Longitudinal Changes in Testosterone, Luteinizing Hormone, and Follicle-Stimulating Hormone in Healthy Older Men

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Cross-sectional studies have demonstrated a decline in testosterone and free and bioavailable testosterone with age. This occurs in a majority of older persons without an increase in luteinizing hormone (LH), suggesting that a component of the testosterone decrease is due to secondary hypogonadism. To determine whether these findings could be duplicated in a longitudinal study, we measured testosterone, LH, follicle-stimulating hormone (FSH), and sex hormone–binding globulin (SHBG) levels in 77 men participating in the New Mexico Aging Process Study who had sera available in 1980 or 1981 and two or more serial samples in 1982, 1984, 1989, and/or 1994. Thirty-nine subjects had samples available from both 1980 and 1994. The age at entry into the study ranged from 61 to 87 years. Testosterone levels decreased over the 15 years of the study. In persons who were alive for the duration of the study, testosterone levels were significantly lower 5 years before termination of the study ($P < .05$). Testosterone levels did not differ at entry into the study among those who died and those who were alive at the end of the study period. Eight of 77 subjects (10%) had LH levels above the normal range at some time during the study. In contrast, 43% of subjects had elevated FSH levels. Both LH and FSH increased significantly with age. SHBG levels were measured in 1980 and 1994 and increased significantly with age ($P < .0001$). LH and FSH were highly correlated with one another, but neither correlated with testosterone. This study demonstrated a longitudinal decline in testosterone and an increase in LH and FSH in older men. The average rate of decrement in testosterone concentration was 110 ng/dL every decade.

THE DECREASE in testosterone and the concomitant finding of normal to low gonadotropins with advancing age have predominantly been studied in a cross-sectional fashion. The majority of these studies found that testosterone decreased with age,[1-6] although the Baltimore Longitudinal Aging Study failed to find a decrease in testosterone in cross-sectional data.[7] The latter study was criticized because samples were obtained predominantly in the afternoon when testosterone values are at the nadir, and thus, young and old subjects showed no statistically significant difference in testosterone concentration.[8] Cross-sectional studies using bioavailable or free testosterone have tended to demonstrate even greater differences between healthy young and healthy elderly individuals.[9,10]

Luteinizing hormone (LH) levels fail to increase appropriately in response to the decreasing testosterone levels with aging.[3,9,11-14] This has led to the suggestion that older men develop secondary hypogonadism due to decreased function of the hypothalamus and/or pituitary. Recent interventional studies have suggested that gonadal failure may have functional consequences such as decreased upper-body strength, decreased hematocrit, and altered bone metabolism.[15-18]

To more fully define the natural history of testosterone and gonadotropin changes with aging, we have examined the alterations in these hormones in a group of elderly men participating in the New Mexico Aging Process Study.[19-21]

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SUBJECTS AND METHODS

Subjects

Seventy-seven men participating in the New Mexico Aging Process Study who had blood taken in 1980 or 1981 and had two or more serial samples available for analysis in 1982, 1984, 1989, and/or 1994 were studied. Samples were drawn between 8 and 11 AM. Thirty-nine subjects had samples available from both 1980 and 1994. The New Mexico Aging Process Study is a longitudinal study of nutrition and aging that was begun in 1979. The age at entry to the study ranged from 61 to 87 years, with the majority of subjects between 66 and 80 years of age. They were white men with above average income and education who resided predominantly in the area of Albuquerque, NM. Ninety-six percent of the participants were non-Hispanic whites, and 4% were of Hispanic origin. This cohort is not representative of a population-based sample of Albuquerque, which is approximately one third Hispanic. The entrance criteria for the study excluded subjects with serious diseases such as cancer (other than skin cancer) within the previous 5 years, recent myocardial infarction, chronic obstructive pulmonary disease, and use of chemotherapeutic, cardiac, respiratory, or antipsychotic medications. Diuretics were used by 8% of the men. None of the men were current smokers. Continued participation in the study was not contingent upon the maintenance of good health. Overall, the subjects would be considered to be in better than average health. All participants provided informed consent, and the longitudinal study was approved by the Human Subjects Research Review Committee of the University of New Mexico School of Medicine.

Hormone Measurements

Measurements were made on single serum samples in the College of American Pathologists (CAP)-certified Geriatric Medicine laboratory at St Louis University. Samples were stored at −70°C. All specimens from a single individual for a specific hormone were analyzed in the same assay. Testosterone level was measured by radioimmunoassay using a kit from DPC (Los Angeles, CA). The intraassay coefficient of variation (CV) was 5.8% and the interassay CV 10.4%. Bioavailable testosterone or free testosterone levels were not measured, since in our experience

these measurements are markedly altered after 6 or more months of storage. LH level was measured by radioimmunoassay using a kit from ICN Pharmaceuticals (Costa Mesa, CA). The intraassay CV was 9.1% and the interassay CV 8.2%. Follicle-stimulating hormone (FSH) level was measured by radioimmunoassay using a kit from DPC. The intraassay CV was 5.2% and the interassay CV 7.4%. Sex hormone–binding globulin (SHBG) level was measured by radioimmunoassay using a kit from DSL (Webster, TX). The intraassay CV was 2.9% and the interassay CV 10.9%. CVs for 20 samples assayed in our laboratory 3.5 years apart were 4.6% for testosterone, 4.8% for FSH, and 7.3% for LH. Based on samples in healthy young males in our laboratory, the normal ranges are 278 to 749 ng/dL for testosterone (n = 20) and 30 to 102 nmol/L for SHBG (n = 20).

Statistical Methods

Repeated-measures ANOVA ($P < .05$) was used for basic analyses. Bonferroni pairwise comparison was used to compare means within groups. For some comparisons, subjects were divided into cohorts based on the starting age, in groups of 61 to 65, 66 to 70, 71 to 75, and older than 75 years.

Pearson's correlation was used to compare hormonal variables with one another and with age when the underlying population variables were normally distributed. Spearman's rank correlations were used when the underlying population variables were not normally distributed.

All data are presented as the mean ± SEM.

RESULTS

Testosterone

Testosterone levels in the older cohort decreased with age ($r = -.2099$, $P < .0001$; Fig 1). When each age cohort was examined individually, there was a significant decline in testosterone in groups aged 66 to 70 ($F_{4,122} = 5.63$, $P < .0004$), 71 to 75 ($F_{4,116} = 2.94$, $P < .02$), and older than 75 years ($F_{4,43} = 3.39$, $P < .01$) over 15 years of the study (Fig 2). The failure to observe a decline in the other group was related to the small n value for that group. At entry into the study, the lowest testosterone level was 303 ng/dL. By 1989, two subjects had developed testosterone levels less than the lower limit of normal for young males for the assay (245 ng/dL). By 1994, one third of the individuals had testosterone concentrations less than this level. To ensure that the longitudinal decrease in testosterone was not due to the subjects with elevated baseline testosterone levels not remaining in the study, we examined testosterone values in 39 individuals who had samples in both 1980 and 1994 (Table 1). In this cohort, testosterone levels decreased significantly ($F_{4,182} = 5.89$, $P < .0002$). Testosterone values in 1989 and 1994 both differed significantly from the entry value in 1980 ($P < .05$). The average rate of decline was 110 ng/dL every 10 years.

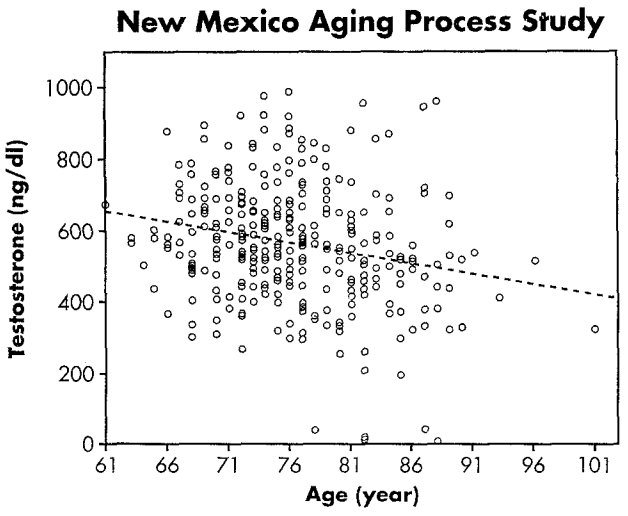

Fig 1. Correlation between serum testosterone and age in all the samples. $r = -.2099$, $P < .0001$.

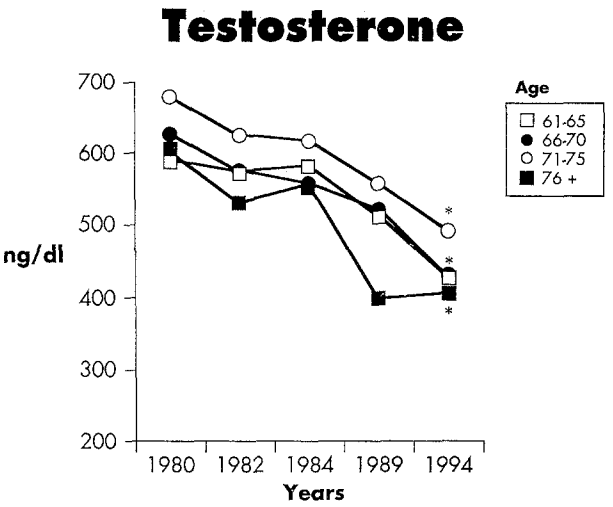

Fig 2. Longitudinal alterations in serum testosterone levels from 1980 to 1994 in 4 age cohorts. *$P < .05$. Age groups refer to age at entry to the study. P values refer to significant change from entry value. n = 77 for 1980, n = 63 for 1982, n = 71 for 1984, n = 68 for 1989, and n = 39 for 1992.

LH

At entry into the study, none of the subjects had a LH level greater than the upper limit of normal for the assay (ie, >20 mIU/L). By 1984, three subjects had elevated LH levels. Two further subjects developed elevated LH levels in 1989. In 1994, five of 39 samples (12.5%) available for analysis had elevated LH levels. Of the original 79 subjects, eight (10%) developed elevated LH levels at some stage during the study.

In the total group, LH increased with age ($r = .2686$, $P < .000001$; data not shown) and LH increased with time in the different age cohorts (Fig 3). LH also increased when only individuals with samples available in both 1980 and 1994 were

Table 1. Longitudinal Alterations in Hormone Values in Subjects With Samples Available Both in 1980 and in 1994

Year	Testosterone (ng/dL)	LH (mIU/mL)	FSH (mIU/mL)
1980	633 ± 22	9.4 ± 0.4	14.1 ± 1.0
1982	594 ± 26	10.3 ± 0.6	15.5 ± 1.1
1984	570 ± 25	10.3 ± 1.1	19.1 ± 2.8*
1989	515 ± 29*	10.7 ± 0.7	20.2 ± 2.1*
1994	464 ± 33*	13.7 ± 1.3*	27.4 ± 3.4*
	$F_{4,182} = 5.49$, $P < .002$	$F_{4,182} = 3.40$, $P < .01$	$F_{4,182} = 5.09$, $P < .007$

*$P < .05$ *v* 1980.

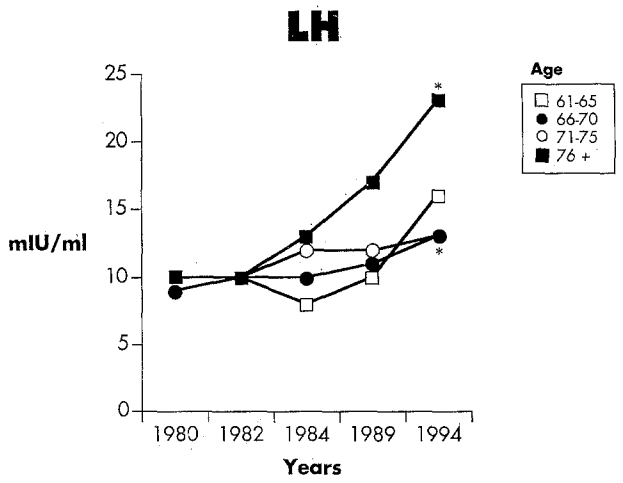

Fig 3. Longitudinal alterations in serum LH levels from 1980 to 1994. *$P < .05$. (See Fig 2.)

examined ($F_{4,181} = 3.4$, $P < .01$). Samples obtained in 1994 significantly differed from those obtained in 1980 ($P < .05$).

FSH

At entry into the study, 18% of subjects had FSH levels greater than the upper limit of normal (20 mIU/mL). By the end of the study period, 43% of subjects had elevated FSH levels. Overall, FSH levels increased with age ($r = .2823, P < .000001$) and increased with time in the different age cohorts (Fig 4). FSH increased significantly in the cohort with samples available in both 1980 and 1994 ($F_{4,178} = 5.09$, $P < .0007$; Table 1). The 1994 cohort differed from all other groups ($P < .05$), and the 1984 and 1989 cohorts differed from the 1980 and 1982 cohorts ($P < .05$).

SHBG

Because of the shortage of sample volumes, SHBG level was only measured in 1980 and 1994 samples when sufficient sample was available. SHBG values for 1980 were 84.4 ± 2.5 nmol/L, and for 1994, 104.5 ± 3.6 nmol/L ($P < .0001$).

Hormone Correlations

Using D'Agostino's test of normality,[22] LH and FSH were found not to be normally distributed, and therefore, Spearman rank correlations were used for calculations involving these hormones. LH and FSH were highly correlated with one another ($r = .4542$, $P < .00001$; Fig 5), whereas testosterone did not correlate with either LH ($r = -.0316$) or FSH ($r = -.0900$). SHBG correlated with age ($r = .5310$, $P < .00001$). Grubb's test for outliers failed to identify any outliers.[23]

Mortality

A total of 18 subjects died during the study period. Serum testosterone levels at initiation into the study for those who died were 651 ± 38 ng/dL, compared with 639 ± 18 ng/dL for those who did not die.

DISCUSSION

This study demonstrated a longitudinal decline in testosterone with aging. Testosterone levels declined by more than the assay CVs in 77% of subjects. In the group for whom samples were available over the whole study period, a significant decline in testosterone occurred 5 years before the last sampling period, suggesting that this decline was not due to significant illness. Various studies have demonstrated that illness can result in either primary or secondary hypogonadism (see Morley and Melmed[24] for a review). As a whole, this group of men were healthier than average. Overall, this longitudinal study confirms the decrement in testosterone with age reported in cross-sectional studies.[1-6,9] Preliminary data from the Baltimore Longitudinal Study of Aging have also suggested a longitudinal decline in testosterone with aging.[25]

Much of the cross-sectional data had suggested that the primary defect with aging is due to a failure of the hypothalamic-pituitary unit to appropriately respond to the decrease in testosterone.[3,9,11-14] It has been suggested that there is increased

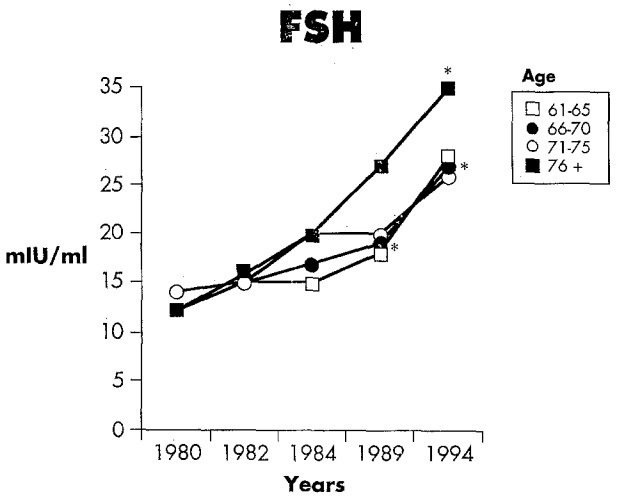

Fig 4. Longitudinal alterations in serum FSH levels from 1980 to 1994. *$P < .05$. (See Fig 2.)

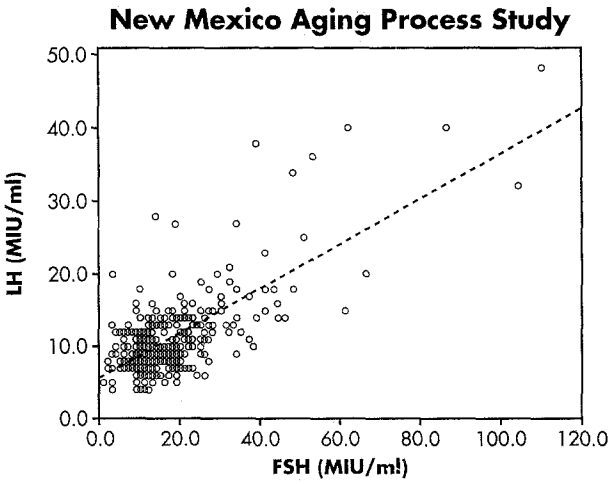

Fig 5. Correlation between serum LH and FSH levels in all the samples. $r = .4542$, $P < .00001$.

sensitivity to the negative-feedback effect of testosterone associated with aging.[26] LH does increase with aging.

FSH levels increased dramatically with aging. Nearly half of the subjects had elevated FSH levels by the end of the study. This confirms Sertoli cell failure with advancing age,[12,27,28] and is likely associated with a decrease in inhibin and thus disinhibition of FSH. The correlation of LH with FSH is not unexpected, since both gonadotropins are released by gonadotropin-releasing hormone.

This study clearly demonstrated an increase in SHBG with age. Previously, Field et al[29] have shown in a cross-sectional study that SHBG increased with age. The increase in SHBG suggests that had we been able to measure free or bioavailable testosterone levels in these samples, these would have decreased to an even greater extent than was observed with total testosterone levels.

In conclusion, this study has demonstrated a longitudinal decline of testosterone with aging. Although LH increases with aging, it did not correlate with testosterone, suggesting an alteration in the normal feedback relationship of these hormones. FSH increased with aging, supporting the concept that there is decreased inhibin feedback from Sertoli cells with aging. The underlying mechanism(s) of these alterations remains to be elucidated.

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