

CLINICAL STUDY

Endogenous sex hormones in men aged 40–80 years

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Abstract

Objective: To determine the relationship between aging, life-style factors and health-related factors and endogenous sex hormone levels.

Design: Cross-sectional study of 400 independently living men between 40 and 80 years of age.

Methods: After exclusion of subjects who were not physically or mentally able to visit the study center, 400 men were randomly selected from a population-based sample. Total testosterone (TT), bioavailable testosterone (BT) (i.e. not bound to sex hormone-binding globulin (SHBG)), SHBG, estradiol (E₂) and dehydroepiandrosterone-sulfate (DHEA-S) were investigated for their relationship with age, body mass index (BMI), waist circumference, smoking, physical activity and general health status. Multivariate models using ANCOVA analyses were used to examine the contribution of life-style factors to sex hormone variability.

Results: TT, BT and DHEA-S decreased with age; 0.2, 0.7 and 1.2%/year respectively. SHBG showed an increase with age of 1.1%/year. No changes with age were found for E₂. General health status modified the association of TT and SHBG with age (*P* interaction 0.10 and 0.002 respectively). Increased BMI and waist circumference were associated with decreased TT, BT, SHBG and DHEA-S and increased E₂ (all *P* < 0.01). Current smoking, lower alcohol intake and a higher physical activity score were associated with higher TT and SHBG levels.

Conclusion: This study showed the important determinants of sex hormones were age, BMI, waist circumference, smoking, general health status and physical activity. Furthermore, it can be concluded that general health status modified the effect between sex hormones and age. For future observational studies it should be taken into account that the above-mentioned determinants may alter the association between sex hormones and diseases and related conditions.

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Introduction

Unlike women, men do not experience an abrupt reduction in endogenous sex hormone production. Still, age-associated decreases in the levels of (bioactive) androgens occur (1–4). The fact that men lack a major identifiable change in hormonal status, comparable with menopause, makes the characterization of male endocrine status particularly difficult.

Contradictory findings suggesting that testosterone levels remain stable with age in healthy men (5–8) raised questions regarding the relative roles of chronic age-related illness and other factors vs aging *per se* in changes in androgen levels. Research on the effect of aging on endogenous androgens is impeded by the complex interrelations of sex hormones with other hormone systems, with common diseases, in the elderly, and with associated conditions and behavior (9–11). Growth hormone (GH), insulin-like growth factor I (IGF-I), insulin and thyroid hormone levels appear to be determinants of testosterone and sex

hormone-binding globulin (SHBG) levels (9, 12). Acute critical illness, surgical injury or acute myocardial infarction cause a profound, generally transient, decrease of testosterone levels (10, 11). As far as chronic diseases are concerned, decreased testosterone levels are observed in men with endocrine disorders, renal failure, malignancy, coronary atherosclerosis and diabetes mellitus (12, 13).

Although the effects of age and body mass index (BMI) on total testosterone (TT) and SHBG concentrations are well studied, the determinants of bioavailable testosterone (BT) (i.e. not bound to SHBG), estradiol (E₂) and dehydroepiandrosterone sulfate (DHEA-S) concentrations in men are less known. There is some evidence that life-style factors such as smoking, alcohol consumption and exercise affect androgen levels (14–17), but results are inconsistent.

In a large-scale cross-sectional study we have assessed the influence of aging, life-style factors and health-related factors on endogenous sex hormone levels in men.

Subjects and methods

Study population and procedures

This is a cross-sectional, single-center study in 400 independently living men aged 40–80 years. The subjects were recruited in two ways. First by means of asking female participants of other studies conducted by the department by letter whether they knew a possible interested male volunteer between the age of 40 and 80 years. Invitation letters were sent to 770 female participants, and 240 men volunteered for participation. Due to this indirect way of recruiting it was not possible to assess the exact participation rate.

Secondly, names and addresses of a randomly selected male population aged 40–80 years were drawn from the municipal register of Utrecht, a town in the middle part of The Netherlands; 1230 invitation letters were sent to a selection. From this group, 390 men volunteered for participation (participation rate of 32%).

From the total 630 volunteers we excluded those who did not live independently and subjects who were not physically or mentally able to visit the study center independently ($n = 16$). No additional health-related eligibility criteria were used. Of the remaining 614 men, eventually 400 were randomly selected to participate. To yield equal numbers in each age decade from the age of 40 to 80, we sampled 100 men in each decade. All participants gave written informed consent before enrollment in the study and the institutional review board of the University Medical Center Utrecht approved the study.

Data collection took place between March 2001 and April 2002, and participants visited the research center twice. A medical doctor obtained information on prevalent diseases, medication use and life-style factors from a specified medical history. A venepuncture was performed between 0800 and 1000 h, and fasting blood samples were obtained. Platelet-free serum was obtained by centrifugation and immediately stored at -20°C .

Life-style variables

Smoking was estimated from self-report and categorized into current, former and never smokers. Alcohol consumption was assessed by a validated food frequency questionnaire, designed to estimate regular intake of 178 food items in the year prior to enrollment (18, 19). Daily alcohol intake was categorized as no, 0–20, 20–40 or ≥ 40 g/day. Height and weight were measured in the standing position without shoes. BMI was calculated as the weight in kilograms divided by the square of the height in meters, and was categorized as < 25 , 25–30 and ≥ 30 kg/m². Waist circumference was measured midway between the lower rib margin and the iliac crest with participants in a standing position without heavy outer garments and with emptied pockets, breathing out gently, and was categorized in

tertiles. Physical activity was assessed using the Voorrips questionnaire, a questionnaire validated in an elderly population, and was categorized in quartiles (20). Low scores represent low physical activity.

Clinical parameters and prevalence of disease

Diseases were classified using the International Classification of Diseases, 10th revision. Participants were asked about current use of medications; these reports were checked by examining labels of drugs brought to the clinic. Peripheral blood pressure was measured twice in the left and right brachial artery, with the subject in a supine position, with a semi-automated oscillometric method. The average of the two measurements of systolic and diastolic blood pressure was used for analysis and further calculation. Hypertension was defined as systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 95 mmHg or use of anti-hypertensive medication (21). Diabetes mellitus was defined as treatment with insulin or oral hypoglycemic agents or fasting plasma venous glucose > 6.9 mmol/l. Cardiovascular disease (CVD) is a pooled condition including coronary heart disease, peripheral artery disease and stroke, which was defined as present when men reported a history of these conditions with diagnosis and treatment. Chronic disease or medication use was defined as presence of CVD, pulmonary disease, cancer, hypertension, diabetes and/or chronic use of medication. Healthy and/or healthy life-style was defined as absence of chronic disease (CVD, pulmonary disease, cancer, hypertension, diabetes), chronic use of medication, severe alcohol consumption (≥ 40 g/day), current smoking and obesity (BMI ≥ 30 kg/m²).

Laboratory measurements

An automatic enzymatic procedure was used to determine serum total cholesterol (Synchron LX Systems; Beckman Coulter, Mydrecht, The Netherlands). Fasting blood glucose was measured by using a reagent-strip glucose oxidase method, using a GlucoTouch reflectometer (LifeScan, Inc., Benelux, Beerse, Belgium). Venous whole blood was immediately applied to the test strip. Levels of steroids were measured in serum. TT was measured after diethyl ether extraction using an in-house competitive RIA employing a polyclonal anti-testosterone (Dr JH Pratt, Indianapolis, IN, USA). [1α , 2α -³H]Testosterone (DuPont NEN, Boston, MA, USA) was used as a tracer following chromatographic verification of its purity. The lower limit of detection was 0.24 nmol/l and inter-assay variation was 6.0, 5.4 and 8.6% at 2.1, 5.6 and 23 nmol/l respectively ($n = 85$). SHBG was measured using an immunometric technique on an Immulite Analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA). The lower limit of detection was 5 nmol/l and inter assay variation was 6.1, 4.9 and 6.9% at 11.6, 36 and 93 nmol/l

respectively ($n = 30$). DHEA-S was measured on an Advantage Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The lower limit of detection was $0.1 \mu\text{mol/l}$ and inter assay variation was 5.2, 5.6 and 4.2% at 1.0, 4.9 and $14.2 \mu\text{mol/l}$ respectively ($n = 19$). BT was calculated from SHBG and TT using the method of Vermeulen *et al.* (22). E_2 was measured after diethyl ether extraction and Sephadex chromatography using an in-house competitive RIA employing a polyclonal anti- E_2 antibody (Dr F de Jong, Erasmus MC, Rotterdam, The Netherlands). [$2,4,6,7\text{-}^3\text{H}$] E_2 (Amersham Nederland BV) was used as a tracer following chromatographic verification of its purity. The lower limit of detection was 20 pmol/l (2 ml sample) and interassay variation was 10.0 and 3.1% at 81 ($n = 24$) and 660 pmol/l ($n = 17$) respectively.

Data analyses

Subjects were grouped by age decades, sex hormone levels were expressed as means and 95% confidence intervals (95% CI, $\pm 1.96 \times \text{S.E.}$) and tested for trends over age categories, by linear regression analysis. Multivariate models using ANCOVA analyses were used to estimate mean hormone levels with a 95% CI across categories of age, life-style variables and health status. Trend analyses were done using linear regression models. To analyze the modifying effect of general health status on the association between age and hormone levels, interaction terms were included in the linear regression models. Where significant interaction terms were found, ANCOVA analyses were repeated in strata of health status. Estimates were adjusted for age, BMI, physical activity, smoking (current, else), and alcohol consumption. Data analyses were performed using SPSS statistical software (Version 11.0; SPSS, Chicago, IL, USA).

Results

Mean age of the study population was 60.2 years (range 40–80), mean BMI 26.3 kg/m^2 (range 17–43), mean waist circumference 99 cm (range 74–133) and mean physical performance score 18.1 (range 1–44). Twenty-two percent of the population reported they were never smokers, 54% were former smokers and 20% were current smokers. Concerning

the prevalence of diseases and risk factors, 11% of the population had diabetes mellitus, 13% had CVD, 12% had a BMI ≥ 30 and 27% had hypertension. Forty-four percent of the population reported a chronic disease (CVD, chronic destructive pulmonary disease, hypertension, cancer, diabetes) or used medication, and 33% of the population were defined as healthy or having a 'healthy lifestyle' (absence of chronic disease, chronic use of medication, severe alcohol consumption, current smoking and obesity).

Age

TT, BT and DHEA-S showed a decline with age. BT declined in a more pronounced way (0.7%/year) than the TT serum level (0.4%). The annual reduction in DHEA-S was 1.2%. Serum SHBG concentrations showed an increase with age of 0.8%/year and E_2 did not change with age (Table 1). After adjustment for BMI, current smoking, physical activity and alcohol intake the rates of decline of TT, BT and DHEA-S were 0.2, 0.7 and 1.2%/year. The annual decline rate of TT attenuated from 0.4 to 0.2%/year. After adjustment SHBG showed an increase of 1.1%/year (Table 2).

Lifestyle factors

After adjustment for age, current smoking, BMI and physical activity, participants with the highest alcohol intake ($> 40 \text{ g/day}$) were found to have lower TT and SHBG levels. Increases of DHEA-S levels were associated with higher alcohol intake ($P_{\text{trend}} < 0.001$). Current smokers were found to have higher TT and SHBG levels, after adjusting for age, BMI, alcohol intake and physical activity. After adjustment for age, current smoking, alcohol intake and physical activity a significant inverse linear trend was found for TT, BT, SHBG and DHEA-S in association with BMI and waist circumference categories. A direct association was found for E_2 levels and BMI and waist circumference. Increases of TT, BT and SHBG levels were associated with a higher score on the physical activity questionnaire (Table 2).

Health status

Adjusted mean TT concentrations were lower in men with two or more disorders, 17.0 nmol/l (95% CI

Table 1 Circulating sex hormone levels by age category and the P for the linear trend across age categories. Data represent means (95% CI).

Age (years)	TT (nmol/l)	BT (nmol/l)	SHBG (nmol/l)	DHEA-S ($\mu\text{mol/l}$)	E_2 (pmol/l)
40–50	20.2 (19.1–21.3)	9.9 (9.4–10.4)	34.7 (32.0–37.4)	9.0 (8.5–9.6)	88.6 (94.4–92.7)
51–60	18.5 (17.4–19.5)	8.4 (8.0–8.7)	38.0 (35.4–40.6)	7.5 (6.9–8.2)	92.2 (87.8–96.5)
61–70	17.9 (16.8–19.0)	7.4 (7.0–7.7)	43.6 (40.6–46.5)	5.4 (4.9–5.9)	94.2 (89.0–99.4)
71–80	17.6 (16.7–18.6)	7.0 (6.7–7.3)	46.1 (43.5–48.7)	4.8 (4.3–5.3)	90.1 (89.0–93.5)
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.51$

Table 2 Multivariate adjusted mean levels and 95% CIs of TT, BT, SHBG, DHEA-S and E₂ by category of age, smoking, BMI, waist and physical activity, and the *P* for the linear trend across categories. Variables were adjusted for age, BMI, smoking status, alcohol intake and physical activity (Voorrips score) in quartiles (Q) and presence/absence of chronic disease. For age categories no adjustment was made for age; for alcohol categories no adjustment was made for alcohol; for smoking no adjustment was made for smoking status; for BMI categories and waist categories in tertiles (T), no adjustment was made for BMI; for physical activity quartiles no adjustment was made for Voorrips score.

	TT (nmol/l)	BT (nmol/l)	SHBG (nmol/l)	DHEA-S (μmol/l)	E ₂ (pmol/l)
Age (years)					
40–50	19.3 (18.3–20.2)	9.7 (9.3–10.1)	32.6 (29.9–35.2)	8.9 (8.3–9.5)	88.8 (84.2–94.5)
51–60	19.3 (18.3–20.2)	8.5 (8.1–8.9)	39.8 (37.2–42.4)	7.6 (7.1–8.2)	90.8 (86.3–95.4)
61–70	17.6 (16.7–18.6)	7.3 (6.9–7.7)	42.9 (40.4–45.5)	5.3 (4.8–5.9)	93.6 (89.1–98.1)
71–80	17.7 (16.7–18.7)	7.0 (6.6–7.4)	46.5 (43.7–49.0)	4.9 (4.3–5.5)	90.3 (85.7–94.9)
	<i>P</i> = 0.004	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> = 0.48
Alcohol (g/day)					
0	19.0 (17.8–20.1)	8.2 (7.7–8.6)	41.6 (38.6–44.6)	5.8 (5.1–6.5)	88.7 (83.3–94.1)
0–20	18.3 (17.6–19.1)	8.0 (7.7–8.3)	41.3 (39.3–43.2)	6.5 (6.1–6.9)	91.1 (87.7–94.6)
2–40	19.0 (18.1–20.0)	8.3 (8.0–8.7)	41.1 (38.6–43.7)	7.1 (6.5–7.6)	92.9 (88.4–97.5)
> 40	17.2 (15.9–18.4)	8.1 (7.6–8.6)	35.3 (31.9–38.6)	7.7 (7.0–8.4)	89.5 (83.6–95.4)
	<i>P</i> = 0.16	<i>P</i> = 0.73	<i>P</i> = 0.01	<i>P</i> < 0.001	<i>P</i> = 0.65
Smoking					
Former/never	18.2 (17.7–18.8)	8.1 (7.9–8.3)	39.6 (38.2–41.0)	6.6 (6.3–6.9)	90.7 (88.1–93.2)
Current	19.4 (18.3–20.5)	8.2 (7.8–8.6)	43.7 (40.8–46.6)	7.1 (6.5–7.7)	91.8 (86.7–97.0)
	<i>P</i> = 0.05	<i>P</i> = 0.71	<i>P</i> = 0.01	<i>P</i> = 0.17	<i>P</i> = 0.69
BMI (kg/m²)					
< 25	20.2 (19.4–21.0)	8.6 (8.2–8.9)	43.7 (41.6–45.8)	7.2 (6.8–7.7)	86.9 (83.1–90.7)
25–30	17.9 (17.2–18.5)	8.0 (7.7–8.3)	39.5 (37.7–41.2)	6.5 (6.1–6.8)	92.2 (89.0–95.3)
≥ 30	15.8 (14.4–17.2)	7.5 (6.9–8.0)	34.5 (30.8–38.2)	6.0 (5.2–6.8)	97.6 (91.0–104.3)
	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> = 0.003	<i>P</i> = 0.003
Waist (cm)					
T1 < 95	20.0 (19.1–20.9)	8.4 (8.1–8.8)	44.2 (41.9–46.5)	7.1 (6.7–7.6)	85.4 (81.4–89.4)
T2 95–102	18.5 (17.7–19.4)	8.3 (7.9–8.6)	39.8 (37.6–42.0)	6.9 (6.4–7.4)	90.6 (86.8–94.5)
T3 ≥ 102	16.9 (16.0–17.7)	7.7 (7.4–8.1)	37.4 (35.2–39.6)	6.0 (5.6–6.5)	96.5 (92.6–100.5)
	<i>P</i> < 0.001	<i>P</i> = 0.006	<i>P</i> < 0.001	<i>P</i> = 0.002	<i>P</i> < 0.001
Physical activity					
Q1 < 12.5	17.8 (16.8–18.7)	8.0 (7.7–8.4)	38.0 (35.4–40.6)	7.3 (6.7–7.8)	93.1 (88.5–97.6)
Q2 12.5–17.5	17.7 (16.8–18.7)	7.9 (7.5–8.3)	39.3 (36.8–41.8)	6.3 (5.7–6.8)	86.2 (81.7–90.6)
Q3 17.5–23.1	18.7 (17.7–19.6)	8.0 (7.7–8.4)	42.1 (39.6–44.6)	6.8 (6.2–7.3)	89.6 (85.2–94.1)
Q4 ≥ 23.1	19.6 (18.7–20.6)	8.5 (8.1–8.9)	42.2 (39.7–44.8)	6.5 (5.9–7.0)	94.8 (90.3–99.3)
	<i>P</i> = 0.003	<i>P</i> = 0.07	<i>P</i> = 0.01	<i>P</i> = 0.16	<i>P</i> = 0.38

15.7–18.3 nmol/l), compared with one or no disorders in the chronic disease category, 18.6 (95% CI 17.7–19.5 nmol/l) and 18.7 (95% CI 18.0–19.4 nmol/l) respectively. No differences were found for BT, SHBG, DHEA-S and E₂ (data not shown). Adjustments were made for age, BMI, physical activity, current smoking and alcohol intake. When subdividing the group into 'healthy lifestyle' and 'other', a near zero slope for the age-related change in TT was found in the group with healthy life-style ($\beta = 0.00$, 95% CI -0.08 to 0.08). In the rest of the group the following age-related change in TT was found: $\beta = -0.11$ (95% CI -0.16 to -0.06). The *P* for interaction for the TT-age association was 0.10 (Fig. 1A). The steepest slope for the age-related change in SHBG was found in the 'healthy-lifestyle' group, $\beta = 0.71$ (95% CI 0.51–0.91) (Fig. 1B). For the rest of the group the age-related change in SHBG was $\beta = 0.29$ (95% CI 0.15–0.42) (*P* interaction = 0.002). Concerning the age-related change in BT no differences were found between the 'healthy-lifestyle' group and others (*P*interaction = 0.52, Fig. 1C).

Discussion

The present data showed a continuous decline in serum sex hormones in independently living men between 40 and 80 years of age. Besides aging, important determinants of sex hormone concentrations were BMI, waist circumference, current smoking, general health status and physical activity. General health status modified the effect between TT and age, i.e. TT remained stable with age in healthy participants whereas TT declined with age in the remainder of the study group. A modifying effect of general health status was also found for the association between SHBG and age (Fig. 1).

It is generally accepted that aging in men is accompanied by a decrease in testosterone levels. Our results are in line with several large cross-sectional studies which have come to a consensus on a 0.5–2% annual decline in TT, BT and DHEA-S and annual increase in SHBG around 1% (1, 3, 4, 9, 23, 24). Whether the fall in testosterone levels is primary or secondary is debated. It seems that declining gonadal function in aging males has both testicular and

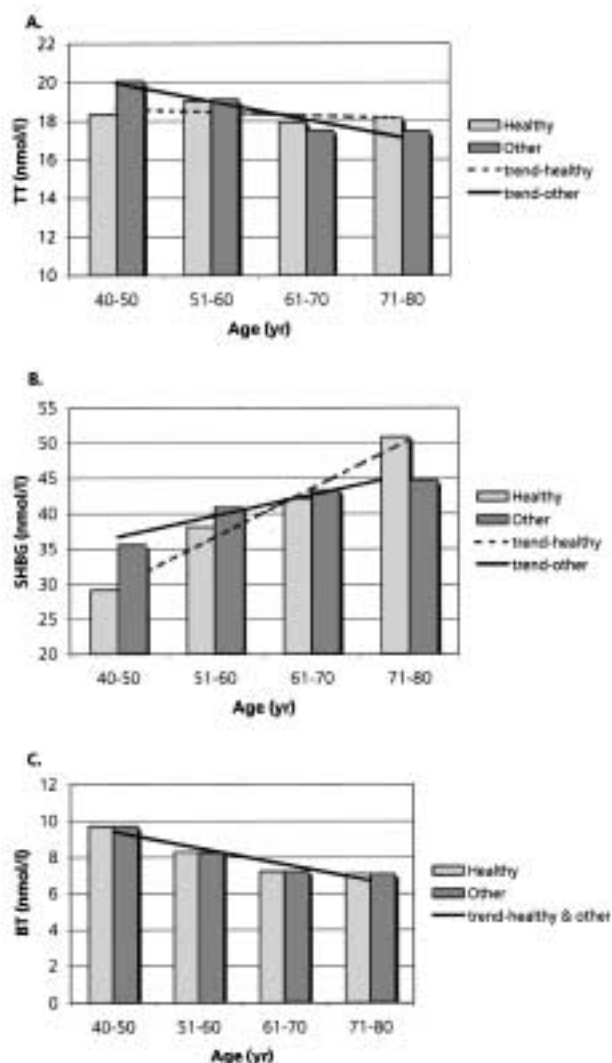


Figure 1 (A) Multivariate adjusted mean levels of TT and trend-lines for healthy and non-healthy participants over the different age categories. P interaction = 0.10. (B) Multivariate adjusted mean levels of SHBG and trend-lines for healthy and non-healthy participants over the different age categories. P interaction = 0.002. (C) Multivariate adjusted mean levels of BT and trend-lines for healthy and non-healthy participants over the different age-categories. P interaction = 0.52. Healthy (healthy life-style) was defined as absence of chronic disease, chronic use of medication, severe alcohol consumption (≥ 40 g/day), current smoking and obesity ($BMI \geq 30$ kg/m²). Adjustments were made for alcohol consumption (g/day), BMI (kg/m²) and physical activity (Voorrips score).

hypothalamo–pituitary elements (25). The cause of the increase in SHBG levels with age remains unclear. Possibly the age-associated decrease in GH and IGF-I levels might contribute to the increased SHBG levels (9). GH treatment of adult men with isolated GH deficiency results in a decrease of SHBG and TT levels (9). As GH therapy induces insulin resistance, it might be hypothesized that part of the effect of GH on testosterone and SHBG levels is mediated by hyperinsulinism

(26). As for DHEA-S, annual rates of decline around 2%/year have been found (9, 27). The decline in DHEA-S levels seems to be caused by a selective decrease in the number of zona reticularis cells in the adrenal cortex (25). Until recently little attention has been paid to the role of estrogens in elderly men. The studies which have been reported so far show no change of total E₂ levels with age in men (1, 9, 28), or a decrease of E₂ levels only in old age (29, 30). In the present study no changes with age were found. However, in a cross-sectional study in men aged 25–85 years bioavailable E₂ decreased significantly with age (31). The bioavailable sex steroids comprise the fractions that have rapid access to target tissues; measurement of TT and E₂ levels does not accurately reflect the actual levels available to tissues (31).

Some early researchers, who found that plasma levels of testosterone remain fairly constant up to 80 years of age, challenged this age-related decrease in testosterone. This finding that TT levels did not fall significantly with age in healthy men (5–8) raised the question of the relative roles of chronic age-related illness and other subject characteristics vs aging *per se* in producing the observed decreases. Studies have suggested that age, illness, medication use, smoking, alcohol use and physical activity all exert independent effects on androgens during aging (6, 8–11, 13–16, 32, 33). Despite a non-significant interaction term, our results suggest that the age-related decline of TT is modified by general health status (Fig. 1), which might be an explanation for the differences found in testosterone decline in the different studies.

It is known that severe alcohol consumption alters gonadal function especially when liver cirrhosis has already developed, but there is controversy regarding the pathogenesis of this alteration (34). In this study an alcohol intake >40 g/day is associated with a decline in testosterone and SHBG levels. These findings are in line with findings of other studies (8, 16, 17), which points to the fact that only severe alcohol consumption leads to decreased levels of testosterone. Our finding of increased DHEA-S levels with increasing alcohol consumption has been reported by other investigators (16, 35).

Our data confirm that smokers have higher TT and SHBG levels than non-smokers (9, 15, 16, 36, 37). SHBG has been shown to be significantly correlated with serum cotinine levels, a measure of nicotine exposure from cigarette smoking (14, 16).

We have confirmed in our data that obesity in males is accompanied by a significant decline in testosterone and SHBG levels and an increase in E₂ levels. An increasing waist circumference, considered to be a more accurate reflection of abdominal adipose tissue than the waist:hip ratio (38), is also associated with a reduction in testosterone and SHBG and an increase of E₂. However, after adjusting for BMI, it was only additionally associated with increased E₂ levels. It has

been reported that E₂ levels are highly related to body fat mass and more specifically to subcutaneous abdominal fat (39). As BMI varies with age in our population, failure to adjust for BMI may lead to an overestimation of the age-associated decline of testosterone levels.

It is suggested that endogenous testosterone levels are acutely and substantially influenced by physical exercise; however, this exercise-induced testosterone increase is smaller in the elderly (40). The increase in circulating testosterone occurs within a short period of time of the onset of exercise and fades again afterwards (40). On the other hand, it could be suggested that low testosterone levels induce reduced physical fitness (29, 41). Physical activity measured in this study, however, represents physical activity during the past year. The increase in TT, BT and SHBG found in the high activity group in this study is probably a reflection of a favorable general health state rather than a high acute physical activity level.

To appreciate these findings, some issues need to be addressed. Strengths of the present study include that this cross-sectional study is one of the largest to date to investigate the associations between life-style factors, general health status and circulating sex hormone concentrations in independently living men across a wide age range. Another issue that needs to be considered is that TT was measured using an RIA with an extraction method, which, compared with direct assays, is more reliable and reduces the cross-reaction of assay antibodies with other hormone-like substances (22, 42).

The interpretability of the results may be limited by several factors inherent to the cross-sectional community-based design, which limits conclusions regarding within-person change or direction of causality. Studies have suggested that low androgen levels are associated with an increase in morbidity (25, 40). Younger subjects, who are still too young to have suffered significant morbidity, will demonstrate the full range of values of androgens. Because morbidity is more common in older subjects, selective non-response may distort findings in the old (43). This might explain previous reports that longitudinal age trends appear steeper than cross-sectional trends (2, 3, 44).

The best methods for determining free testosterone or BT are equilibrium dialysis and ammonium sulfate precipitation respectively. These are, however, time-consuming techniques which are not easily automated. Calculation of BT from TT and SHBG, as used in this study, yields values that are in good agreement with values obtained by indirect methods (22).

In conclusion, the findings in the present large-scale cross-sectional study show that age, obesity, smoking, physical activity and general health status are associated with serum sex hormones. It is important to determine the exact pattern of sex hormone changes with age and to assess which determinants alter these hormone levels, because of the central role these

hormones play in etiology, diagnosis and management of several diseases. For future observational studies, it should be taken into account that the above-mentioned determinants may alter the association between circulating sex hormones and chronic diseases and associated conditions. Therefore failure to adjust for these determinants may lead to both an under- and overestimation of the association between sex hormones and chronic diseases. Furthermore, these determinants could be used to define the target populations for male hormonal replacement therapy.

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