein(s), or factors in aging serum that interfere with the bioassay. Data from other studies (15, 27–30, 33) suggest that secretion of an altered FSH molecule is the most likely reason for the diminished FSH B/I ratio in aging men.

CC is frequently used to stimulate the hypothalamic-pituitary mechanisms controlling gonadotropin secretion (21). The exact mechanism of gonadotropin stimulation induced by CC is not known, but CC is thought to have its major effect on gonadotropin secretion by binding to hypothalamic estrogen receptor sites and blocking estrogen negative feedback (34, 35). CC was used in this study to evaluate the bioactivity of FSH produced in response to pituitary stimulation. CC also has a dose-dependent direct inhibitory effect on the testes (36), but this effect occurs at much higher drug levels than expected from oral dosing (37). Furthermore, addition of 10^{-7} M CC (a concentration much greater than expected in the serum of men in our study) to the GAB assay system did not alter FSH bioassay results.

Both young and elderly men had a significant increase in their serum bioactive and immunoreactive FSH levels in response to CC stimulation, consistent with preserved hypothalamic and pituitary responsiveness to this compound in elderly men. FSH B/I ratios did not significantly change in either group after CC administration, since FSH bioactivity and immunoreactivity increased similarly in the two groups. Of note is the finding that baseline FSH B/I ratios in the subset of men in both age groups who underwent 24-h study were similar, unlike the FSH B/I baseline ratios in the same age groups using single sample data. The possibility that this difference could be due to alterations in FSH B/I ratio across a 24-h period cannot be ruled out. However, this seems unlikely because single samples were obtained in both age groups at varying times, including early morning and later evening, without any noticeable trend in values with time of day.

FSH is needed for quantitatively normal spermatogen-

esis (10). Since spermatogenesis quantitatively decreases with normal aging in men (1–3), it was important to assess the age-related changes in bioactive FSH. Despite lower single sample FSH B/I ratios, elderly men, both before and after CC stimulation, had total serum FSH bioactivity levels similar to those of young men. This finding suggests that the previously described testicular changes with normal aging are not the direct result of a chronic FSH deficiency, but, rather, are a reflection of primary testicular changes (such as germ cell or Sertoli cell loss or dysfunction, or alterations in intratesticular growth or inhibitory factors). The results also suggest that elderly men may have altered hypothalamic-pituitary regulation, since their end-organ dysfunction, as demonstrated by the finding of lower serum T levels, was not accompanied by higher serum bioactive FSH levels than those found in young men. That the elderly men were capable of sustaining higher than baseline bioactive FSH levels was demonstrated by the CC results.

In conclusion, we found that serum FSH bioactivity levels were similar in healthy young and elderly men, but the FSH B/I ratio was lower in elderly men compared to that in their younger counterparts. Furthermore, CC stimulation resulted in similar increases in bioactive and immunoreactive FSH levels in young and elderly men. From these results we conclude 1) that the age-related decline in testicular function cannot be explained by a primary decrease in pituitary production of bioactive FSH and is more indicative of primary testicular insufficiency; 2) that aging may be associated with changes in the circulating form of FSH; and 3) that hypothalamic-pituitary responsiveness to CC is preserved in elderly men.

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