0021-972X/00/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 85, No. 9
Printed in U.S.A.

Measures of Bioavailable Serum Testosterone and Estradiol and Their Relationships with Muscle Strength, Bone Density, and Body Composition in Elderly Men*

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ABSTRACT

In the present cross-sectional study of 403 independently living elderly men, we tested the hypothesis that the decreases in bone mass, body composition, and muscle strength with age are related to the fall in circulating endogenous testosterone (T) and estrogen concentrations. We compared various measures of the level of bioactive androgen and estrogen to which tissues are exposed.

After exclusion of subjects with severe mobility problems and signs of dementia, 403 healthy men (age, 73–94 yr) were randomly selected from a population-based sample. Total T (TT), free T (FT), estrone (E_1), estradiol (E_2), and sex hormone-binding globulin (SHBG) were determined by RIA. Levels of non-SHBG-bound T (non-SHBG-T), FT (calc-FT), the TT/SHBG ratio, non-SHBG-bound E_2, and free E_2 were calculated. Physical characteristics of aging included muscle strength measured using dynamometry, total body bone mineral density (BMD), hip BMD, and body composition, including lean mass and fat mass, measured by dual-energy x-ray absorptiometry.

In this population of healthy elderly men, calc-FT, non-SHBG-T, E_1, and E_2 (total, free, and non-SHBG bound) decreased significantly with age. T (total and non-SHBG-T) was positively related with muscle strength and total body BMD (for non-SHBG-T, respectively, $\beta = 1.93 \pm 0.52$, $P < 0.001$ and $\beta = 0.011 \pm 0.002$, $P < 0.001$). An inverse association existed between T and fat mass ($\beta = -0.53 \pm 0.15$, $P < 0.001$). Non-SHBG-T and calc-FT were more strongly related to muscle strength, BMD, and fat mass than TT and were also significantly related to hip BMD. E_1 and E_2 were both positively, independently associated with BMD (for E_2, $\beta = 0.21 \pm 0.08$, $P < 0.01$). Non-SHBG-bound E_2 was slightly strongly related to BMD than total E_2. The positive relation between T and BMD was independent of E_2. E_1 and E_2 were not related with muscle strength or body composition.

In summary, bioavailable T, E_1, total E_2, and bioavailable E_2 all decrease with age in healthy old men. In this cross-sectional study in healthy elderly men, non-SHBG-bound T seems to be the best parameter for serum levels of bioactive T, which seems to play a direct role in the various physiological changes that occur during aging. A positive relation with muscle strength and BMD and a negative relation with fat mass was found. In addition, both serum E_1 and E_2 seem to play a role in the age-related bone loss in elderly men, although the cross-sectional nature of the study precludes a definitive conclusion. Non-SHBG-bound E_2 seems to be the best parameter of serum bioactive E_2 in describing its positive relation with BMD. (*J Clin Endocrinol Metab* **85:** 3276–3282, 2000)

THROUGHOUT ADULT life, all physiological functions gradually decline. In men, part of these age-related physiological changes (loss of muscle size and strength, loss of bone, and increase in fat mass) may be related to the decrease in serum levels in bioavailable testosterone (T) with aging (1, 2). Some studies report a positive relation between estimates of serum T levels and bone density in older men (1, 3, 4), although this has not been a universal finding (5, 6). Androgen administration to older men with low plasma T levels results in increases in lean body mass, bone density, and/or muscle strength (7–10).

Recently it has been suggested that estrogens may play an important role in the development and maintenance of the male skeleton (11). Several studies have reported positive relations between serum estradiol (E_2) concentrations and bone mineral density (BMD) or bone turnover markers in men (12).

Serum T as well as E_2 are mainly bound to sex-hormone binding globulin (SHBG) and albumin. It has been suggested that the fraction of T bound to albumin has access to target tissues (13). It remains to be established whether total, non-SHBG-bound (albumin bound and free) or free T (FT) levels are the best representation of the bioactive hormone concentrations. The same holds true for serum E_2 concentrations.

Therefore, in the present cross-sectional study of 403 independently living elderly men, we tested the hypothesis that the decreases in bone mass, body composition, and muscle strength with age are related to the fall in circulating endogenous T and estrogen concentrations. We compared various measures of the level of bioactive androgen to which tissues are exposed: total T (TT) and FT levels in serum, the TT/SHBG ratio, calculated free and non-SHBG-bound T levels and TT adjusted for SHBG. In addition, relationships with total, non-SHBG-bound, and free estradiol (fE_2) were compared.

Received July 21, 1999. Revision received December 29, 1999. Rerevision received May 15, 2000. Accepted June 5, 2000.

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* Supported by Schering Ltd. Berlin.

Subjects and Methods

Subjects

The study is a cross-sectional, single-center study in 403 independently living men, 70 yr of age and higher. Names and addresses of all male inhabitants 70 yr and older were drawn from the municipal register

of Zoetermeer, a medium sized town in the midwestern part of The Netherlands. A total of 1567 men were invited, and after exclusion of subjects who did not live independently and subjects who were not physically or mentally able to visit the study center independently, eventually 403 men participated (25.7%). A total of 886 men did not respond to the mailed invitation in which it was already mentioned that subjects who did not live independently or with severe mobility problems would not be allowed to participate. The main reason not to participate among the respondents was because they were already being seen by a specialist or general practitioner at the moment (28%), whereas 16% were excluded on the basis of physical (10%) or mental (6%) problems. Participants signed an informed consent. The study has been approved by the Medical Ethics Committee of the Erasmus University Hospital Rotterdam. No additional health-related eligibility criteria were used. A number of participants were taking medications for chronic illnesses, like hypertension (n = 96) and mild congestive heart failure (n = 28). However, none of these medications, in retrospect, influenced the relations described in this study. Some of the illnesses, for example mild knee pain (n = 79), influenced the physical characteristics measured, but, in retrospect, they did not change the relations between the physical characteristics nor between the circulating hormone levels and the physical characteristics reported in this study.

Hormone measurements

Blood samples were collected in the morning after an overnight fast. The period of storage at −40C varied from 0–5 months. Serum concentrations of TT (nmol/L), FT (nmol/L), and SHBG (nmol/L) were all measured by RIA using commercial kits (Diagnostic Systems Laboratories, Webster, TX). The intra assay coefficients of variation (CV) for these assays were 8.1%, 6.2%, and 3.0%, respectively. The interassay CV were 10.5%, 9.7%, and 4.4%. The FT RIA uses an [I-^{125}]-labeled T analog, which has a low affinity for SHBG and albumin. This analog competes with the unbound T in the test sample for binding to specific anti-T polyclonal antibodies that have been immobilized on the assay tube. This competitive binding format allows direct estimation of unlabeled FT levels in unextracted samples. Furthermore, as measures of biologically active T, the TT/SHBG ratios were calculated, as well as FT (calc-FT, nmol/L) and non-SHBG-bound T (non-SHBG-T, nmol/L, is calc-FT plus albumin-bound T) (Table 1) (14). In these calculations, the possible binding of other steroids to SHBG was disregarded. Finally, TT adjusted for SHBG in a multiple regression analysis was used as a measure of non-SHBG-bound T.

Serum concentrations of estrone (E_1; nmol/L) and E_2 (nmol/L) were also measured by RIA using commercial kits (Diagnostic Systems Laboratories). The intra-assay CV were, respectively, 5.6% and 5.3%. The interassay CV were, respectively, 10.2% and 8.1%. As measures of biologically active E_2, fE_2, and non-SHBG-bound E_2 were calculated according to the method described by Södergård *et al.* (14), taking the concentration of T into account. Albumin (g/L) was measured by photometry using a commercial kit (ALB; Boehringer, Mannheim, Germany).

Physical characteristics of aging

Muscle strength. Isometric grip strength (IGS) was measured using an adjustable hand held dynamometer (JAMAR dynamometer) at the nondominant hand (15). Each test was repeated three times, and the average was used in the analyses. Leg or knee extensor strength (LES) was measured as described previously (16, 17), using the Hoggan MicroFET hand-held dynamometer. To obtain one main outcome measurement for LES, "maximum LES" (maxLES) was defined as the maximum strength for the right or left leg, whichever is largest, in a position of 120-degree extension. Statistical analyses were based on the physical unit momentum (Nm), obtained by multiplying the maximum strength (in Newton) and the distance of the dynamometer to the knee joint (in meters).

BMD and body composition

Total body BMD (TBBMD) was measured using dual-energy x-ray absorptiometry (Lunar, Madison, WI), as were hip BMDs at the femoral neck, trochanter, and Ward's triangle (18). In addition, total and trunk lean body mass and fat mass were measured (19, 20). Quality assurance for dual-energy x-ray absorptiometry, including calibration, was performed every morning, using the standard provided by the manufacturer.

Height and weight were measured in standing position without shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. The waist circumference was measured at the level of the umbilicus, and the hip circumference was measured at the level of the greater trochanter. The average of two readings was used in the analyses. Waist to hip ratio, which represents a measure of upper body adiposity, was calculated from these two measurements.

Data analyses. Results were expressed, unless otherwise stated, as mean and SD with the interquartile range. Comparisons between groups were made by using Student's *t* test. Differences were given together with the 95% confidence interval. Relations between variables were assessed using linear regression for continuous variables and logistic regression for binary variables and described as the linear regression coefficient (β) and its SE. Multiple regression analysis was used to adjust for age and BMI, as well as to assess the contribution of different independent variables to the dependent variable. To assess the contribution of different variables on the dependent parameter, we used standardized regression, described as the standardized regression coefficient (B). Bs are regression coefficients normalized by the ratio of the SD of the dependent variable. Correlations between variables were assessed by

TABLE 1. Measurements and calculations for the various concentrations of T and E_2

Steroid variables	Measurements and calculations
TT (nmol/L)	Measured by RIA (Diagnostic Systems Laboratories-4100, Webster, TX)
FT (nmol/L)	Measured by RIA (Diagnostic Systems Laboratories-4900)
calc-FT (nmol/L)	According to Södegård *et al.* (14): calc-FT = $[-b + \sqrt{(b^2 + 4a[\Sigma TT])}]/2a$ $a = K^A + K^T + (K^A \times K^T)$ ([SHBG] + [albumin] − [ΣTT]) $b = 1 + K^T$[SHBG] + K^A[albumin] − $(K^A + K^T)$[ΣTT]
Non-SHBG-T (nmol/L)	According to Södegård *et al.* (14): non-SHBG-T = $(K^A \times$ [albumin] × [calc-FT])/(1 + $K^A \times$ [calc-FT]) + [calc-FT]
TT/SHBG ratio	Measured TT/measured SHBG ratio
TT adjusted SHBG, nmol/L	Measured TT Adjusted for measured SHBG in a multiple regression analysis
E_2 (nmol/L)	Measured by RIA (Diagnostic Systems Laboratories-4400)
fE_2 (nmol/L)	According to Södegård *et al.* (14): $fE2 = [-b - \sqrt{(b^2-4ac)}]/2a$ $a = (K^A \times$ [albumin] + 1)(K^E) b = ([ΣE2] × K^E) − ($K^{AE} \times$ [albumin] + 1)(1 + K^T[SHBG] × calc-FT) − ($K^E \times$ [SHBG]) c = [ΣE2] × (1 + K^T[SHBG] × calc-FT)
Non-SHBG-E_2 (nmol/L)	According to Södegård *et al.* (14): non-SHBG-E_2 = [ΣE2] − ($K^E \times$ [SHBG] × fE2/(1 + $K^E \times$ fE2 + K^T[SHBG] × calc-FT)

K^A, Association constant for binding of T to albumin; K^T, association constant for binding of T to SHBG; K^{AE}, association constant for binding of E_2 to albumin; K^E, association constant for binding of E_2 to SHBG.

using Pearson's correlation coefficient *r*. All analyses were done using STATA Statistical Software, Release 5.0 (Stata Corporation, Texas).

Results

Descriptive data of the physical characteristics, as well as age and BMI are shown in Table 2. Muscle strength, BMD, lean body mass, and fat mass all decreased with age in our study group (Table 3). Because virtually all parameters were related with age, all further analyses were done after adjustment for age. Descriptive data of the hormone measurements are shown in Table 4.

Relations of the hormone concentrations with age

The relations of the hormone measurements with age are shown in Table 5. In our population, all T measurements, except TT, significantly decreased with age. The relation between non-SHBG-bound T and age is illustrated in Fig. 1. Serum SHBG and percentages of SHBG-bound T and E_2 increased with age. E_1 and total E_2 concentrations were inversely related with age. Calculated fE_2 and calculated non-SHBG-bound E_2 (non-SHBG-E_2) decreased relatively more with age compared with total E_2 (Bs were, respectively, -0.15, -0.15, and -0.12).

Relations between physical characteristics

Lean mass, fat mass, and maxLES were all positively, independently related with TBBMD [respectively, $\beta = 0.0060 \pm 0.0008$ kg/(g/cm^2), $P < 0.001$; $\beta = 0.0063 \pm 0.0008$ kg/(g/cm^2), $P < 0.001$; and $\beta = 0.0011 \pm 0.0002$ Nm/(g/cm^2), $P < 0.001$]. Lean mass and maxLES were both positively associated ($\beta = 0.12 \pm 0.01$ Nm/kg, $P < 0.001$).

Relations between hormone concentrations

Mean values of measured FT were significantly lower than calc-FT (difference 0.16 ± 0.003 nmol/L, confidence interval = 0.156–0.166, $P < 0.001$). Results of the different T measurements correlated well with each other (Table 6). Correlations between serum levels of T, E_1, and E_2 are shown in Table 7. E_1 and E_2 were positively related with each other, and with the various measures of T.

Relation between T concentrations and physical characteristics

Because BMI was significantly related to levels of T, maxLES, IGS, and BMD, all analyses including these parameters were done after adjustment for BMI.

TABLE 2. Descriptive data of the study population

	Mean	±SD	Interquartile range	
Age (yr)	77.8	3.58	75	80
BMI	25.5	3.0	23.3	27.3
IGS (kg)	34.3	6.9	30.0	38.7
maxLES (Nm)	103.2	20.9	89.43	117.12
Total fat mass (kg)	21.2	6.4	17.4	24.5
Trunk fat mass (kg)	10.6	2.6	9.0	12.4
Total lean mass (kg)	51.7	5.6	47.8	55.5
TBBMD (g/cm^2)	1.17	0.10	1.11	1.23
Femoral neck BMD (g/cm^2)	0.88	0.14	0.78	0.97
Femoral ward BMD (g/cm^2)	0.72	0.16	0.60	0.82
Femoral trochanter BMD (g/cm^2)	0.85	0.15	0.76	0.94

TABLE 3. Relationship between physical characteristics and age

	Age (yr)		
	β	±SE	*P*
IGS (kg)	−0.53	0.09	<0.001
maxLES (Nm)	−1.30	0.28	<0.001
Total fat mass (kg)	−0.18	0.08	0.02
Trunk fat mass (kg)	−0.10	0.04	0.007
Total lean mass (kg)	−0.38	0.08	<0.001
TBBMD (g/cm^2)	−0.003	0.001	0.006
Femoral neck BMD (g/cm^2)	−0.002	0.002	0.25
Femoral ward BMD (g/cm^2)	−0.003	0.002	0.17
Femoral trochanter BMD (g/cm^2)	−0.004	0.002	0.04

TABLE 4. Summarized values of hormone levels in serum from 403 men with ages between 73 and 94 yr

	Mean ± SD		Interquartile range	
TT (nmol/L)	8.83	2.98	7.19	10.66
FT (nmol/L)	0.03	0.01	0.024	0.038
SHBG (nmol/L)	31.5	14.4	23.18	37.46
Calculated FT (nmol/L)	0.19	0.06	0.16	0.23
Non-SHBG-T (nmol/L)	5.68	1.89	4.62	6.76
Percentage SHBG-bound T (%)	35.0	9.51	29.2	41.1
Percentage albumin-bound T (%)	62.8	9.23	56.9	68.6
Percentage free T (%)	2.21	0.31	2.03	2.39
TT/SHBG ratio	0.32	0.15	0.23	0.39
E_1 (nmol/L)	0.102	0.039	0.075	0.126
E_2 (nmol/L)	0.098	0.058	0.059	0.126
Calculated fE_2 (pmol/L)	2.5	1.4	1.5	3.2
Non-SHBG-E_2 (nmol/L)	0.077	0.045	0.045	0.100
Percentage SHBG-bound E_2 (%)	21.9	7.40	17.2	26.1
Percentage albumin-bound E_2 (%)	75.5	7.21	71.4	80.2
Percentage fE_2 (%)	2.56	0.24	2.42	2.72

All T measurements were positively related to TBBMD after adjustment for age (Table 8). Calc-FT, non-SHBG-T, the TT/SHBG ratio, and TT adjusted for SHBG were also significantly positively related to all regions of proximal femur BMD. As an example, the relationships between the different T measures and BMD of the Ward's triangle are shown in Table 8.

None of the T measurements, with the exception of the TT/SHBG ratio, was related with lean mass (data not shown). Fat mass was negatively associated with all T measurements, except with the TT/SHBG ratio (Table 8).

All measurements for T were positively related with maxLES (Table 8) and with IGS and after adjustment for age (with the exception that FT was not related with IGS). T was independently related to both muscle strength and BMD.

The SHBG-bound fraction of T was not related to any of the physical characteristics, with the exception of fat mass ($\beta = -1.03 \pm 0.18$, $P < 0.001$). The strength of the relations of the albumin-bound fraction of T to the physical characteristics was equal to those for non-SHBG-T.

Serum SHBG concentrations were inversely related to lean mass [$\beta = -0.07 \pm 0.02$ kg/(nmol/L), $P < 0.001$], fat mass [$\beta = -0.08 \pm 0.02$ kg/(nmol/L), $P < 0.001$], and BMD [*e.g.* TBBMD; $\beta = -0.001 \pm 0.0003$ (g/cm^2)/(nmol/L), $P = 0.01$].

Relations between estrogens and physical characteristics

Serum E_1 and E_2 concentrations were not related to muscle strength. In this elderly male population, serum E_1 levels

TABLE 5. Relationship between age and hormone levels

	Age (yr)		
	β	±SE	P
TT (nmol/L)	−0.04	0.04	0.37
FT (nmol/L)	−0.001	0.0001	<0.001
SHBG (nmol/L)	0.92	0.20	<0.001
Calculated FT (nmol/L)	−0.002	0.001	0.02
Non-SHBG-T (nmol/L)	−0.07	0.03	0.01
Percentage SHBG-bound T (%)	0.66	0.13	<0.001
Percentage albumin-bound T (%)	−0.64	0.12	<0.001
Percentage fT (%)	−0.029	0.004	<0.001
TT/SHBG ratio	−0.009	0.002	<0.001
E_1 (nmol/L)	−0.005	0.0005	<0.001
E_2 (nmol/L)	−0.002	0.0008	0.01
Calculated fE_2 (pmol/L)	−0.060	0.020	0.003
Non-SHBG-E_2 (nmol/L)	−0.002	0.001	0.002
Percentage SHBG-bound E_2 (%)	0.51	0.10	<0.001
Percentage albumin-bound E_2 (%)	−0.50	0.10	<0.001
Percentage fE_2 (%)	−0.013	0.003	<0.001

β, Coefficient of linear regression represents changes in unit per year (*e.g.* non-SHBG-T decreases 0.07 nmol/L per year).

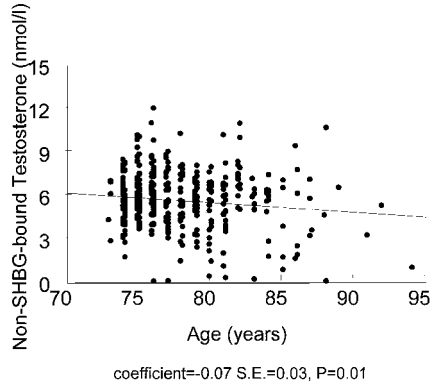

FIG. 1. Relationship between non-SHBG-bound T and age. Coefficient denotes the linear regression coefficient. F.e. non-SHBG-bound T decreases 0.07 nmol/L per year.

were positively associated with TBBMD [$\beta = 0.36 \pm 0.12$ $(g/cm^2)/(nmol/L)$, $P = 0.004$, age adjusted]. Serum E_2 concentrations (total, free, and non-SHBG bound) were positively related to TBBMD as well as to hip BMD (Table 9). Serum E_1 contributed slightly more to the variation in TBBMD, as shown by the Bs (respectively, 0.10 for E_2 and 0.13 for E_1). To investigate whether the above described effect of T on BMD might be mediated through its aromatization to estrogens, a multiple regression analysis was performed, including BMD as the dependent variable and E_2 and T as the independent variables. E_2 and TT remained independently related to TBBMD and contributed equally to the variation of TBBMD, as shown by the Bs (both 0.11). Again, serum E_1 contributed to a slightly greater variation in TBBMD compared with TT (Bs, respectively, 0.12 and 0.10). Furthermore, with regard to hip BMD, E_2 and non-SHBG-T remained independently, significantly related to BMD. Similar results were obtained when non-SHBG-E_2 was the independent variable.

Measures of E_1 and E_2 were not related to body composition nor to lean mass, total or trunk fat mass. Nor were they related to fat mass after adjustment for T. A weak positive association was found between E_1 and waist to hip ratio [$\beta = 0.13 \pm 0.07/(nmol/L)$, $P = 0.05$].

Discussion

In this population, circulating non-SHBG-bound T and FT, E_1, and E_2 (total, free, and non-SHBG-bound) levels all declined with age. Serum TT did not change with age, whereas the SHBG-bound fraction of both T and E_2 increased with age. Serum concentrations of the non-SHBG-T were most strongly related to several physical characteristics of aging; positively with muscle strength and BMD, and inversely with fat mass. Serum E_1 and E_2 concentrations were positively associated with BMD, but not with muscle strength. Free and non-SHBG-bound E_2 were more strongly related to BMD than total E_2.

Serum T concentrations as found in this study seem to be relatively low. Furthermore, calc-FT concentrations were significantly higher than measured FT. It has to be questioned whether the FT assay provides a good method to measure the biologically active fraction of circulating T. Our findings that FT (both measured and calculated), non-SHBG-T, and the TT/SHBG ratio decrease with increasing age are in agreement with previous studies (2, 21, 22). TT levels did not decrease with age, but TT levels in this population were below the normal range for middle-aged adults, suggesting that T levels had already declined in comparison with younger men. To our knowledge, no reference values for percentage of SHBG bound, albumin bound and free fractions of T exist in the elderly. The decrease of bioavailable T is probably due to the increase of SHBG-bound T with age, in combination with the decrease of the albumin-bound fraction to a similar extent.

Until recently, not much attention has been paid to the role of estrogens in elderly men. The studies that have been reported so far show no change of total E_2 levels with age in men (23–25) or a decrease of E_2 levels only at old age (26). In the present study, serum total E_2 was inversely associated with age. Bioavailable and fE_2 concentrations decreased with age to an even greater extent than total E_2. Two earlier studies also reported a decrease of bioavailable E_2 (12, 25). We studied, however, an older and larger population compared with these studies. As with T, the SHBG-bound fraction of serum E_2 increased with age, whereas the albumin fraction decreased. In addition, serum E_1 concentrations decreased with age.

A number of clinical problems prevalent in older men may be related to androgen deficiency, including reduced muscle strength (27, 28), changes in body composition, and loss of BMD (1, 3, 4). It should be of interest to know whether the decrease in bone mass with age is causally related with the decrease in lean mass and/or in serum T or E_2 concentrations. Also, it would be important to investigate whether the fall in lean mass with age is caused by the decrease in muscle strength and/or serum T or E_2 concentrations. Finally, also the reverse could be the case, in which the decrease in muscle strength with age is caused by the fall in lean mass, and/or serum T levels or other factors. Unfortunately, the cross-sectional nature of our study does not allow a differentiation between cause and effect. All the factors are statistically related, making it impossible to differentiate which factor explains another. Therefore, no conclusions can be drawn

TABLE 6. Age-adjusted relations between different measures of T concentrations

	TT (nmol/L)		TT/SHBG ratio		Calc-FT (nmol/L)	
	$\beta \pm$ SE	r	$\beta \pm$ SE	r	$\beta \pm$ SE	r
Measured FT (nmol/L)	181.1 ± 7.19	0.78[a]	0.03 ± 0.004	0.39[a]	0.15 ± 0.007	0.75[a]
Calculated FT (nmol/L)	42.80 ± 1.00	0.91[a]	0.29 ± 0.02	0.68[a]		
Non-SHBG-T (nmol/L)	1.42 ± 0.04	0.89[a]	8.98 ± 0.45	0.71[a]		
TT/SHBG ratio	8.04 ± 0.92	0.40[a]				

[a] $P < 0.001$.

TABLE 7. Age-adjusted relations between serum T and estrogens levels

	TT (r)	Non-SHBG-T (r)	calc-FT (r)	E_1 (r)	E_2 (r)	fE_2 (r)
E_1	0.28[a]	0.28[a]	0.29[a]			
E_2	0.17[a]	0.18[a]	0.14[b]	0.24[a]		
fE_2	0.14[b]	0.20[a]	0.16[b]	0.24[a]	0.98[a]	
Non-SHBG-E_2	0.13[b]	0.20[a]	0.18[a]	0.24[a]	0.99[a]	0.995[a]

[a] $P < 0.001$.
[b] $P < 0.01$.

TABLE 8. Relations between T measurements and physical characteristics of successful aging

	maxLES (Nm)			TBBMD (g/cm^2)			Ward BMD (g/cm^2)			Fat mass (kg)		
	β	SE	B	β	SE	B	β	SE	B	β	SE	B
TT (nmol/L)	0.99	0.33	0.14[a]	0.004	0.001	0.13[a]	0.005	0.003	0.08[b]	−0.49	0.09	−0.13[c]
FT (nmol/L)	151.1	76.6	0.10[d]	0.69	0.35	0.09[d]	0.88	0.63	0.07[b]	−86.0	21.8	−0.10[c]
Calculated FT (nmol/L)	55.5	15.4	0.17[c]	0.28	0.07	0.18[c]	0.35	0.13	0.14[a]	−16.6	4.5	−0.13[c]
Non-SHBG-T (nmol/L)	1.93	0.52	0.17[c]	0.011	0.002	0.20[c]	0.012	0.004	0.14[a]	−0.53	0.15	−0.13[c]
TT/SHBG ratio	25.5	6.56	0.18[c]	0.14	0.03	0.22[c]	0.14	0.05	0.13[a]	−1.77	1.93	−0.12[b]
TT adjusted for SHBG (nmol/L)	1.11	0.34	0.16[c]	0.005	0.002	0.16[c]	0.007	0.003	0.12[d]	−0.42	0.10	−0.14[c]

β, coefficient of linear regression; B, standardized linear regression coefficient.
All analyses were done after adjustment for age and BMI.
[a] $P \leq 0.01$.
[b] $P > 0.05$.
[c] $P \leq 0.001$.
[d] $P \leq 0.05$.

TABLE 9. Relation between serum E_2 concentrations and BMD

	E_2 (nmol/L)		fE_2 (pmol/L)		Non-SHBG-E_2 (nmol/L)	
	$\beta \pm$ SE	B	$\beta \pm$ SE	B	$\beta \pm$ SE	B
TBBMD (g/cm^2)	0.21 ± 0.08	0.13[a]	0.009 ± 0.003	0.14[a]	0.31 ± 0.10	0.14[a]
Femur neck BMD (g/cm^2)	0.26 ± 0.12	0.11[b]	0.011 ± 0.005	0.11[b]	0.36 ± 0.16	0.11[b]
Femur ward BMD (g/cm^2)	0.29 ± 0.14	0.11[b]	0.013 ± 0.006	0.11[b]	0.42 ± 0.18	0.12[b]
Femur trochanter BMD (g/cm^2)	0.32 ± 0.12	0.12[a]	0.013 ± 0.005	0.13[a]	0.41 ± 0.15	0.13[a]

β, Coefficient of linear regression; B, standardized linear regression coefficient.
All analyses were done after adjustment for age and BMI.
[a] $P \leq 0.01$.
[b] $P \leq 0.05$.

concerning the physiological pathway(s). For example, also physical activity *per se* might be a factor contributing to the relationships found. A longitudinal study is necessary to answer these questions. From the results of our study, we can, however, confirm previous findings that serum T was, independent of age, positively related to both IGS and maxLES. In addition, we found a positive, age-independent relation between estimates of serum T levels and bone density. Furthermore, in agreement with previous findings, T was inversely associated with fat mass, but not with lean body mass (1). Serum non-SHBG-bound T concentrations are not only related to TBBMD, but also to all regions of the proximal femur BMD.

In this study, we established whether free T, the non-SHBG-bound fraction of T, or TT measured in plasma represent the bioactive hormone best. Pardridge (13) suggested that albumin-bound T is available for uptake by most tissues, whereas SHBG-bound T is not. Considering the fact that non-SHBG-T as well as the albumin-bound fraction were strongly related to the physical characteristics of aging, it is possible that the albumin-bound fraction of T is available for uptake by tissues and can exert biological effects. Furthermore, in agreement with the suggestion of Pardridge (13), SHBG-bound T was not related to any physical characteristic. Serum non-SHBG-T concentrations were stronger related to the physical characteristics than TT, as shown by the Bs, and slightly stronger than serum calc-FT concentrations. In addition, non-SHBG-T levels were related to proximal femur BMD, whereas TT levels were not.

There was a considerable difference in the relationships

between non-SHBG-bound T calculated according to the method described by Södergård *et al.* (14), and the TT/SHBG ratio as a measurement of non-SHBG-bound T, on the one hand, and the physical characteristics, on the other hand. The TT/SHBG ratio probably reflects in part the non-T-dependent inverse association of SHBG with these characteristics (demonstrated in the relation with lean mass, fat mass, and BMD). Furthermore, use of the ratio obfuscates the absolute levels of the components of the quotient. TT adjusted for SHBG as a measure of non-SHBG-bound T is probably a better parameter than TT/SHBG, because the inverse associations of SHBG are not taken into account. However, as judged by the Bs, it shows no clear advantage compared to non-SHBG-T. Thus, the calculated bioavailable T concentration, according the method described by Södergård *et al.* (14), seems to be an easy, inexpensive, and informative measure in representing the bioactive fraction of circulating T.

Most of the E_2 and about 20% of E_1 produced in normal men is formed by extraglandular aromatization of circulating androgens. A smaller part of the circulating E_2 is derived from a direct secretion by the testicles (29). Most of the E_1 in elderly men is produced by the adrenals. Recently it has been demonstrated that estrogens play an important role in maintaining BMD in healthy older men (5, 30). Trabecular bone is generally thought to be more responsive to gonadal steroids than cortical bone. However, we did not measure spinal BMD, because degenerative arthritis influences the outcome of measurements too strongly. In our population, serum total E_2 was independent of age, strongly positively related to BMD at all sites measured. Serum non-SHBG-bound E_2 was slightly stronger related to BMD at all sites measured compared with total E_2, as shown by the Bs. This suggests that the non-SHBG-bound fraction of E_2 is the best representation of bioactive hormone, although to a lesser extent than is the case for T, possibly because E_2 is more dissociable from SHBG than T. These findings are in agreement with those described by Khosla *et al.* (12). Also serum E_1 concentrations were related to bone density, although to TBBMD only. However, this relation was independent of E_2 and T and contributed to an even greater extent to the variation in TBBMD than E_2 or T levels. This might imply that E_1 has an effect on BMD directly, or that it is locally converted to E_2. Serum T and E_2 both contribute equally to the variation in BMD, suggesting that the effect of T on bone is not only attributed to the aromatization to estrogens (31).

Although it is generally accepted that estrogens and fat mass, especially trunk fat, are strongly related in postmenopausal women (32), we did not find a significant relation between E_1 or E_2 and (trunk) fat mass in these elderly men, before or after adjustment for T. This confirms other findings that aromatase activity is also present in other tissues apart from fat (33–36).

In summary, together with a decline in muscle strength, bone density, and body composition, bioavailable T, E_1, total E_2, and bioavailable E_2 all decrease with age in healthy old men. In this cross-sectional study in healthy elderly men, non-SHBG-bound T seems to be the best parameter for serum levels of bioactive T, which seems to play a direct role in the various physiological changes that occur during aging through its positive relation with muscle strength and BMD and its negative relation with fat mass. In addition, both serum estrone and estradiol seem to play a role in the age-related bone loss in elderly men, although the cross-sectional nature of this study precludes definitive conclusion. Non-SHBG-bound E_2 seems to be the best parameter for serum levels of bioactive E_2 in describing its positive relation with BMD.

Acknowledgments

We thank Annette Bak for help in initiating the study. Andro Medical Research, Rotterdam, facilitated the investigation by offering assistance and its study-center in Zoetermeer, The Netherlands. Finally, the cooperation of the city board and the general practitioners is appreciated.

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