CLINICAL STUDY

Serum estradiol is associated with lean mass in elderly Swedish men

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Abstract

Objective: Association studies in men have shown that androgens are inversely related to fat measures, while the relation between sex steroids and lean mass remains unclear. We, therefore, investigated the associations between serum sex steroid levels and body composition in elderly men with a main focus on lean mass measures.

Design and methods: A cross-sectional survey of a population-based cohort of 3014 elderly men, aged 69–80 years (Osteoporotic Fractures in Men study, Sweden). Serum levels of testosterone and estradiol (E_2) were measured by mass spectrometry, sex hormone-binding globulin (SHBG) levels were measured by IRMA, and measures of body composition were obtained by dual-energy X-ray absorptiometry.

Results: Total as well as free serum testosterone associated independently inversely ($P < 0.001$), while total as well as free serum E_2 associated independently directly ($P < 0.001$) with total body fat mass and trunk fat mass. Serum SHBG associated independently inversely with central fat distribution. Serum E_2 and free E_2 but not serum testosterone or free testosterone levels associated positively with lean mass ($P < 0.01$). Elderly men within the lowest quartile of free E_2 had 0.5 kg less lean mass in the legs than subjects within the highest quartile, while the subjects in the different quartiles of free testosterone did not differ in lean mass.

Conclusions: Serum E_2 , but not serum testosterone, is directly associated with lean mass in this large study of elderly Swedish men. In addition, serum SHBG is associated with central fat distribution and we confirmed that serum testosterone is inversely associated with fat mass.

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Introduction

Part of the age-related physiological changes in men, including loss of muscle size and strength, loss of bone mass and increase in fat mass, is thought to be related to the decrease in serum sex steroid levels with aging (1). This hypothesis has greatly focused interest on testosterone supplementation in the increasing elderly male population, especially in the United States, where sales have increased by 400% since 1999 (2). However, there is a lack of large, placebo-controlled trials demonstrating the efficacy and safety of testosterone supplementation (3, 4). Moreover, since studies focusing on the associations between age-related outcomes and serum estradiol (E_2) levels in men are scarce, it remains unknown whether these testosterone effects occur only through androgen receptor (AR) activation or also via estrogen receptor (ER) activation following aromatization.

Several cross-sectional association studies in men examining the relation between serum sex steroid levels and fat mass consistently reported that serum testosterone is inversely correlated to body mass index (BMI), waist circumference, and total body fat mass (1, 5–14). In addition, several studies have shown that serum testosterone levels associated inversely with abdominal fat mass and regional abdominal fat deposits (6, 7, 15, 16). Studies determining the association between serum E_2 levels and fat mass in men are less consistent, with some describing a positive correlation (9, 15), whereas others report no significant association (7, 8, 10).

The association between serum sex steroids and lean mass in older men is unclear. Serum testosterone levels were found to be directly related to lean mass in a few studies (17–19), but this was not always confirmed (8, 11). The few studies investigating the association between serum E_2 levels and lean mass in men reported no significant correlation between these two variables (8, 18).

It is well established that serum sex hormone-binding globulin (SHBG) levels increase with age (1). Serum SHBG levels were reported to be inversely correlated with total body fat mass (7, 8) as well as with abdominal fat areas (6, 7, 15) in both young and older men.

Some of the conflicting results regarding the association between serum sex steroid levels and body composition measurements reported so far could be due to the use of immuno-based techniques for the measurement of serum sex steroid levels. These commercially available assays, especially those for serum E₂, are thought to have a reduced specificity at lower concentrations (20, 21). In this study, we have determined the serum levels of sex steroids by the specific gas chromatography–mass spectrometry (GC–MS) technique in a large, well-characterized cohort of elderly men. The aim of our study was to investigate the associations between serum sex steroid levels and body composition in elderly men with a main focus on lean mass measures.

Methods

The Osteoporotic Fractures in Men study (MrOS) is a multicenter study including elderly men in Sweden ($n=3014$), Hong Kong (≈ 2000), and the United States (≈ 6000). The MrOS Sweden cohort consists of three subcohorts from three different Swedish cities ($n=1005$ in Malmö, $n=1010$ in Gothenburg, and $n=999$ in Uppsala) and the study subjects (men aged 69–80 years) were randomly identified using national population registers. To be eligible for the study, the subjects had to be able to walk without aids and were not allowed to have bilateral hip prosthesis. There were no other exclusion criteria. A total of 45% of the subjects who were contacted participated in the study (Table 1) (22). Informed consent was obtained from all the study participants in the MrOS Sweden study. The study was approved by the ethics committee at the Universities of Gothenburg, Uppsala, and Lund.

Anthropometrical measurements

Height was measured using a wall-mounted Harpenden stadiometer, and weight was measured by an electric scale. The coefficient of variation (CV) was below 1% for these measurements. BMI was defined as weight in kilograms divided by the square of height in meters,

Table 1 Baseline characteristics of the study subjects.

MrOS Sweden ($n=3014$)		
Age (years)	75.4 ± 3.2	
Height (cm)	174.8 ± 6.5	
Weight (kg)	80.8 ± 12.1	
BMI (kg/m ²)	26.4 ± 3.6	
SHBG (nmol/l)	43.3 ± 21.9 (44.1 ± 22.1)	
Serum (GC–MS)	$n=2639$ ($n=1830$)	
E ₂ (pg/ml)	20.9 ± 7.9 (21.4 ± 8.0)	
FE ₂ (pg/ml)	0.36 ± 0.15 (0.37 ± 0.15)	
Testosterone (ng/ml)	4.50 ± 1.87 (4.63 ± 1.90)	
FT (ng/ml)	0.081 ± 0.034 (0.083 ± 0.035)	
DEXA	Lunar ($n=2004$)	Hologic ($n=1010$)
Total body fat mass (kg)	23.7 ± 7.9	18.5 ± 5.7
Total body fat (%)	28.8 ± 6.5	22.7 ± 4.8
Trunk fat mass (kg)	14.2 ± 5.0	9.7 ± 3.6
Central fat distribution (%)	59.5 ± 5.2	51.3 ± 6.2
Total body lean mass (kg)	53.7 ± 6.0	59.3 ± 6.8
Legs lean mass (kg)	17.4 ± 2.3	18.6 ± 2.4

Values are given as means ± s.d. DEXA, dual-energy X-ray absorptiometry. Central fat distribution is calculated as trunk fat mass/total body fat mass × 100. Values within brackets for serum SHBG and sex steroids are for the subjects with morning samples before 1000 h.

with BMI < 25 kg/m² regarded as normal weight, BMI ≥ 25 and < 30 kg/m² as overweight, and BMI ≥ 30 kg/m² as obese.

Dual-energy X-ray absorptiometry

Fat mass and lean mass of the whole body and fat and lean mass of the trunk, as well as lower extremities, were assessed using the Lunar Prodigy dual-energy X-ray absorptiometry (DEXA; GE Lunar Corp., Madison, WI, USA; in Malmö and Uppsala) or Hologic QDR 4500/A-Delphi (Hologic, Waltham, MA, USA; in Gothenburg).

Assessment of sex hormones in serum

For the current study, all the subjects with at least 1 ml serum stored from a blood draw at the baseline visit were included ($n=2639$). Serum levels of testosterone and E₂ were analyzed at the Laboratory of Molecular Endocrinology and Oncology, Laval University Hospital Research Center, Québec, Canada, as previously described (23, 24). Briefly, the validated GC–MS system was used for the analyses of testosterone (limit of detection 0.05 ng/ml, intra-assay CV 2.9%, inter-assay CV 3.4%)

Table 2 Anthropometric data.

$n=2639$ ($n=1830$)	E ₂	FE ₂	Testosterone	FT	SHBG
Body weight	0.05 (0.03)	0.13 (0.10)	−0.29 (−0.28)	−0.16 (−0.16)	−0.26 (−0.25)
Height	0.03 (0.01)	0.02 (0.01)	−0.01 (−0.02)	−0.01 (−0.03)	0.00 (−0.01)
BMI	0.04 (0.03)	0.13 (0.11)	−0.32 (−0.31)	−0.17 (−0.17)	−0.29 (−0.28)

Pearson's correlation coefficients (r) are shown for serum levels of sex steroids versus anthropometric data. Values in brackets indicate subjects with morning samples before 1000 h. Bold indicates $P < 0.05$.

Table 3 Body composition analyzed by DEXA.

	Hologic DEXA n=943					Lunar DEXA n=1635 (n=830)				
	E ₂	FE ₂	Testosterone	FT	SHBG	E ₂	FE ₂	Testosterone	FT	SHBG
Total body fat mass	-0.06	0.01	-0.35	-0.26	-0.20	0.02 (0.00)	0.10 (0.07)	-0.37 (-0.36)	-0.22 (-0.23)	-0.28 (-0.27)
Total body fat percentage	-0.09	-0.05	-0.35	-0.31	-0.15	-0.01 (-0.03)	0.07 (0.04)	-0.36 (-0.34)	-0.22 (-0.23)	-0.26 (-0.25)
Trunk fat mass	-0.05	0.04	-0.35	-0.22	-0.25	0.01 (0.00)	0.11 (0.08)	-0.37 (-0.35)	-0.21 (-0.21)	-0.31 (-0.29)
Central fat distribution	0.01	0.11	-0.22	-0.05	-0.29	0.00 (0.02)	0.08 (0.09)	-0.19 (-0.17)	-0.04 (-0.03)	-0.25 (-0.24)
Total body lean mass	0.07	0.15	-0.10	0.03	-0.20	0.10 (0.10)	0.13 (0.12)	-0.09 (-0.08)	-0.01 (-0.03)	-0.14 (-0.14)
Legs lean mass	0.10	0.16	-0.05	0.05	-0.14	0.12 (0.12)	0.15 (0.13)	-0.04 (-0.02)	0.01 (0.00)	-0.09 (-0.08)

Pearson's correlation coefficients (*r*) are shown for serum levels of sex steroids versus body composition parameters. Central fat distribution is calculated as trunk fat mass/total body fat mass $\times 100$. DEXA, dual-energy X-ray absorptiometry. Values within brackets for the Lunar DEXA subcohort are for the subjects with morning samples before 1000 h. All the subjects analyzed by the Hologic DEXA had morning samples before 1000 h. Bold indicates $P < 0.05$.

and E₂ (limit of detection 2.00 pg/ml, intra-assay CV 1.5%, inter-assay CV 2.7%). The analytes and internal standard were detected using a HP5973 quadrupole mass spectrometer equipped with a chemical ionization source. Serum SHBG was measured using IRMA (Orion Diagnostics, Espoo, Finland; limit of detection 1.3 nmol/l, intra-assay CV 3%, inter-assay CV 7%). Free testosterone (FT) and free E₂ (FE₂) were calculated according to the method described by Vermeulen *et al.* (25) and Van den Beld *et al.* (8) taking the concentrations of total testosterone and SHBG into account and assuming a fixed albumin concentration of 43 g/l.

Statistical analysis

Variables not normally distributed (i.e. weight, BMI, and DEXA parameters) were log transformed. However, log transformation did not improve the distribution of E₂, FE₂, testosterone, FT, or SHBG and, therefore, these were transformed to normally distributed standardized variables: an empirical distribution function was made which was then applied for the calculation of the inverse of the standardized normal distribution. The relationships between serum sex steroids and body composition measurements were evaluated using Pearson's correlation coefficients (Tables 2 and 3). Logistic regression examined predictive values of overweight and obesity for prevalence of hypogonadism, and differences in the serum levels of testosterone, FT and SHBG according to BMI status were calculated using ANOVA followed by Tukey's *post hoc* test (Table 4). The independent associations between serum sex steroids and different measures of body composition were calculated using multiple linear regression models (Tables 5–7). Age and height were added as confounding variables since both were associated with lean mass and fat mass measures (data not shown). Multiple linear regression models were used to test the independent associations either between serum levels of testosterone, E₂ and SHBG and measures of body composition (Tables 5 and 6) or between serum levels of FT and FE₂ (without including testosterone, E₂, and SHBG) and measures of body composition (Table 7 and data not shown). Standardized β values are shown in Tables 5–7. Standardized β values are expressed as s.d. units and, thus, are directly comparable because they are not dependent on the units of measurement of the variable. As such, they provide an indication of the relative importance of the independent variables in the model. The standardized β value gives the number of s.d. the outcome/dependent variable will change as a result of one s.d. change in the predictor/independent variable. Differences in lean mass between quartiles of FE₂ and FT were assessed using ANOVA followed by Tukey's *post hoc* test (Fig. 1). All calculations were performed with the SPSS Statistical Software (version 13.0, SPSS, Chicago, IL, USA). Values are given as means $\pm$ s.d., unless otherwise indicated.

Table 4 Hypogonadism in relation to body mass index (BMI) status for the subjects with morning serum samples ($n=1830$).

	All subjects	Normal weight (<25)	Overweight (≥ 25 and <30)	Obese (≥ 30)	P value
Hypogonadal (testosterone <3 ng/ml)	292/1830 (16.0%)	63/704 (8.9%)	155/887 (17.5%)*	74/239 (31.0%)*	
Testosterone (ng/ml)	4.63 ± 1.90	5.15 ± 1.91	$4.48 \pm 1.81^\dagger$	$3.66 \pm 1.71^\dagger$	<0.001
FT (ng/ml)	0.083 ± 0.035	0.087 ± 0.035	$0.083 \pm 0.033^\dagger$	$0.071 \pm 0.035^\dagger$	<0.001
SHBG (nmol/l)	44.1 ± 22.1	50.4 ± 24.8	$41.3 \pm 19.0^\dagger$	$36.5 \pm 20.4^\dagger$	<0.001

Hypogonadism was defined as serum testosterone <3 ng/ml (10.4 nmol/l) (5). Normal weight = BMI <25 kg/m² ($n=704$ (38%)), overweight = BMI ≥ 25 and <30 kg/m² ($n=887$ (48%)), obese = BMI ≥ 30 kg/m² ($n=239$ (14%)). Differences in prevalence of hypogonadism according to BMI status were assessed using logistic regression. * $P<0.01$ versus normal weight. Differences in serum levels of testosterone, FT, and SHBG according to BMI status were calculated using ANOVA followed by Tukey's *post hoc* test. $^\dagger P<0.01$ versus normal weight.

Results

The general characteristics of the elderly male cohort (mean 75 years of age) are shown in Table 1. The lean mass and fat mass levels are clearly distinct when comparing the subjects analyzed by the Hologic DEXA and the Lunar DEXA and, therefore, all subsequent analyses of the associations between serum sex steroids and DEXA-derived parameters were performed separately for the Hologic and Lunar subcohorts (Tables 3 and 5–7, Fig. 1). In general, all significant associations between serum sex steroid levels and the three primary outcome measures (fat mass versus testosterone, lean mass versus E₂, and central fat distribution versus SHBG) showed a similar pattern in both the Hologic and Lunar subcohorts. All the subjects in the Hologic cohort had morning serum samples before 1000 h ($n=943$). Slightly more than half of the subjects in the Lunar cohort (830/1635) had morning samples before 1000 h, while the remaining samples in the Lunar cohort were drawn around noon (between 1000 and 1500 h, average 1300 h for the noon samples). Serum levels of E₂ (-7.1%), FE₂ (-5.4%), testosterone (-9.1%), FT (-7.0%), and SHBG (-5.6%) were slightly lower in 31% of the subjects not having morning samples compared with the 69% of the subjects with morning samples. The associations between serum sex steroids and body composition for the Lunar cohort are, therefore, shown both for all the subjects and for the subjects with morning samples (the latter within brackets; Tables 2, 3, 5–7, Fig. 1). All major associations between serum sex steroids and body composition parameters reported in this study were similar for the whole cohort and the subcohort only including the subjects with morning samples (Tables 2, 3, 5–7, Fig. 1).

Associations between serum levels of sex steroids and BMI

To investigate the correlations between serum levels of sex steroids and parameters reflecting body composition, univariate association analyses were performed. Serum levels of both testosterone and FT associated robustly negatively with body weight and BMI

(r^2 ranging from 2.6 to 10.2%), with serum testosterone levels explaining 10.2% of the variance (r^2) in BMI (Table 2). Serum levels of FE₂ (r^2 1.7%), but not E₂, associated weakly, but statistically significantly, with body weight and BMI. SHBG levels correlated inversely with body weight and BMI. Height was not associated with serum sex steroid levels (Table 2).

Elevated proportion of hypogonadism among obese men

When using serum testosterone <3 ng/ml (10.4 nmol/l) as a threshold for hypogonadism (5), 16% of the elderly men with morning serum samples were classified as hypogonadal (Table 4). Using this threshold, obese men (BMI ≥ 30 kg/m²) were more often classified as hypogonadal than men with normal weight (BMI <25 kg/m²;

Table 5 Independent associations between serum levels of sex steroids and parameters reflecting fat mass and fat distribution.

	Hologic $n=943$	Lunar $n=1635$ ($n=830$)
Total body fat mass		
E ₂	0.17	0.29 (0.26)
Testosterone	-0.43	-0.51 (-0.48)
SHBG	0.06	-0.03 (-0.03)
Total body fat percentage		
E ₂	0.19	0.30 (0.26)
Testosterone	-0.49	-0.52 (-0.48)
SHBG	0.08	-0.04 (-0.03)
Trunk fat mass		
E ₂	0.17	0.29 (0.25)
Testosterone	-0.38	-0.47 (-0.44)
SHBG	0.01	-0.06 (-0.06)
Central fat distribution		
E ₂	0.04	-0.01 (0.02)
Testosterone	0.00	0.05 (0.06)
SHBG	-0.18	-0.16 (-0.15)

Linear regression analysis of the association between serum sex steroid levels and fat parameters as analyzed by dual-energy X-ray absorptiometry (DEXA). The regression model consisted of a fat mass parameter as the dependent variable and age, height, a corresponding lean mass parameter, and serum E₂, testosterone and SHBG as independent variables. Central fat distribution is calculated as trunk fat mass/total body fat mass $\times 100$. Central fat distribution was also adjusted for total body fat mass. Values within brackets for the Lunar DEXA are for the subjects with morning samples before 1000 h. All the subjects analyzed by the Hologic DEXA had morning samples before 1000 h. Standardized β values are given. Bold indicates $P<0.05$.

Table 6 Independent associations between serum levels of sex steroids and lean mass.

	Hologic <i>n</i> =943	Lunar <i>n</i> =1635 (<i>n</i> =830)
Total body lean mass		
E ₂	0.08	0.10 (0.12)
Testosterone	0.05	−0.04 (−0.07)
SHBG	− 0.13	− 0.06 (−0.04)
Legs lean mass		
E ₂	0.13	0.12 (0.13)
Testosterone	0.00	−0.04 (−0.05)
SHBG	− 0.12	− 0.05 (−0.03)

Linear regression analysis of the association between serum sex steroid levels and lean mass as analyzed by dual-energy X-ray absorptiometry (DEXA). The regression model consisted of a lean mass parameter as the dependent variable and age, height, a corresponding fat mass parameter, and serum E₂, testosterone and SHBG as independent variables. Values within brackets for the Lunar DEXA are for the subjects with morning samples before 1000 h. All the subjects analyzed by the Hologic DEXA had morning samples before 1000 h. Standardized β values are given. Bold indicates $P<0.05$.

odds ratio, OR 4.6, 95% confidence interval, CI 3.1–6.7). Serum testosterone was 29% lower in the obese men than in men with normal weight. As serum SHBG was lower in the obese men than in men with normal weight (−28%), the reduction in FT was less pronounced (−18%) than the reduction in total testosterone (Table 4).

Associations between serum levels of sex steroids and fat measurements

Since BMI is composed of a lean and a fat component, we further explored the associations between serum levels of sex steroids and lean and fat mass. Serum testosterone and FT associated robustly negatively in a consistent manner with fat parameters in both subcohorts (e.g. total body fat mass, r^2 ranging from 4.8 to 13.7%, Table 3). No consistent association was seen between E₂ or FE₂ and fat parameters, except for a weak, but statistically significant, positive association between serum FE₂ and fat parameters in the sub-cohort investigated by the Lunar DEXA (Table 3).

We next evaluated the independent association between serum sex steroids and various fat parameters using multiple linear regression analyses (including age, height, a corresponding lean mass parameter, and serum E₂, testosterone, and SHBG as covariates). Serum testosterone associated independently inversely (standardized β values ranged from −0.38 to −0.52, $P<0.001$), while serum E₂ associated independently directly (standardized β values ranged from 0.17 to 0.30, $P<0.001$) with total body fat mass, total body fat percentage and trunk fat mass (Table 5). Multiple linear regression models including FT and FE₂ (and not including testosterone, E₂, and SHBG) showed similar results with an independent inverse association between FT and fat mass and an independent direct association between FE₂ and fat mass (data not shown).

Associations between serum levels of SHBG and fat measurements

SHBG levels correlated negatively with total body and trunk fat mass (Table 3). When looking at central fat distribution, defined as the ratio of trunk fat mass over total body fat mass, no independent (adjusted for total body fat) associations were found with serum levels of E₂ or testosterone (Table 5). In contrast, serum levels of SHBG associated negatively in a robust manner with central fat distribution in both subcohorts (r^2 : 8.4 and 6.2%, Table 3). This correlation remained independently (also after adjustment for total body fat) negative after multiple regression analysis (Table 5).

Associations between serum levels of sex steroids and lean mass

Serum levels of E₂ and FE₂ correlated weakly, but statistically significantly, positively in a consistent manner with total body lean mass and legs lean mass (r^2 ranging from 1.0 to 2.6%) in both the Hologic and Lunar cohorts (Table 3). In contrast, neither serum testosterone levels nor serum FT levels associated positively with lean mass. Serum SHBG levels associated negatively with lean mass (Table 3).

To evaluate the independent associations between serum sex steroid levels and lean mass, multiple linear regression analyses, including age, height, a corresponding fat mass parameter, and serum E₂, testosterone and SHBG as covariates, were performed. Serum levels of E₂ associated independently directly with lean mass measurements (Table 6). To account for non-linear terms, further adjustment not only for testosterone but also for testosterone² was performed, but this did not alter the positive associations between serum levels of E₂ and lean mass (data not shown). When analyzing levels of FE₂ and FT in the same multiple regression model, only serum FE₂ levels associated positively with total body and legs lean

Table 7 Independent associations between serum free sex steroid levels and lean mass.

	Hologic <i>n</i> =943	Lunar <i>n</i> =1635 (<i>n</i> =830)
Total body lean mass		
FE ₂	0.10	0.11 (0.13)
FT	0.05	−0.04 (−0.07)
Legs lean mass		
FE ₂	0.16	0.14 (0.14)
FT	−0.01	−0.05 (−0.06)

Linear regression analysis of the association between serum free sex steroid levels and lean mass as analyzed by dual-energy X-ray absorptiometry (DEXA). The regression model consisted of a lean mass parameter as the dependent variable and age, height, a corresponding fat mass parameter, and serum FE₂ and FT as independent variables. Values within brackets for the Lunar DEXA are for the subjects with morning samples before 1000 h. All the subjects analyzed by the Hologic DEXA had morning samples before 1000 h. Standardized β values are given indicates. Bold indicates $P<0.05$.

Figure 1 Serum FE₂ but not FT is associated with lean mass in elderly men. Mean legs lean mass (kg $\pm$ s.e.m.) in MrOS Sweden subjects analyzed by either the Hologic (A and B) or the Lunar DEXA (C and D), adjusted for age, height, fat mass, and either FE₂ (B and D) or FT (A and C) according to quartiles of serum FE₂ (A and C) or quartiles of serum FT (B and D). Limits of serum levels of FE₂ were ≤ 265 for quartile 1, > 265 and ≤ 349 for quartile 2, > 349 and ≤ 437 for quartile 3, and > 437 fg/ml for quartile 4. Limits of serum levels of FT were ≤ 61 for quartile 1, > 61 and ≤ 79 for quartile 2, > 79 and ≤ 99 for quartile 3, and > 99 pg/ml for quartile 4. Statistical analysis was performed by ANOVA followed by Tukey's *post hoc* test. * $P < 0.05$ versus quartile 1; † $P < 0.05$ versus quartile 2. P values in bar charts are linear trend for quartiles. NS, non significant.

mass (Table 7). To further explore the association between serum levels of FE₂ and lean mass, legs lean mass was plotted against quartiles of FE₂. Elderly men within the lowest quartile of FE₂ had significantly lower legs lean mass (-0.5 kg, $P < 0.01$; Fig. 1A and C) than the subjects within the highest quartile of FE₂. In contrast, legs lean mass did not significantly differ between men within the different quartiles of FT (Fig. 1B and D).

Discussion

Sex steroids might be involved in the regulation of body composition in men. The age-associated alterations in male body composition, including loss of lean mass and bone mass and increased fat mass, are thought to be related to the decrease in serum sex steroid levels with aging (1). Only a few studies have addressed the association between serum sex steroid levels and body composition in elderly men. In particular, the association between serum sex steroid levels and lean mass in elderly men remains unclear. We, herein, made the novel observation that serum levels of E₂, but not testosterone, associated directly with lean mass in elderly men. In addition, we confirmed previous studies demonstrating that serum levels of testosterone were

inversely associated with several parameters reflecting fat mass in men. Finally, serum SHBG was found to be an independent marker of central fat distribution.

Few large studies have examined the association between serum sex steroids and lean mass in elderly men. In this study, we found no significant independent association between serum total testosterone or FT and lean mass in elderly men. A few previous studies found a positive correlation between serum free androgens and muscle mass (17–19), but the absence of an association between lean mass and serum bioavailable testosterone in a large subgroup of elderly men in the MrOS US cohort (11) supports our present data. However, it should be emphasized that both in the MrOS US cohort and in the present study, circulating levels of testosterone were measured, while the availability or action of testosterone on muscle cell ARs could not be evaluated. Importantly, serum E₂, on the other hand, was positively associated with lean mass in the elderly men of MrOS Sweden, both unadjusted and after adjustment for confounding variables. Elderly men within the lowest quartile of FE₂ had 0.5 kg less lean mass in the legs than the subjects within the highest quartile of FE₂. Szulc *et al.* previously showed that older men from the MINOS study within the lowest quartile of bioavailable E₂ had lower lean body mass, although this association

did not hold after adjustment for covariates (10). In the present study, total body lean mass was assessed by DEXA, whereas actual muscle mass was not measured. However, when measuring legs lean mass, which more closely reflects real muscle mass, similar or even stronger associations were found. The cross-sectional design of this study did not allow us to investigate the possible causal relationship between serum E_2 and lean mass in elderly men. It is well established that androgens increase muscle mass in men (26–28), while the possible role of estrogens and ERs for muscle mass in men remains unclear. Clinical trials investigating the effects of aromatase inhibition in older men showed no effect of the intervention on body composition or muscle strength (29, 30) and, thus, did not support an important role of serum E_2 levels for lean mass in men. Nevertheless, animal studies have indicated that not only AR activation but also ER activation modulates lean mass (31–34). It was recently demonstrated that ER α activation is required for normal muscle mass in male mice (31). In addition, there are indications that both ER α and ER β activation modulate muscle mass and/or function in mice (32, 33). We also previously showed that E_2 , but not dihydrotestosterone, increased lean mass in orchidectomized, aged male rats (34). Finally, it is well established that estrogen treatment results in an anabolic effect on muscle mass in cattle (35). Further studies are therefore required to determine if the stimulatory effect of testosterone on muscle mass in elderly men does not only involve AR activation but also aromatization followed by ER activation. However, the positive association between serum E_2 and lean mass in the present study may also be the result of aromatase activity in muscle (36, 37). Alternatively, serum E_2 may merely be a general indicator of good health in elderly men. Supporting the possible importance of estrogens in elderly men is the fact that older men with low serum E_2 have reduced bone mineral density (38, 39) and increased risk of fractures (40, 41). Interestingly, it was recently demonstrated that serum E_2 was strongly associated with proximal femur strength in men, an association that was partially mediated by body composition (42).

Several previous studies have shown that circulating testosterone levels correlate inversely with waist circumference, BMI, total body fat mass, abdominal fat mass, and regional abdominal fat depots both in young adults and in older men (6–16). We confirm these findings here in our large cohort of elderly men by showing that serum testosterone associated inversely with several measures of fat mass. We found that the proportion of hypogonadal men, defined by using a threshold of serum testosterone < 3 ng/ml (10.4 nmol/l) (5), was significantly greater in the obese men (OR 4.6) than in men with normal weight. The obese men clearly had reduced serum SHBG levels and, therefore, FT was less reduced than the total testosterone. Thus, our data support previous reports

suggesting that, in order not to classify too many obese men as hypogonadal, adjustments for serum SHBG, such as the use of a threshold for FT, should be considered as additional criteria for hypogonadism (5, 12–14).

Regarding serum E_2 levels, we found that these levels, after adjustment for covariates, correlated directly with measures of fat mass in men. Similar associations were reported earlier in young adult men participating in the Odense Androgen Study (15) and in older Dutch men (9), whereas other studies reported no association between serum E_2 levels and fat mass measures in men (7, 8, 10).

In the present study, serum SHBG associated independently and inversely with central fat distribution in elderly men. In contrast, neither serum testosterone nor serum E_2 levels associated independently with this parameter, suggesting that serum SHBG is a specific indicator of central fat mass distribution. This is of particular interest with regard to the increased prevalence of abdominal obesity in the western population and the associated increased risk for cardiovascular morbidity and mortality, and the development of type 2 diabetes (43, 44). In this context, already more than 20 years ago serum SHBG was proposed as a risk factor for cardiovascular disease and death (45). Most recently, prospective studies of men and women showed that higher serum SHBG levels were strongly associated with a decreased risk for type 2 diabetes (46).

The major strength of the present study is that the serum levels of testosterone and E_2 were measured with the validated and highly specific GC–MS method. To our knowledge, this is the first study in which sex steroids have been analyzed using GC–MS, with the aim to study associations between androgens, estrogens, and body composition in men. Another strength of this study is the population-based nature and the large number of elderly subjects investigated. Limitations of the present study include its cross-sectional design. In addition, morning samples were only available for 69% of the MrOS Sweden subjects, while the remaining 31% of the subjects had serum samples drawn at 1300 h on average. Therefore, for all the parameters investigated, the associations between serum sex steroids and body composition parameters are given both for the subjects with morning samples and for the whole cohort. Importantly, all major associations reported were similar for the whole cohort and the subcohort only including subjects with morning samples. Another possible limitation is that different DEXA equipment (Hologic and Lunar) was used with different methodologies for assessing body composition within MrOS Sweden. We, therefore, decided to present the associations between serum sex steroids and body composition parameters separately for the Hologic and Lunar subcohorts. We believe that the rather similar associations between serum sex steroids and body composition parameters found for the Hologic and

Lunar subcohorts support the validity of the findings in the present study. We acknowledge that multiple testing should be considered in our study. However, we were interested in the general pattern of associations and, therefore, we only considered the primary associations that were consistently found in both subcohorts (fat mass versus testosterone, lean mass versus E_2 , and central fat distribution versus SHBG) for further analysis and discussion. If we would have combined both subcohorts, we would have had a lower number of tested associations as well as more significant associations found (combined P value for both subcohorts <0.01). Yet, we preferred to keep both subcohorts separated to be able to show the original data and demonstrate that similar data were obtained in both subcohorts using different DEXA methodology. Furthermore, our results are limited to Caucasian men and, thus, may not apply to other populations.

In conclusion, we made the novel observation that serum E_2 but not serum testosterone was associated with lean mass in this population-based, cross-sectional study of elderly Swedish men. In addition, serum SHBG was found to be associated with central fat distribution, and we confirmed previous studies demonstrating that serum testosterone was inversely associated with fat mass.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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