0021-972X/01/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 86, No. 2
*Printed in U.S.A.*

# Longitudinal Effects of Aging on Serum Total and Free Testosterone Levels in Healthy Men

S. MITCHELL HARMAN, E. JEFFREY METTER, JORDAN D. TOBIN, JAY PEARSON, AND MARC R. BLACKMAN

*The Intramural Research Program, National Institute on Aging, National Institutes of Health (S.M.H., E.J.M., J.D.T., J.P.) and Department of Medicine (M.R.B.), The Johns Hopkins University School of Medicine, Baltimore, Maryland 21224*

**ABSTRACT**

Many studies have shown cross-sectional (and two small studies, longitudinal) declines in total and/or free testosterone (T) levels, with age, in men. The extent to which decline in T is the result of the aging process *per se*, as opposed to chronic illness, medication use, and other age-related factors, remains controversial. The frequency with which aging leads to T levels consistent with hypogonadism has also not been defined. These issues bear on the potential use of T replacement in aging men, because aging and hypogonadism have, in common, reduced bone and lean body mass and muscle strength and increased total and abdominal fat. We measured T and sex hormone-binding globulin (SHBG), by RIA, in stored samples from 890 men in the Baltimore Longitudinal Study on Aging. Using a mixed-effects model, we found independent effects of age and date of sampling to reduce T levels. After compensating for date effects, which investigation suggested was artifactual, we observed significant, independent, age-invariant, longitudinal effects of age on both T and free T index (free T index = T/SHBG), with an average change of −0.124 nmol/L·yr and −0.0049 nmol T/nmol SHBG·yr. T, but not free T index, also decreased with increasing body mass index. Use of β−blocking drugs was associated with higher T and higher free T index levels. Using total T criteria, incidence of hypogonadal T levels increased to about 20% of men over 60, 30% over 70 and 50% over 80 yr of age, and even greater percentages when free T index criteria were employed. Our observations of health factor independent, age-related longitudinal decreases in T and free T, resulting in a high frequency of hypogonadal values, suggest that further investigation of T replacement in aged men, perhaps targeted to those with the lowest serum T concentrations, are justified. (*J Clin Endocrinol Metab* **86:** 724–731, 2001)

NUMEROUS CROSS-SECTIONAL INVESTIGATIONS have demonstrated lower concentrations of circulating testosterone (T) and/or free T in older men (1–9). However, the finding, in some studies, that T levels did not fall significantly with age in exceptionally healthy men (10–12) raised the question of the relative roles of chronic age-related illness *vs.* aging *per se* in producing the observed decreases. Studies of this issue have suggested that age, illness, and smoking all exert independent effects on T and free T levels during aging (8, 13, 14). In the absence of studies of longitudinal change in T with age, it is uncertain to what extent confounds such as secular or cohort effects (15) may have influenced the above results. Two small-scale longitudinal investigations have observed decreases, with aging, in total T (16, 17); but no large longitudinal series has been reported to date. The above studies suggest that T levels decline at a more or less constant rate, with age, in men, with no period of accelerated decline. However, no quantitative evaluation of the longitudinal influence of aging on the incidence of hypogonadism has yet been reported.

These issues are of more than theoretical concern because aging in men is associated with decreases in bone mineral density (BMD) (18, 19), lean body and muscle mass (20, 21), strength (22, 23) and aerobic capacity (24), as well as with increases in total and abdominal body fat, low-density lipoprotein cholesterol, and/or low-density lipoprotein/high-density lipoprotein cholesterol ratios (25–28), all of which also occur in nonelderly hypogonadal men (29, 30). These changes in body composition and metabolism predispose to musculoskeletal frailty, to osteoporotic fractures [a major health problem in elderly men (31)], and to cardiovascular disease.

Because of the potential for using T replacement to prevent or ameliorate age-related osteoporosis, sarcopenia, and the abdominal obesity/glucose intolerance/hyperlipidemia syndrome (syndrome X) in men, a more thorough understanding of the rate and extent of longitudinal changes in T with age and how such changes are related to other variables (such as disease and smoking) is needed. In the current study, we measured concentrations of T and sex hormone binding globulin (SHBG), and we calculated free T indices in sequential serum samples obtained from a large group of men well-characterized with regard to health-related variables, in the Baltimore Longitudinal Study of Aging (BLSA).

## Materials and Methods

### *Subjects and samples*

All men were participants in the BLSA, a largely middle class, 87% Caucasian population, whose characteristics have been described (32). The BLSA, an open registration study of the physiology of aging, has, for more than 40 yr, accumulated data on men studied, at approximately 2-yr intervals, during visits to the NIA's Gerontology Research Center in Baltimore. The BLSA investigative protocol is approved by the combined Institutional Review Board of the Johns Hopkins Bayview Medical Center and the Gerontology Research Center. All subjects studied signed

Received May 8, 2000. Revision received October 4, 2000. Accepted October 13, 2000.

Address all correspondence and requests for reprints to: S. Mitchell Harman, M.D., Ph.D., Director, Kronos Research Foundation, 4455 East Camelback Road, Suite B135, Phoenix, Arizona 85018. E-mail: harman@kronofoundation.org.

IRB-approved informed-consent documents. Each man receives an extensive interim medical and psychological history and physical examination at each visit, and serum samples are banked for future investigation. Blood samples were obtained from BLSA subjects in the morning between 0700 and 0930 h, after an overnight fast. Before 1992, samples were stored at −20 C. Samples collected after 1992 were kept at −80 C. All men (n = 890) from whom serum samples of adequate volume were available were included in the study. The BLSA men studied were somewhat, but not significantly, younger (mean age, 58.8 ± 15.8 *vs.* 61.8 ± 17.1) than the remaining 821 BLSA men who were not studied, and were seen, on average, 8 yr more recently than the BLSA men who were not included in the study. However, there is no reason to believe that the men who were studied differed in any substantive way from those who were not. Samples assayed in this study were selected from the frozen serum bank as follows: During a 6-month period in 1995, sera from each subject's most recent and previous 3 visits and from visits closest to 10, 15, 20, 25, and 30 yr from the most recent one were retrieved and sent to Covance Laboratories, Inc. (Vienna, VA) for assay of T and SHBG. The number of samples assayed was 3763, with 3565 producing acceptable assay results for T and 3537 for both T and SHBG. The number of samples per subject ranged from 1–10 (mean 4 ± 1.9), with samples stored from less than 1, up to 33, yr (mean 10 ± 8 yr).

### *Assays*

T levels were determined, in duplicate, on aliquots of 100 μL serum, using $^{125}$I, double antibody RIA kits obtained from Diagnostic Systems Laboratories, Inc. (Webster, TX). Minimum detectable T levels averaged 0.42 nmol/L, with intra- and interassay coefficients of variance, respectively, of 4.8% and 5.7% at concentrations of 7.74 and 7.29 nmol/L, and 3.3% and 6.4% at concentrations of 44.7 and 42.9 nmol/L. SHBG concentrations were measured in 50-μL aliquots using RIA kits purchased from Radim (Liege, Belgium) which employ $^{125}$I labeled SHBG and PEG-complexed second antibody. The sensitivity of the SHBG assay was approximately 10 nmol/L. The CV at 5 nmol/L was 22%; and at 25 nmol/L, 5%; with intra- and interassay coefficients of variance, respectively, of 3.4 and 10.8% at concentrations of 22 and 19 nmol/L, and 1.8% and 7.7% at concentrations of 117 and 118 nmol/L.

### *Confounds and validation*

Preliminary analysis of data from 3565 samples, stored between 1961 and early 1995, revealed a significant increase in T level with length of storage, independent of age, so that mean levels of T in samples taken from 1961 to 1975 were, on average, about 40% higher than levels in samples stored between 1985 and 1995. To investigate whether this finding was an artifact or a true secular trend in the population, we first assayed an additional set of 120 serum samples from later visits (1995–1998) for T and SHBG, by the same methods used for the original samples. Some of these samples were from men whose samples were included in the first set of assays; others were not. We detected no apparent discontinuity between the more recent set of samples and those immediately preceding them, for any of the above measures (Fig. 1). Next, we reassayed 221 of the previously examined samples, evenly distributed across the entire date range, using a different RIA method at Endocrine Sciences, Inc. Laboratories (Calabasas Hills, CA). This latter assay employed $^{125}$I-labeled T and second antibody-coated tubes after extraction and column chromatography of samples (33). The minimum detectable level of T was 1.04 nmol/L; and intraassay CV's were, respectively, 9.4, 5.3, and 7.1% at concentrations of 3.61, 9.65, and 17.7

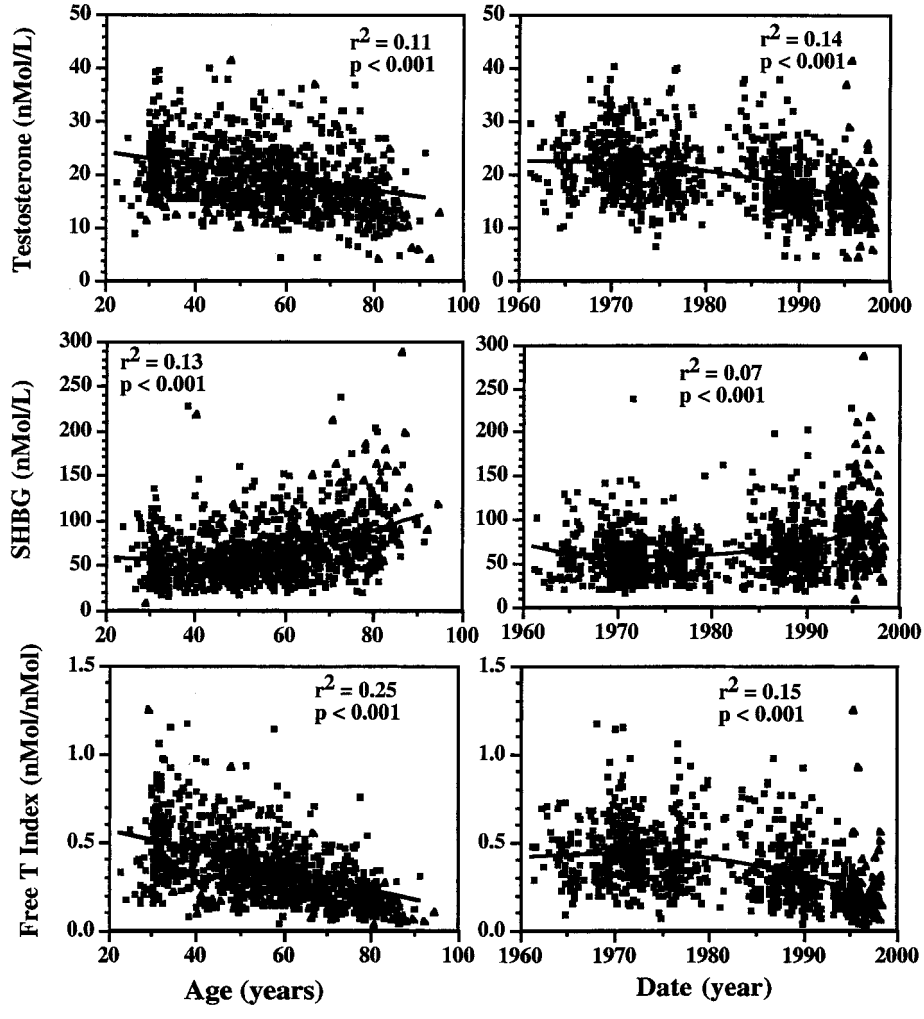


FIG. 1. Effects of age (*left panels*) and date (*right panels*) on serum T, SHBG, and free T index. Individual data points for T (*upper panel*), SHBG (*middle panel*), and free T index (*lower panel*) from the earliest available (usually first) visits during 1963–1994 are shown as *closed squares*, and the additional set of 120 serum samples from later visits (1995–1998) are plotted as *closed triangles*, against age (*left panels*) and sample date (*right panels*). Best-fit regression lines, $r^2$ and *P* values for the 1963–1994 data are shown. Total T concentrations and free T index values decreased linearly with increasing age, whereas SHBG exhibited a curvilinear increase with age, rising at a slightly greater rate in the older, than in younger, men. With progressively more recent sample date, total T concentrations fell, SHBG level was higher, and free T index decreased, all with significant curvilinear relationships. There were no apparent discontinuities between the original samples and the post-1994 samples assayed later.

ng/dL, with interassay CV's of 5.7, 2.8, and 7.0% at concentrations of 3.12, 8.84, and 16.1 nmol/L. By the new assay, T showed no significant correlation with visit date ($r = -0.088$, $P > 0.15$), whereas the T values derived from the original assays on the same 221 samples were inversely related to visit date ($r = -0.199$, $P < 0.01$). Using date intervals that divided the samples into equal numbers (1963.9–1986.8; 1986.8–1988.3; and 1988.3–1994.6), the mean T values from the repeat assay were significantly higher in each time period, compared with T values in the original assay [being, respectively, 18.5 ± 7.1 *vs.* 15.5 ± 5.7 ($P < 0.01$); 19.1 ± 6.2 *vs.* 14.1 ± 4.3 ($P < 0.001$); and 17.4 ± 6.1 *vs.* 13.0 ± 4.3 ($P < 0.001$); with a mean difference of 4.2 nmol/L, even after adjustment for date effects]. To detect evaporation of samples as a possible cause of higher T concentrations in the older samples, we determined serum $Na^+$ levels, by a routine laboratory method (automated spectrophotometry at Endocrine Sciences, Inc. Laboratories) in 191 of these same 221 samples. Sodium concentrations were elevated (>145 mmol/L) in only 12 samples distributed fairly evenly over the full range of dates, and low (<135 mmol/L) in 18 samples, with 11 of 18 values occurring in the oldest samples. There was no significant relationship between either sample date ($r^2 = 2 \times 10^{-4}$, $P > 0.8$) or serum T level ($r^2 = 0.003$, $P > 0.4$) and $Na^+$ concentration, by linear regression analysis. Finally, we added either pure T in charcoal-extracted female serum, in amounts sufficient to increase the T concentrations by about 6.9 nmol/L, or an equal amount of blank serum, to duplicate aliquots of 24 samples stored between 1968 and 1972 and to 24 samples from visits between 1988 and 1992. These matched aliquots were then coded and assayed blindly at Covance Laboratories, Inc. using the original reagents and method. This assay read an average increase of 9.0 ± 1.0 nmol/L in the older samples and 6.5 ± 1.2 nmol/L in the more recent samples, a 29% difference ($P < 0.001$). This discrepancy is of an order of magnitude similar to the difference in estimated T levels by date in the original assays and, along with the above findings, strongly suggests the presence of a date (*i.e.* storage time)-related assay artifact.

### *Statistical analysis*

The sample size consisted of 3651 samples analyzed for T and SHBG, after inclusion of the 120 newer samples used in assessing the confounds. Thirty of the FTI estimates were unreasonably high (>3.3 SD above the mean) and were excluded, producing a final sample size of 3621 FTI measurements. Descriptive statistics used to characterize the data were calculated using SPSS, Inc. Version 9 (SPSS, Inc., Chicago, IL). Cross-sectional analyses used simple and multiple regression to examine the effects of age and date. Best-fit regression lines are shown for each relationship (either simple linear plots or, where higher order equations significantly improved the $r^2$ values, curvilinear plots).

The longitudinal analyses were based on mixed effects models using the statistical package MLWIN (34) to examine the effect of age on T and free T index. We first corrected all values of T by adding 4.2 nmol/L to adjust for the constant and systematic underestimates, compared with the standard (extraction) assay. The model considered initial age, year, and elapsed time from first measurement in the prediction. The form of the equation was: $T = (b0+b0i)+b1 \times \text{first-age}+(b2+b2i) \times \text{time}+(b3+b3I) \times \text{time}^2+b4 \times \text{first-date}+b5 \times \text{first-date}^2+b6 \times \text{time} \times \text{first-age}+bj \times \text{covariatej}+\text{error}$ (where b0i, b2i, and b3i are random effects that reflect individual variation from the mean effect). To account for the above described effect of date on apparent T concentrations, date-adjusted estimates were developed by setting the date to 1995, the most recent date from which the original samples assayed were obtained. A similar approach was used to estimate free T index. The date effect we observed was systematic, so that adjustment of total T levels to a common date of 1995 resulted in a distribution in which mean T for men 20–45 was 17.6 nmol/L (508 ng/dL) and the 2.5th percentile (used by convention to define hypogonadism) was at 11.3 nmol/L (325 ng/dL). These values resemble those generally accepted for normal young adults (35, 36), suggesting that the adjustment was appropriate.

Based on the models, individual regression equations were developed for all men with two or more determinations (n = 782), and the equations were used to establish grouped average effects based on the age decade at first evaluation. Initial age, slopes, and time were calculated for each age decade group of men and were subsequently plotted. For analyses that examined the impact of covariates, the additional terms were added to the equations as fixed effects.

## Results

### *Characterization of study population*

Table 1 shows the means and ranges for the age, weight, height, and body mass index (BMI = $kg/m^2$) for the men at date of entry into this study. To investigate whether effects of medications, use of tobacco and alcohol, or chronic illness interacted with the those of aging *per se*, we classified men, with regard to each of these variables, by review of computerized visit records. At each visit, subjects were identified as to whether they were taking glucocorticoids, β-blockers, or psychotropic medications, all of which can alter reproductive hormone dynamics. Men were also classified as never, former, or current smokers and as users of less than or more than 2 oz of alcohol daily, and their illnesses were identified by review of diagnostic classifications in visit charts. Percentages of men in each classification are depicted in Table 1. The ages ranged from the third through the ninth decades. Men varied in body fatness from very lean to severely obese, with a mean BMI value in the overweight zone according to the new NHLBI and WHO classification scheme (37, 38). Of the medications classified, only β-blockers were used by a high-enough proportion of the population (~15%) to exert a significant effect. Men were about evenly divided between never and former smokers. The former smokers

**TABLE 1.** Cross-sectional description of men in sample at entry

| Continuous variables | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|
| Age (yr) | 53.8 | 16.0 | 22.5 | 91.3 |
| Weight (kg) | 80.2 | 12.3 | 53.3 | 152.1 |
| Height (cm) | 176.8 | 6.8 | 156.1 | 197.6 |
| BMI ($kg/m^2$) | 25.6 | 3.2 | 18.3 | 43.0 |

| Classification variables | Percentage | | Percentage |
|---|---|---|---|
| Medication usage during study period | | Diseases present during study period | |
| Glucocorticoids | 4.4 | Cancer | 9.4 |
| Beta blockers | 14.7 | Coronary disease | 20.3 |
| Psychiatric | 5.6 | Diabetes | 16.3 |
| Smoking | | Stroke | 4.9 |
| Never | 40.8 | Renal | 0.2 |
| Former | 41.3 | Liver | 0.4 |
| Current | 17.9 | Thyroid | 4.3 |
| Alcohol (>2 gm/day) | 6.1 | COPD | 12.8 |

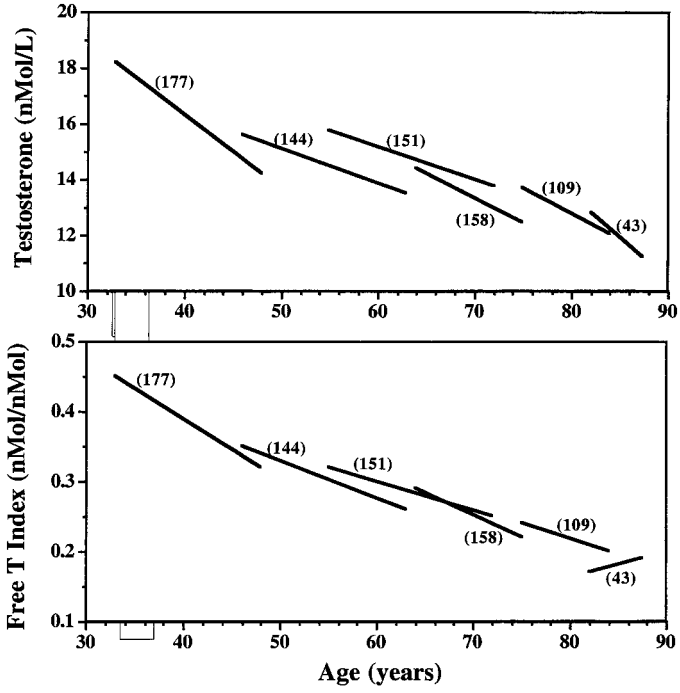


FIG. 2. Longitudinal effects of aging on date-adjusted T and free T index. Linear segment plots for total T and free T index *vs.* age are shown for men with T and SHBG values on at least two visits. Each linear segment has a slope equal to the mean of the individual longitudinal slopes in each decade, and is centered on the median age, for each cohort of men from the second to the ninth decade. *Numbers in parentheses* represent the number of men in each cohort. With the exception of free T index in the ninth decade, segments show significant downward progression at every age, with no significant change in slopes for T or free T index over the entire age range.

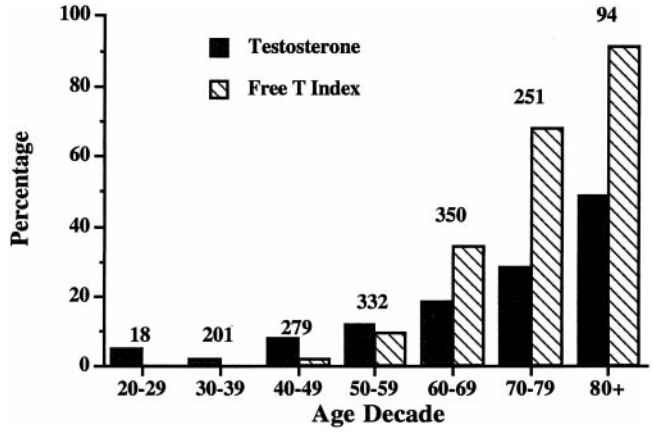


FIG. 3. Hypogonadism in aging men. *Bar height* indicates the percent of men in each 10-yr interval, from the third to the ninth decades, with at least one T value in the hypogonadal range, by the criteria of total T < 11.3 nmol/L (325 ng/dL) (*shaded bars*), or T/SHBG (free T index) < 0.153 nmol/nmol (*striped bars*). *Numbers above each pair of bars* indicate the number of men studied in the corresponding decade. The fraction of men who are hypogonadal increases progressively after age 50 by either criterion. More men are hypogonadal by free T index than by total T after age 50, and there seems to be a progressively greater difference, with increasing age, between the two criteria.

tended to be older than the other two smoking groups (56.0 yr for former smokers, 53.6 for nonsmokers, and 48.0 for current smokers). Relatively few men used excessive amounts (>2 ounces/day) of alcohol. The most common chronic diseases were coronary artery disease, diagnosed as either history of a clinical event (myocardial infarction, angina, heart failure) or definitely abnormal treadmill exercise testing, and diabetes mellitus, which is probably present at a relatively high rate in the BLSA, compared with the general population, because all BLSA volunteers undergo glucose tolerance testing, and thus the detection rate can be assumed to be nearly 100%. Cancer (other than nonserious skin cancers) and chronic obstructive pulmonary disease (COPD) were present in significant numbers of subjects as well. Each of the other chronic illnesses classified occurred in less than 5% of the population studied.

*Cross-sectional analysis of T, SHBG, and free T index*

Figure 1 illustrates values, from the earliest available (usually first) visit for each man, for the total T concentrations, SHBG levels, and free T indices (calculated as total T/SHBG) plotted against age of the subjects and against the date on which the sample was obtained. Total T concentrations and free T index values decreased linearly with age, whereas SHBG exhibited a curvilinear relationship with age, increasing at a slightly greater rate in the older than in younger men. Total T, SHBG, and free T index all showed significant curvilinear relationships with sample date as well.

*Longitudinal analysis*

Figure 2 illustrates linear segment plots for date-adjusted total T and free T index *vs.* age, based on samples from all men with sera available for at least two visits (n = 782). Using mixed effects models to predict individual equations, we plotted segments that represent the longitudinal trend (mean slope) for cohorts of men, in each decade from the 30's to the 80's. These segments show significant downward progression at every age, with no significant differences among slopes for T or the free T index. The apparent exception was the slope of free T index for the relatively small number (n = 43) of men in their 80's, which was not significantly negative or positive and had a large SE. The earliest point of each segment was within acceptable limits, relative to the point at the corresponding age for men in the previous decade, to exclude significant discontinuities. Thus, there was no evidence of secular effects on these date-adjusted measures.

*Multivariate analysis*

A mixed-effects model analysis (Table 2) confirmed independent longitudinal effects of age on both T and free T index, with respective coefficients of −0.110 nmol/L·yr and −0.005 nmol T/nmol SHBG·yr (Z = 14.5 and 14.2, $P < 0001$). Date and date$^2$ were also significant contributors to estimates of T, requiring adjustment of T values to a common date, as noted above. In this analysis, T decreased (−0.350 nmol/L·kg·m$^2$) with increasing BMI, independent of age, whereas there was a small (0.003 nmol/L·kg·m$^2$), but significant, increase in the free T index with increasing BMI. In this population, smoking did not contribute significantly to either T levels or the free T index, whereas alcohol consumption decreased the free T index. Of the medications studied, only

**TABLE 2.** Coefficients and their SD's for mixed effects models with testosterone or free testosterone index as dependent variables (only significant, $P < 0.05$, coefficients are shown)

| Independent Variable | Testosterone | | Free testosterone index | |
|---|---|---|---|---|
| | Coefficient | SD | Coefficient | SD |
| Constant | 15.8 | (0.26)[a] | 0.1890 | (0.026)[a] |
| Initial age deviation from mean initial age | −0.110 | (0.01) | −0.0050 | (0.000) |
| Date deviation from 1995 | −0.510 | (0.03) | −0.0180 | (0.001) |
| Date$^2$ deviation from 1995 | −0.010 | (0.001) | −0.0004 | (0.0000) |
| Time | −0.170 | (0.03)[a] | −0.0050 | (0.000)[a] |
| Time × Time | 0.006 | (0.001)[a] | | |
| Time × Initial Age deviation | −0.002 | (0.001) | | |
| BMI deviation from mean BMI | −0.350 | (0.03) | 0.003 | (0.001) |
| Alcohol | | | −0.023 | (0.011) |
| Smoking | | | | |
| Psychiatric medications | | | | |
| Glucocorticoids | | | | |
| Beta blockers | | | 0.0290 | (0.001) |
| Renal disease | | | | |
| Liver disease | | | | |
| Thyroid disease | | | | |
| COPD | | | | |
| Cancer | −0.0920 | (0.01) | | |
| Diabetes | | | | |
| Stroke | | | | |
| Coronary heart disease | | | | |

[a] Significant random effect.

β-blockers had an apparent effect, with users having higher free T indices than nonusers. Of the disease categories examined, only cancer showed an influence, lowering total T but not the free T index.

*Occurrence of hypogonadism*

We used date-adjusted T and free T index values to calculate the percentages of men in each decade who were hypogonadal, defined as having at least one visit in that age decade at which T was less than 11.3 nmol/L (325 ng/dL) or the free T index was less than 0.153 nmol/nmol (the 2.5th percentile values, defined as: the mean values − 1.96 SD, for men 21–45 yr of age in our study). As shown in Fig. 3, there were progressive increases in the prevalence of hypogonadism, defined by either set of criteria, from relatively low levels for men less than 49 yr of age to 12%, 19%, 28%, and 49% (by total T) or to 9%, 34%, 68%, and 91% (by free T index) in men in their 50's, 60's, 70's, and 80's, respectively. Using the free T index, the percentage of men classified as hypogonadal tended to be lower under age 50 and higher above age 50 than by the total T criterion. Further analysis showed that 78% of the men identified as hypogonadal by a single total T determination, who had subsequent samples evaluated, had low total T levels in all subsequent (mean, 2.6) visits. This percentage was even higher (97%) for men identified as hypogonadal by free T index.

## Discussion

Most (1, 5–9), but not all (10–12), cross-sectional studies have demonstrated a decrease, with age, in total T in men. To date, there have been only two longitudinal studies reported, each of which showed decreases in total T in, respectively, 66 men, 41–61 yr old, followed for 13 yr (16) and 77 men, 61–87 yr old, followed for 15 yr (17). In the present study in 890 generally healthy, middle class, American men in the BLSA, we found that both T and free T index (a calculated value related to free or bioavailable T) decreased progressively at a rate that did not vary significantly with age, from the third to the ninth decades. The magnitude of the decrease in total T averaged 0.110 nmol/L (3.2 ng/dL) per year, was similar whether examined cross-sectionally or longitudinally, and was comparable to the 0.121 nmol/L (3.5 ng/dL) per year reported by Zmuda *et al.* in 66 men, 41–61 yr old, followed for 13 yr (16) but less than the rate of 0.382 nmol/L (11 ng/dL) per year observed in 77 men, 61–87 yr old, followed for 15 yr by Morley *et al.* (17). The decrease in free T index was somewhat steeper than that of total T, owing to a trend for an increase in SHBG with age. These latter results are also consistent with findings in earlier investigations (2–4, 9, 10).

Cohort and secular effects are two potential confounds of cross-sectional studies of aging (15). A cohort effect is one that occurs when individuals of similar age vary significantly from older and/or younger groups, in one or more measured parameters, because of a historical factor common to their generation. A secular effect is one that results when there is progressive alteration of a critical condition in the environment, producing the appearance of a change with age. Our study contained an apparent secular effect that was probably factitious, *i.e.* systematically higher T levels at every age in samples obtained less recently, leading to an apparent decrease in serum T of about 40% from the 1970's to the 1990's. Such an effect could have occurred because of a change in environmental exposure of our study cohort, over time, such as an increase in estrogenic substances in food and/or water, which has been suspected to cause a decrease in sperm counts and a higher incidence of male infertility (39, 40). However, careful evaluation suggested a progressive alteration in the frozen samples, with time in storage, leading to a systematic upward variation in assay estimates of total T.

The latter change may have been attributable to the observed decrease, with increasing storage time, in levels of SHBG. This protein is stable in short-term frozen storage (41) but was progressively depleted in specimens stored for years in our study. If the antibody we employed in the T RIA competed incompletely with SHBG for binding of T, samples with higher SHBG levels would have lower apparent total T estimates. Our finding, that known additions of pure T to older samples assayed as about 30% greater, compared with identical additions to more recent samples, is consistent with such a mechanism.

Another potential confound, common to both cross-sectional and longitudinal studies, is a progressive effect of age-related variables, such as chronic illness or cumulative exposure to alcohol or tobacco, which are independent of the aging process itself (8, 14, 39). In our study, adjustment for illness, medications, and smoking produced little change in the overall effect observed because of aging *per se*. Of the chronic illnesses identified in our study population, only cancer, present (at any time during the period of follow-up) in 9%, was associated with a greater decrease in T levels than occurred with aging alone. This is consistent with our prior observations in men with cancer (14) and accords with other studies suggesting that serious chronic illness is associated with decreased T secretion in men (8, 13). The present finding that coronary artery disease had little or no impact on serum T levels is consistent with our previous prospective study in BLSA men (42), but it contrasts with other studies in which coronary heart disease and/or coronary risk factors have been associated with diminished T levels (43) or a greater rate of decline in serum T over time (16).

We found no significant effects of current smoking on T and free T index. In a previous large cross-sectional study, smokers were found to have somewhat higher levels of total T (8). Moreover, excessive alcohol consumption is a well-known cause of male hypogonadism (12). The apparent lack of effect of smoking and the relatively small alcohol effect on free T index were probably attributable to the relatively small number of smokers and, possibly, to the lighter use of both tobacco and alcohol in this health-conscious population. The use of β-blocking agents was associated with a trend to somewhat higher levels of T and free T index. Whether this association was caused by an effect of the drugs themselves, or some characteristic of men more likely to be prescribed these drugs, is currently unknown. Hypertensive men treated with β-blocking agents often experience sexual dysfunction and have mild reductions in serum T levels (44, 45). Although the mechanism(s) by which β-blocking agents might increase serum T is unknown, propranolol or nadolol have been reported to prevent melatonin-induced short photoperiod gonadal regression in male hamsters (46).

Our finding that total T, but not free T index, tended to decrease with greater BMI is consistent with prior studies showing that obesity is associated with decreases in both SHBG and total T, with an unchanged T-to-SHBG ratio (47), but contrasts with other studies showing diminished free, as well as total, T in with increasing total (48) or abdominal (49) obesity in men.

Our analysis of date-adjusted T and free T index levels, by decade, showed that relatively high numbers of older men in this generally healthy population had at least one hypogonadal value (defined as below the 2.5th percentile for young men). By the free T index criterion, this fraction rose from nearly 35% of men in their 60's to approximately 90% of men in their 80's. Using a total T criterion, the rates of hypogonadism were still significant but somewhat less dramatic. The issue of how properly to define hypogonadism, or indeed any hormone deficiency, remains problematic. The conventional definition for T levels is statistical (values more than 2 SD below the mean), rather than functional. Such a definition does not reflect clinical realities, such as the existence of characteristic individual set points for circulating hormone levels, below which one, but not another, individual may develop metabolic changes of hormone deficiency; nor does it address the concept of reserve capacity, the possibility that persons with hormone levels 2 SD below the population mean still may have adequate hormone concentrations to meet their metabolic needs. Using a dependent indicator hormone (*e.g.* LH for gonadal function) is helpful, to some extent, but is also potentially circular (*i.e.* the issue becomes that of determining what is the upper limit of normal for an LH level). It would clearly be better to define the lower limit of normal for a hormone as: the blood level at which metabolic and/or clinical sequelae of hormone deficiency begin to appear, or the level below which definite benefits can be demonstrated for hormone supplementation for a significant proportion of the population. However, insufficient data exist to do this with a high degree of confidence for T. The recent observation that elderly men with T levels less than 200 ng/dL at baseline had significant improvements in BMD after 3 yr of T treatment, whereas men with higher T levels did not (50), is a good beginning, but more information is needed. Higher bioavailable (but not total) T levels have been associated with greater BMD (51, 52) in men and are inversely associated with fat mass (53) in older men. Free or total T level is also a significant independent predictor of lean body mass and muscle strength (53, 54). These findings highlight the importance of determining the extent to which the age-related decrease in T is associated with changes common to pathological hypogonadism (29, 30) and the so-called aging phenotype, including increased total and central body fat (25–28), decreased lean body and skeletal muscle mass and strength (20–23), bone calcium depletion (18, 19, 55), and the metabolic and clinical consequences of these alterations, as well as the increase in erectile dysfunction and loss of libido (56).

Given the likelihood that age-related sarcopenia contributes importantly to frailty, (57) and the importance of osteoporotic fractures as a cause of morbidity and mortality in elderly men (58–60), T replacement is a potentially useful strategy for reducing age-associated disabilities in some aged men (61). Three months of T enanthate injections increased lean body mass in men over 60 yr of age (62); T treatment improved hamstring and quadricep muscle strength, after 4 weeks, in healthy older men (63); and administration of T for 2 yr produced a gain in bilateral grip strength in elderly hypogonadal men (64). In contrast, 3 yr of T replacement, using transdermal patches, failed to improve muscle strength in men more than 65 yr of age, despite significant increases in lean body mass (65).

To our knowledge, the current study is the largest longitudinal evaluation of the effects of normal aging on male gonadal hormone function reported to date. Results strongly support the concept of an effect of aging to lower both total and bioavailable circulating T levels at a relatively constant rate, independent of obesity, illness, medications, cigarette smoking, or alcohol intake. In addition, our findings suggest that a significant proportion of men over 60 yr of age have circulating T concentrations in the range conventionally considered to be hypogonadal. Whether such men may benefit from T replacement therapy deserves further investigation.

## Acknowledgments

We gratefully acknowledge the expert nursing and administrative assistance of the BLSA staff, especially Mr. Howard Baldwin; Mr. Norman Beaudry of Covance Laboratories, Inc. for performance of T and SHBG RIAs; and Drs. Reubin Andres and Adrian Dobs for constructive and critical reviews of this manuscript.

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