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Sex Hormones and Age: A Cross-sectional Study of Testosterone and Estradiol and Their Bioavailable Fractions in Community-dwelling Men

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The role of endogenous sex hormones in many diseases makes understanding factors that influence levels of these hormones increasingly important. This study examined age-associated variations in total and bioavailable testosterone and estradiol levels among community-dwelling Caucasian men in Rancho Bernardo, California. Plasma samples obtained from 810 men aged 24–90 years in 1984–1987 were analyzed in 1993 using radioimmunoassay. Analyses of age–hormone associations, adjusting for weight, body mass index, alcohol ingestion, smoking, physical activity, caffeine intake, specimen storage time, and disease status, were undertaken. Bioavailable testosterone and bioavailable estradiol levels decreased significantly with age independently of covariates. Total testosterone and estradiol levels decreased with age only when analyses were controlled for confounders. The importance of the age-associated decline in endogenous sex hormone levels, particularly levels of bioavailable testosterone and bioavailable estradiol, and their relation to disease and function in men deserve further research. *Am J Epidemiol* 1998;147:750–4.

aging; estradiol; hormones; men; testosterone

Diseases as diverse as cardiovascular disease (1, 2), hypertension (3), cancer (4–6), osteoporosis (7), stroke (8), and diabetes mellitus (9, 10) have been linked to levels of endogenous sex hormones. It is therefore important to understand the effects of age, obesity, and behavior on circulating levels of bioavailable sex steroids. Studies of the association between age and endogenous sex steroids in men have yielded inconsistent results (11–14), possibly reflecting sample selection, time of day of blood sampling, type of hormonal assay, or failure to control for confounding factors (15, 16). Most studies have measured total hormone levels rather than their bioavailable fractions, and to our knowledge no previous report has measured bioavailable estradiol in men.

This study was unique because of its 1) large, geographically defined sample of men, 2) ability to evaluate the effect of age independently of numerous major variables (including body mass index, waist : hip ratio, alcohol use, cigarette smoking, exercise intensity, caffeine intake, and chronic disease) that may covary with age and alter endogenous hormone levels, and 3) measurement of both total and bioavailable estradiol and testosterone.

MATERIALS AND METHODS

Eighty-one percent of surviving men from the Rancho Bernardo Study, an ongoing community-based study of lifestyle and aging among middle- and upper middle-class Caucasian adults, participated in a follow-up evaluation in 1984–1987 involving a clinic visit and administration of a standardized questionnaire on physical activity, smoking, caffeine intake, and alcohol consumption in the 2 weeks prior to the interview. Approximately half of the sample also completed a questionnaire adapted from the 1985 Health Interview Survey (17, 18) that assessed participation in 15 leisure-time activities during the preceding 2 weeks, which were classified using intensity codes established and validated by the Minnesota Heart Survey (19). Height, weight, and waist and hip girth were measured with participants wearing light clothing without shoes; body mass index (weight (kg)/height^2 (m^2)) and waist : hip ratio were then calculated. Diabetes mellitus was defined by medical history and the results of an oral glucose tolerance test, using World Health Organization criteria (20). Plasma glucose was measured in a hospital diagnostic laboratory using a hexokinase method for true glucose. Coronary heart disease was defined by medical history

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and the presence of major Q waves on an electrocardiogram. Medical record review of a 30 percent sample confirmed the reported diagnosis in 85 percent (diabetes) to 95 percent (heart attack) of cases. Venipuncture was performed between 7:00 a.m. and 10:00 a.m. after a 12-hour fast.

Plasma was stored in polypropylene tubes at −70°C until they were first thawed for sex hormone determinations in 1993 (mean storage time = 100.5 months (standard deviation 8.7 months)). Total testosterone and total estradiol levels were determined by radioimmunoassay (21); bioavailable testosterone and estradiol levels were determined by an experienced laboratory technician using the method of Tremblay and Dube (22) in a University of California, San Diego, endocrinology research laboratory. Less than 1 percent of men had hormone levels below the level of assay sensitivity; values for undetectable levels were converted to values slightly below the assay sensitivity level for analysis. Eleven men with testosterone levels more than three standard deviations above the mean were included after medical record review revealed no explanatory medical condition. Intra- and interassay coefficients of variation and sensitivity were as follows: for testosterone, 4.00 percent, 6.78 percent, and 37 pg/ml, respectively; for estradiol, 5.87 percent, 7.44 percent, and 6 pg/ml; for bioavailable testosterone, 5.80 percent, 7.61 percent, and 37 pg/ml × percent free; and for bioavailable estradiol, 3.70 percent, 5.18 percent, and 6 pg/ml × percent free.

Hormone data were available for 914 men. The present analysis includes the 810 men for whom we had complete data on body mass index, waist : hip ratio, current smoking, current alcohol use, caffeine intake, and specimen storage time. These men did not differ significantly by age or hormone level from those excluded. The distribution of hormone values was normal. Additional analyses that controlled for exercise intensity were completed in 391 men. Because specimen storage time was inversely correlated with levels of bioavailable testosterone ($r = -0.20$) and bioavailable estradiol ($r = -0.08$) (but not with total testosterone or estradiol), analyses were adjusted for specimen storage time. Pearson two-tailed correlations were used to determine the cross-sectional associations between age and individual hormone levels. Partial correlations were used to control for body mass index, waist : hip ratio, alcohol use, smoking, caffeine intake, months of storage, and exercise intensity. Age-specific hormone levels were adjusted for body mass index and other covariates using multiple analysis of variance. Two-way analysis of variance, controlling for age and body mass index, was used to test for an interaction between chronic disease (coronary heart disease, diabetes, or cancer) and age. Linear regression was used to estimate the magnitude of hormonal change with age. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, Inc., Chicago, Illinois). Given the multiple comparisons, statistical significance was defined as $p < 0.01$.

TABLE 1. Two-tailed Pearson correlations between behavioral covariates and levels of endogenous sex hormones (pg/ml) in 810 men aged 24–90 years, Rancho Bernardo, California, 1984–1987

Covariate	Testosterone	Bioavailable testosterone	Estradiol	Bioavailable estradiol
Weight (kg)	−0.24***	0.07	0.05	0.18***
Body mass index†	−0.24***	0.02	0.07	0.18***
Waist:hip ratio	−0.13***	−0.05	0.01	0.05
Alcohol intake (g/week)	−0.04	0.06	−0.01	0.04
Cigarette smoking (cigarettes/day)	0.06	0.09	−0.05	−0.03
Caffeine intake (g/month)	−0.06	0.21***	−0.07	0.06

** $p < 0.01$; *** $p < 0.001$.
† Weight (kg)/height² (m²).

RESULTS

The men were aged 24–90 years (mean = 69.6 years (standard deviation 10.9 years)) in 1984–1987. The association of hormone levels with major covariates is shown in table 1. As shown, body size measures were inversely correlated with total testosterone but not bioavailable testosterone and with bioavailable estradiol but not total estradiol. Caffeine intake was positively associated with bioavailable testosterone. There was a strong inverse linear association between age and bioavailable testosterone and bioavailable estradiol in the men (table 2). This association was not

TABLE 2. Two-tailed Pearson correlations between levels of endogenous sex hormones (pg/ml) and age, and partial correlations controlled for body mass index and other covariates, in 810 men aged 24–90 years, Rancho Bernardo, California, 1984–1987

Hormone	Pearson's *r*	Partial correlation, controlled for body mass index†	Partial correlation, controlled for multiple covariates‡
Testosterone			
Total	−0.02	−0.07	−0.12**
Bioavailable	−0.54***	−0.55***	−0.53***
Estradiol			
Total	−0.05	−0.04	−0.09**
Bioavailable	−0.28***	−0.25***	−0.27***

** $p < 0.01$; *** $p < 0.001$.
† Weight (kg)/height² (m²).
‡ Covariates included body mass index, waist:hip ratio, alcohol intake (g/week), smoking (cigarettes/day), sample storage time (months), and caffeine intake (g/month).

materially changed after adjustment for body mass index, waist : hip ratio, alcohol consumption, cigarette smoking, caffeine intake, and specimen storage time. Significant negative associations were also found between age and total estradiol and total testosterone, but only after adjustment for covariates (table 2). These associations were unchanged in a separate analysis that adjusted for exercise intensity (data not shown). Results were similar in men with and without coronary heart disease, cancer, or diabetes.

Figures 1 and 2 show the multiply adjusted hormone levels stratified by 5-year age group. There was a stepwise linear decrease in bioavailable testosterone and estradiol levels which persisted after adjustment for all covariates.

Based on the beta coefficients from linear regression analysis, total testosterone concentrations declined by approximately 1.9 pg/ml for each year of age, while bioavailable testosterone levels declined by 18.5 pg/ml per year of age. Total estradiol concentrations declined by 0.03 pg/ml for each year of age; bioavailable estradiol levels declined by 0.12 pg/ml per year of age.

DISCUSSION

This study found a significant decline in bioavailable estradiol and testosterone with age among men, and a much smaller, though still significant, negative association of total testosterone and estradiol with age when data were adjusted for covariates. Age-related decrements were independent of body mass index, waist : hip ratio, cigarette smoking, alcohol ingestion, caffeine intake, physical activity, and specimen storage time. Negative associations of total testosterone with age in men have been noted in some cross-sectional studies (11–13, 23–26) but not in others (27–29). In 1,408 healthy men aged 20–60 years, Simon et al. (11) reported a stepwise age-associated decrease in total testosterone ($r = -0.25$) with age that was independent of body mass index, subscapular skinfold thickness, and tobacco and alcohol consumption. Similarly, a study of 243 men in the Multiple Risk Factor Intervention Trial (30) reported a negative correlation between age and total testosterone ($r = -0.23$). The Massachusetts Male Aging Study, a study of 1,241 men, showed a weaker inverse association between total testosterone and age ($r = -0.10$) (16), reporting annual declines of 0.4 percent in total testosterone levels among both healthy and ill men (14). A recent meta-analysis of 88 published studies showed a significant inverse association between age and total testosterone levels in men (15).

Age-associated declines in free testosterone (i.e., testosterone that is not bound to sex hormone-binding globulin or albumin) have been reported from only two study centers, and neither group reported levels of total bioavailable testosterone (which includes un-

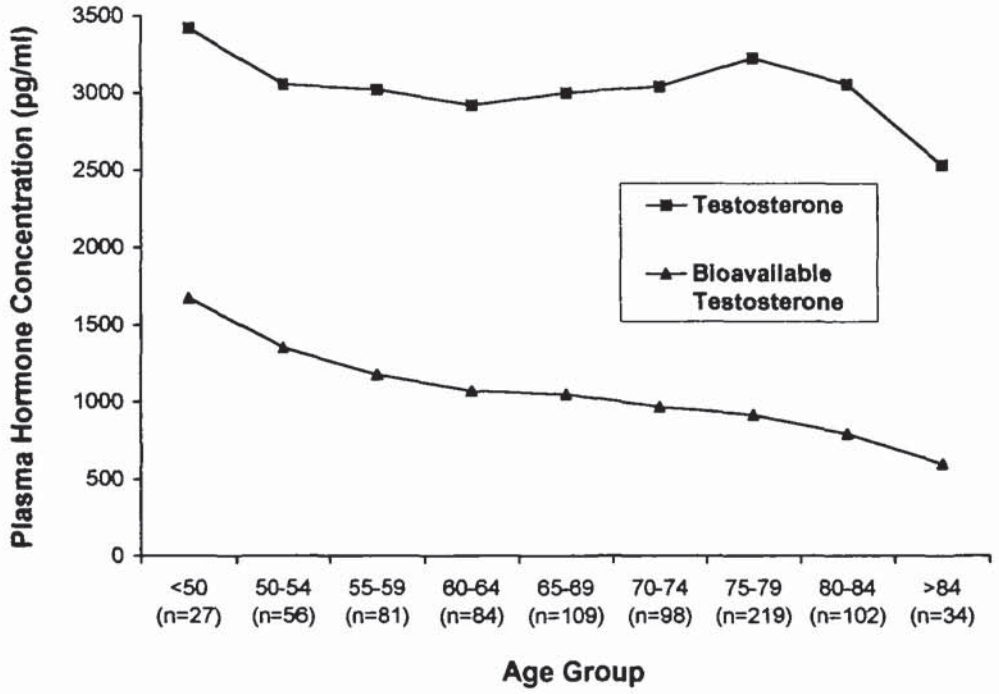

FIGURE 1. Levels of endogenous total and bioavailable testosterone in 810 men aged 24–90 years, by 5-year age group, Rancho Bernardo, California, 1984–1993. Data were adjusted for multiple covariates, including body mass index (weight (kg)/height^2 (m^2)), waist : hip ratio, alcohol intake (g/week), smoking (cigarettes/day), sample storage time (months), and caffeine intake (g/month).

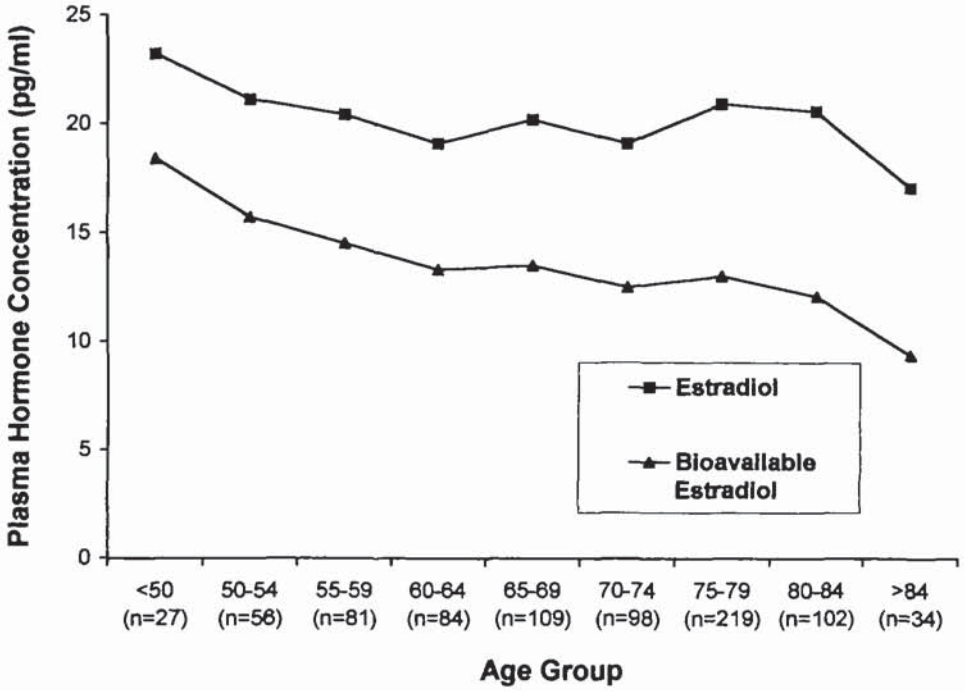

FIGURE 2. Levels of endogenous total and bioavailable estradiol in 810 men aged 24–90 years, by 5-year age group, Rancho Bernardo, California, 1984–1993. Data were adjusted for multiple covariates, including body mass index (weight (kg)/height^2 (m^2)), waist : hip ratio, alcohol intake (g/week), smoking (cigarettes/day), sample storage time (months), and caffeine intake (g/month).

bound as well as albumin-bound hormone). The Massachusetts Male Aging Study reported inverse associations of both free ($r = -0.22$) and albumin-bound ($r = -0.21$) testosterone with age in both ill and healthy men (16). Those investigators estimated annual declines of 1.2 percent in free testosterone and 1.0 percent in albumin-bound testosterone (14). The Multiple Risk Factor Intervention Trial (30) measured only free testosterone levels, which decreased with age independently of body mass index.

The present study showed a weaker association of total testosterone ($r = -0.13$) than of bioavailable testosterone ($r = -0.52$) with age. The latter is the strongest reported independent age–hormone association in the literature. To our knowledge, no previous study has reported on the relation between total bioavailable testosterone and estradiol levels and age in a population-based study. Testosterone that is not bound to sex hormone is thought to be the biologically active component, because testosterone is only weakly bound to albumin. Both decreased production and increased conversion and metabolic clearance of testosterone may explain the age-associated decrease in total testosterone, while a previously reported age-associated increase in sex hormone binding capacity may explain the greater reduction in bioavailable testosterone (31, 32).

In Rancho Bernardo men, the decline in total estradiol with age was small, and it achieved statistical significance only after adjustment for covariates. Previous studies of the relation between aging and estradiol levels have produced mixed results; some reported no age association with estradiol (12, 14, 26, 27) or free estradiol (23); others found higher estradiol levels with age (23, 24); and still others noted reduced levels ($r = -0.10$) with advancing age (11).

In contrast to the weak association between age and total estradiol, there was a strong association of bioavailable estradiol with age. To our knowledge, this association has not been studied previously. The age-associated decrease in bioavailable estradiol among these men may be partially explained by decreasing levels of testosterone, the primary substrate for male estradiol production, coupled with the higher levels of sex hormone-binding globulin in older adults (16).

The accuracy of steroid hormone measurements made in stored plasma samples has been questioned (33). Hormone levels in the present study were somewhat lower than levels reported in Massachusetts (15), possibly reflecting the older age of our cohort, the high sensitivity of our assay, or the use of polypropylene tubes for storage (which may attenuate hormone concentrations). Polypropylene tubes could theoretically bind steroid molecules. This would not alter the ordinal associations of hormone levels with age or other covariates unless there was bias such that older subjects had longer sample storage times. We controlled for the

latter possibility by adjusting statistically for storage time. There is some evidence that the bioavailable fraction of testosterone increases over time, which is compatible with the progressive deterioration of the steroid-protein complex (34). Again, this would not be expected to alter the ordinal associations for the observed levels.

In summary, bioavailable testosterone and estradiol decreased dramatically with age in these community-dwelling men, independently of body size, health behavior, and chronic disease. The relations between this decline in endogenous sex hormone levels and fragility, impaired functioning, and chronic diseases (such as osteoporosis, diabetes, cancer, and heart disease) should be the focus of future research.

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