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CLINICAL STUDY

Measures of bioavailable serum testosterone and estradiol and their relationships with muscle mass, muscle strength and bone mineral density in postmenopausal women: a cross-sectional study

Tineke A C M van Geel, Piet P Geusens[1], Bjorn Winkens[2], Jean-Pierre J E Sels[1] and Geert-Jan Dinant

Department of General Practice, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands, [1]Department of Internal Medicine, Maastricht University Medical Centre, PO Box 5800, 6202 AZ Maastricht, The Netherlands and [2]Department of Methodology and Statistics, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands

(Correspondence should be addressed to T A C M van Geel; Email: t.vangeel@hag.unimaas.nl)

Abstract

Objective: The physiologic role of circulating endogenous testosterone and estrogen concentrations in relation to lean body mass (LBM) and muscle strength is not as well documented in postmenopausal women as in elderly men.

Design: Three hundred and twenty-nine healthy postmenopausal women were randomly selected from a general practice population-based sample aged between 55 and 85 years.

Methods: Total testosterone and estrogen (TT and TE) and sex hormone-binding globulin (SHBG) were determined and estimates of bioavailable testosterone (free androgen index (TT/SHBG, FAI), calculated free testosterone (cFT), and estrogen (TE/SHBG, ESR) were calculated. Examinations included bone mineral density (BMD) of the spine and femoral neck (FN), LBM, maximum quadriceps extension strength (MES) and maximum handgrip strength (MGS), timed up-and-go test (TUGT), osteocalcin (OC), and urinary deoxy-pyridinoline/creatinine (DPyr). Correlations were assessed using Pearson's correlation coefficient (r).

Results: With advancing age, LBM, MES, MGS, BMD, and ESR significantly declined (range r: -0.356 to -0.141) and TUGT, and DPyr significantly increased (range r: 0.135 to 0.282 ($P<0.05$)). After age-adjustment, LBM, MES, and BMD in spine and FN were significantly related to bioavailable testosterone (range r: 0.146 to 0.193, for cFT, and 0.157 to 0.224, for FAI) and to ESR (range r: 0.162 to 0.273). OC and DPyr were significantly inversely related to ESR (r: -0.154 and -0.144 respectively).

Conclusions: Age-related loss of LBM, MES and BMD in postmenopausal women is partly dependent on the presence of endogenous bioavailable testosterone and estrogen.

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Introduction

It is well documented that part of the bone loss that occurs with ageing in postmenopausal women and elderly men is related to a decrease in serum levels of bioavailable estrogen and testosterone (1–5), and that drugs with estrogenic and/or androgenic properties can be used to prevent age-related bone loss (1, 6, 7).

It has been shown that part of the loss of lean (fat-free) body mass, muscle mass, and maximum voluntary strength that occurs with ageing in elderly men is related to bioavailable testosterone (1, 8), and most, though not all, studies have found that testosterone administration to hypogonadal and eugonadal men is associated with dose- and concentration-dependent increase in lean mass, muscle mass, and maximum voluntary strength (7–10). In men, testosterone increases muscle protein synthesis and satellite cell number, probably by stimulating the differentiation of mesenchymal pluripotent cells to the myogenic lineage (10), resulting in muscle fiber hypertrophy and myonuclear number increase (10, 11).

In postmenopausal women, not enough is known about the relation between serum estrogen and the decrease in muscle mass and muscle strength, and the physiological significance of low testosterone in relation to muscle mass and muscle strength remains unclear (12, 13). Data on the effects of estrogen and testosterone administration on muscle mass and muscle strength in postmenopausal women are scarce, much more so than those for elderly men (14, 15).

Serum testosterone and estrogen are mainly bound to sex-hormone binding globulin (SHBG) and albumin. Albumin-bound and free testosterone and estrogen are

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believed to have access to target tissues (1, 16). Several methods to calculate free testosterone from adequately measured testosterone and SHBG by immunoassay are available and some have been shown to be a reliable index of bioavailable testosterone (17). The same holds for estrogen (1).

Therefore, the main analyses in this cross-sectional study of 329 postmenopausal women, focused on the hypothesis that the decreases in muscle mass and muscle strength with age are related to the drop in circulating endogenous testosterone and estrogen concentrations.

Materials and methods

Subjects

Twenty general practitioners from 10 general practice centers participated in the original population-based cohort study. The region consists of two towns in the southern part of the Netherlands, surrounded by suburban villages (18). We drew a random sample of 370 postmenopausal women aged between 55 and 85 years from the 4203 women who were included in the original study. All participating women signed an informed consent form.

Muscle mass and muscle strength

The assessments started in 1998–1999 with weight (in kg), height (in cm) and total body lean and fat mass (including head, LBM and FBM), measured by dual-energy X-ray absorptiometry (DXA; Hologic QDR-1000, Hologic Europe, Brussels, Belgium).

Quadriceps extension strength was measured using the Chatillon DFIS-200 (Largo, FL, USA), and handgrip strength using the Jamar dynamometer (Sammons Preston, Jackson, MI, USA). Quadriceps muscle strength was measured thrice in both legs and the handgrip strength thrice in both hands. Maximum quadriceps extension (MES) and handgrip strength (MGS) were defined as the maximum strength of the left or right leg/hand, whichever was the largest.

Mobility was tested using the timed up-and-go test (TUGT)(19) that measures the time needed to get up from a chair, walk three meters, turn, walk back, and sit down again.

Hormone measurements

Blood samples were collected in the morning after an overnight fast and first morning urine samples were collected as well. All laboratory tests, except for urinary creatinine (performed measured with the methods used in routine practice), were obtained with the IMMULITE device (Diagnostic Products Corporation (DPC), Los Angeles, CA, USA).

RIA was used to measure SHBG, intra-coefficient of variation (CV): 2.4–4.4% and inter-CV: 3.7–7.0%), testosterone (intra-CV of 7.1 to 13.0% and inter-CV of 7.7 to 16.4%) and E_2 (E, intra-CV of 4.3 to 9.9% and inter-CV of 6.7 to 16.0%) (20).

Based on these values, parameters of bioavailable testosterone and estrogen were calculated according to the methods proposed in the endocrine literature (Table 1). These included the free androgen index (FAI) and calculated free testosterone (cFT). cFT was calculated using the value of 'binding of testosterone to SHBG' constant by Vermeulen *et al.* (KTS, Table 1) (17).

Bone turnover markers (BTM) including osteocalcin (OC, intra-CV of 2.2–4.0% and inter-CV of 5.1–9.9%) and deoxypyridinoline/creatinine ratio (DPyr, intra-CV of 3.1–10.0% and inter-CV of 4.3–12.0%) were measured using RIA.

Bone mineral density

Bone mineral density (BMD) was measured at the lumbar spine and at the femoral neck (FN) by the same DXA instrument as used for LBM and FBM.

The quality control is performed in cooperation with the supplier of the DXA devices (Hologic). In addition, daily quality control was performed by the Department of Nuclear Medicine/Radiology. All measurements were performed in one center using the same device.

Statistical analysis

We based our analysis on methods similar to those used by Van den Beld *et al.* (1).

Table 1 Measurements and calculations for the various concentrations of testosterone and estrogen.

	Measurements and calculations
SHBG (nmol/l)	RIA (DPC, IMMULITE2000, Los Angeles, CA)
TT (nmol/l)	RIA (DPC, IMMULITE2000, Los Angeles, CA)
FAI (% M/M)	FAI = 100 * TT/SHBG, with both in nmol/l
cFT (pmol/l)	According to Vermeulen *et al.* (17): $cFT = (-b + \sqrt{(b^2 + 4a\,(TT))})/2a$ a = KTA + KTS + (KTA × KTS) ((SHBG) + (albumin) − (TT)) b = 1 + KTS (SHBG) + KTA (albumin) − (KTA + KTS) (TT)
FTR (% M/M)	FTR = (cFT)/(TT), with cFT in pmol/l, TT in nmol/l
Estradiol (pmol/l)	RIA (DPC, IMMULITE2000, Los Angeles, CA)
ESR (pM/nM)	ESR = (E_2)/(SHBG), with E_2 in pmol/l, SHBG in nmol/l
Osteocalcin (mg/l)	RIA (DPC, IMMULITE2000, Los Angeles, CA)
Dpyr (nM/mM)	Ppyr = (PYR)/(CRE), with PYR in nmol/l, CRE in mmol/l

SHBG, sex-hormone binding globulin; TT, total testosterone; FAI, free androgen index = total testosterone/SHBG; cFT, calculated free testosterone; FTR, free testosterone/total testosterone ratio; KTS, value of the 'binding of testosterone to SHBG' constant (l/mol), 10.0 * t8, per (Vermeulen); KTA, value of the 'binding of testosterone to albumin' constant (l/mol), 3.60 * t4, per (Vermeulen); t4 = 10^4; (17) ESR = E_2/SHBG ratio; DPyr, deoxypyridinoline/creatinine ratio.

The relation between muscle strength, biochemical markers, and BMD with age was assessed using Pearson's correlation coefficient (r). The r shows the number of S.D. that the outcome will change as a result of one S.D. change in age (21). The differences between the highest and the lowest age-groups were calculated using the variables which significantly correlated with age. Partial correlation coefficients (partial r) were analyzed to assess the age-adjusted relation between LBM, muscle strength parameters, and biochemical markers. The age- and body mass index (BMI)–adjusted relation between these variables was also calculated.

SPSS software (version 15.0; SPSS Inc., Chicago, IL, USA) was used for the statistical analysis.

Ethics

The ethics review committee of the Maastricht University and the Maastricht University Hospital approved the study (reference number MEC 94-196.1).

Results

Of the 352 women who agreed to participate (response rate = 95.1%), nine women were excluded from further analyses due to severe mobility problems (e.g., wheel-chair dependent, leg prosthesis, Parkinson's disease, amyotrophic lateral sclerosis). Biochemical markers were available for 329 of the remaining 343 women (95.9%).

Changes in testosterone, estrogen, muscle mass, muscle strength, and BTM with age

Table 2 presents the descriptive data. LBM, MES, and MGS declined with ageing, while the TUGT significantly increased with increasing age (Table 3). Women in the lowest age-group (<60 years, $n=49$, 14.9% of the total) and in the highest age-group (>75 years, $n=45$, 13.7% of the total) were compared in terms of the significant variables. LBM, MES and MGS declined by 4.9% ($P=0.049$), 29.9 and 24.3% ($P<0.001$) respectively, while the TUGT increased by 39.0% ($P<0.001$).

The descriptive data of the hormone concentrations and BTM are presented in Table 4, while Table 5 presents the relation between hormones and BTM and age. The IMMULITE is suitable for measurements at low concentration levels expected for postmenopausal women (20). The intra-assay precision profile (intra CV) for estradiol at a mean of 23.1 pg/ml (84.9/3.671) and total testosterone (TT) at a mean of 43.3 ng/dl (1.5/0.03467) are ~11.5 and 10.8% respectively (22, 23).

ESR significantly decreased with ageing, while DPyr significantly increased with ageing. Again, women in the lowest age-group were compared with the highest age-group in terms of the significant variables. ESR declined by 22.4%, while DPyr increased by 16.7%.

BMI was not significantly related to either MES (partial r: 0.102, $P=0.08$) or MGS (partial r: −0.044, $P=0.423$), but was significantly related to the TUGT (partial $r=0.154$, $P<0.01$). After age-adjustment TT

Table 2 Descriptive data of the study population ($n=329$).

	Mean	S.D.	Interquartile range
BMD lumbar spine (g/cm^2)	0.937	0.170	0.817–1.035
BMD lumbar spine (*T*-score)	−1.29	1.54	−2.40–−0.40
BMD femoral neck (g/cm^2)	0.703	0.119	0.622–0.770
BMD femoral neck (*T*-score)	−1.92	1.19	−2.70–−1.23
Age (years)	66.9	6.51	61.4–71.5
Postmenopausal years	20.1	8.09	14.0–26.0
BMI (kg/m^2)	27.6	4.57	24.7–29.7
LBM (kg)	41.9	4.90	38.4–44.9
Left arm (kg)	2.09	0.35	1.85–2.29
Right arm (kg)	2.20	0.35	1.94–2.39
Trunk (kg)	21.3	2.77	19.4–22.8
Left leg (kg)	6.48	0.90	5.81–7.12
Right leg (kg)	6.73	0.97	6.01–7.42
Head (kg)	3.02	0.28	2.80–3.23
FBM (kg)	25.9	6.93	21.7–30.1
Left arm (kg)	1.78	0.69	1.34–2.04
Right arm (kg)	1.88	0.71	1.39–2.20
Trunk (kg)	12.6	4.16	10.2–14.8
Left leg (kg)	4.27	1.26	3.57–5.20
Right leg (kg)	4.45	1.26	3.57–5.20
Head (kg)	0.90	0.10	0.83–0.97
MES (kg)	23.4	7.77	17.8–28.0
MGS (kg)	26.4	5.90	22.0–30.0
TUGT (s)	13.4	7.78	9.90–14.6

S.D., standard deviation; BMD, bone mineral density; LBM, lean body mass; FBM, fat body mass; MES; maximum extension quadriceps strength; MGS, maximum handgrip strength; TUGT, timed up-and-go test.

Table 3 Relationship between physical characteristics and age ($n=329$).

	Age (years)	
	r	*P* value
BMD lumbar spine (g/cm^2)	−0.152	0.006
BMD lumbar spine (*T*-score)	−0.149	0.007
BMD femoral neck (FN, g/cm^2)	−0.290	<0.001
BMD femoral neck (*T*-score)	−0.300	<0.001
BMI (kg/m^2)	−0.053	0.348
LBM (kg)	−0.166	0.003
Left arm (kg)	−0.150	0.008
Right arm (kg)	−0.149	0.009
Trunk (kg)	−0.149	0.008
Left leg (kg)	−0.138	0.015
Right leg (kg)	−0.127	0.025
Head (kg)	−0.022	0.700
FBM (kg)	0.033	0.557
Left arm (kg)	0.032	0.579
Right arm (kg)	0.039	0.490
Trunk (kg)	0.077	0.173
Left leg (kg)	−0.040	0.487
Right leg (kg)	−0.028	0.628
Head (kg)	0.018	0.757
MES (kg)	−0.318	<0.001
MGS (kg)	−0.356	<0.001
TUGT (s)	0.282	<0.001

r, Pearsons correlation coefficient; *P*, level of significance; BMD, bone mineral density, BMI, body mass index; LBM, lean body mass; FBM, fat body mass; MES, maximum extension quadriceps strength; MGS, maximum handgrip strength; TUGT, timed up-and-go test.

(partial $r=0.075$, $P=0.178$) was not significantly related to BMI, but the FAI (partial $r=0.215$, $P<0.001$) and cFT were significantly related to BMI (partial $r=0.3274$, $P<0.001$).

SHBG and FAI or cFT (partial *r*: −0.440 or −0.348 respectively) were significantly negatively related, while the FAI and cFT or TT (partial *r*: 0.947 or 0.672 respectively) were positively related ($P<0.001$). The relations between estradiol and SHBG (partial *r*: 0.187), ESR and SHBG, FAI or cFT (partial *r*: −0.556, 0.532 or 0.330 respectively) were also significant ($P<0.001$).

Table 4 Summed values of hormone levels in serum ($n=329$).

	Mean	**S.D.**	**Interquartile range**
SHBG (nmol/l)	53.3	30.2	34.4–63.0
TT (nmol/l)	1.50	0.959	0.689–2.00
FAI (% M/M)	3.66	3.41	1.54–4.53
cFT (pmol/l)	22.4	17.4	10.7–28.2
FTR (% M/M)	1.49	0.487	1.17–1.76
Estradiol (E_2, pmol/l)	84.9	47.5	73.4–73.4
E_2/SHBG ratio (ESR, pM/nM)	2.06	1.49	1.22–2.26
Osteocalcin (mg/l)	3.47	2.32	1.68–4.70
DPyr (nM/mM)	6.29	2.34	4.87–7.24

S.D., standard deviation. FAI, TT/SHBG; FTR, cFT10/TT; DPyr, Pyrilinks-D/creatinine; SHBG, sex-hormone binding globulin; TT, total testosterone; FAI, free androgen index; cFT, calculated free testosterone; FTR, free testosterone/total testosterone ratio; DPyr, deoxypyridinoline/creatinine ratio.

Table 5 Relationship between age and hormone levels ($n=329$).

	Age (years)	
	r	*P* value
SHBG (nmol/l)	0.091	0.102
TT (nmol/l)	0.034	0.540
FAI (% M/M)	−0.080	0.153
cFT (pmol/l)	−0.043	0.438
FTR	−0.142	0.011
Estradiol (E_2, pmol/l)	−0.007	0.901
E_2/SHBG ratio (ESR, pM/nM)	−0.141	0.011
Osteocalcin (mg/l)	0.050	0.372
DPyr (nM/mM)	0.135	0.015

r, Pearson's correlation coefficient; SHBG, sex-hormone binding globulin; TT, total testosterone; FAI, free androgen index; CFT, calculated free testosterone; FTR, free testosterone/total testosterone ratio; DPyr, deoxypyridinoline/creatinine ratio.

As regards to testosterone, higher values of FAI and cFT were significantly positively related with LBM, FBM, and MES, after age-adjustment (Table 6).

As regards to estrogen, only higher values of ESR significantly increased LBM and FBM; it was not significantly related to the other muscle strength parameters (Table 6).

In addition, higher values of the BTM osteocalcin significantly increased LBM, (partial $r=-0.117$ ($P<0.05$)), while higher values of DPyr significantly decreased MGS (partial $r=-0.149$ ($P<0.01$)) and significantly increased the TUGT (partial $r=0.126$ ($P<0.05$)).

The relation of testosterone, estrogen, and BTM with BMD

After adjustment for age, higher values of FAI, cFT, estradiol, and ESR were significantly positively related with BMD measured at the lumbar spine and at the FN (Table 6).

Of the BTM, higher values of OC significantly declined BMD measured at the lumbar spine (partial $r=-0.135$ ($P=0.016$)) and at the FN (partial $r=-0.171$ ($P=0.003$)).

Discussion

The results show that age-related loss of LBM, MES, and BMD is partly dependent on the presence of endogenous bioavailable testosterone and estrogen concentrations.

In contrast to the well-documented relations of estrogen and testosterone with BMD in women and men and with muscle strength and mass in men, not much attention has been given to the relations of testosterone and estrogen with muscle mass and muscle strength in postmenopausal women (12, 14). A study involving 20 women aged between 43 and 73 years old found a significant relation between free testosterone

Table 6 Age-adjusted relations between hormone levels and physical characteristics.

	Partial *r*						
	LBM	**FBM**	**MES**	**MGS**	**TUGT**	**BMD L2–L4**	**BMD FN**
SHBG (nmol/l)	−0.282*	−0.210*	−0.083	−0.064	−0.066	−0.094	−0.129‡
TT (nmol/l)	0.016	0.011	0.104	−0.045	−0.051	0.101	0.047
FAI (% M/M)	0.224*	0.148†	0.193*	−0.021	0.005	0.157†	0.138‡
cFT (pmol/l)	0.167†	0.190*	0.178†	−0.017	−0.013	0.146†	0.193*
FTR (% M/M)	0.332*	0.354*	0.103	0.040	0.076	0.138‡	0.258*
Estradiol (E_2, pmol/l)	0.003	0.004	−0.014	−0.005	0.089	0.201*	0.188*
E_2/SHBG ratio (ESR, pM/nM)	0.274*	0.175†	0.100	−0.010	0.071	0.162†	0.168†

SHBG, sex-hormone binding globulin; FAI, total testosterone/SHBG; cFT, calculated free testosterone; FTR, free testosterone/total testosterone ratio; LBM, total lean body mass; FBM, total fat body mass; MES, maximum extension strength; MGS, maximum handgrip strength; TUGT, timed up-and-go test; BMD, bone mineral density; L2–L4, lumbar spine; FN, femoral neck. *$P<0.001$; †$P<0.01$; ‡$P<0.05$; partial *r*, partial correlation.

and muscle mass and strength (13). Another study found that 46 postmenopausal women aged between 46 and 55 years old who used estrogens had decreased bioavailable testosterone and that this decrease was related to a decrease in LBM (24). In our population of 329 postmenopausal women, the age-related decreases in LBM, MES, and BMD in spine and FN were related to bioavailable testosterone (FAI, FTR) and estrogen (ESR).

Changes in testosterone, estrogen, muscle mass, muscle strength, and BTM with age

TT and total estrogen (TE) remained unchanged with ageing in our study. Cappola found a decrease in TT until the age of 80 years, but similar values in 80+ compared with 65–68 years-old women, and no significant relation with the number of years since menopause (12). Risk factors for low testosterone have been documented in women aged 65 years and older and include bilateral oophorectomy, estrogen use, corticosteroid use, and low BMI (12). We found significant relations between bioavailable testosterone and estrogen and BMI.

Although, bioavailable estrogen (ESR) and testosterone (FTR) decreased with age, FAI, which is considered as a reliable index of free testosterone (17), did not change with age, as was also found by Cappola who measured free testosterone directly by dialysis (12).

As expected, we found significant loss of LBM, MES, MGS, and BMD in the spine and hip and an increase in TUGT with increasing age. The loss of MES (−32%) and MGS (−23%) between the ages of <60 years and >75 years was much more pronounced than the loss of LBM (−5%), indicating also a decrease in muscle efficiency with ageing (25). Such losses of muscle mass and strength results in sarcopenia, which is a risk factor for falls and decreased quality of life (25).

Bone resorption (DPyr) increased with age, as has also been found by others (4). OC remained unchanged, while others found an increase in OC with age, but with unchanged bone alkaline phosphatase and carboxy-terminal propeptide of type I procollagen (PICP) (4).

Relations of testosterone and estrogen with muscle mass and muscle strength

LBM and MES was significantly related to FAI and ESR. However, our age-adjusted analysis found FAI to be related to both LBM and MES, while ESR was only related to LBM. In addition, MES was independently related to LBM and FAI, but not to ESR. This indicates that muscle mass is testosterone and estrogen dependent, while muscle strength depends only on testosterone.

Although the effect of testosterone administration on muscle is variable, several intervention studies have reported that it leads to increased LBM, muscle size, and maximum voluntary strength in elderly men (10). The effect of testosterone and estrogen on muscle in postmenopausal women is still unclear (14). One confounder in the search for the relation between estrogen and muscle in intervention studies is the finding that estrogen replacement in postmenopausal women leads to decreased testosterone concentrations, and this decrease in testosterone is associated with a decreased LBM (14). By contrast, the combination of testosterone and estrogen leads to increased muscle mass (10).

Unlike MES, MGS was not related to any total or bioavailable testosterone or estrogen values. This is surprising, as MES, MGS, and LBM were highly intercorrelated. The decrease in muscle mass, strength, and power with ageing is caused by atrophy of muscle fibers, particularly those of type IIa. Testosterone binds to the androgen receptor in muscle tissue and stimulates protein synthesis in both type I (slow, aerobic) and type II (fast, anaerobic) muscle fibers. It is possible that the differences in the association of testosterone with MES and MGS are the result of a greater effect of testosterone in the most disused muscle, i.e., the quadriceps, in which the loss of maximum strength has been found to be 49% larger than the loss of MGS (26). Another possibility is that androgens mainly affect proximal limb muscles. Proximal muscle weakness is a typical clinical feature of Kennedy's disease, a genetic neurodegenerative disorder related to the androgen receptor, and in a

knock-out animal model of this disease, mutant males exhibited androgen-dependent neuromuscular weakness (27). These findings suggest that measuring proximal limb muscle strength such as MES is mandatory in prospective studies of the effect of estrogen and testosterone on muscle strength.

No relation was found between testosterone and estrogen and TUGT, although the TUGT was significantly related to LBM and MES. The TUGT is, however, not only dependent on muscle mass and strength but also on coordination and balance. In a recent study, in elderly men testosterone supplementation did increase LBM but without any effect on the TUGT. Additional studies will be needed to analyze the relation between testosterone and estrogen and muscular functional repercussions.

Relation of testosterone and estrogen with BMD

The results of our study confirm the known relations between bone and circulating estrogen and testosterone in postmenopausal women (5). Circulating estrogen and testosterone levels are associated with BMD (3, 4) and bone size at most skeletal locations (2). Circulating estrogen levels are also related to hip fracture risk and height loss (28), but not to vertebral fractures (29). We found that both the FAI and ESR were independently related to FN BMD, as has also been reported by others for postmenopausal women (4) and in elderly men (1). Prospective studies in elderly men indicate that testosterone is an independent risk factor for future fracture, but data for women are not available.

The BTM (OC and DPyr) were negatively related to ESR and FTR, but not to the FAI. This relation has also been found by others for estrogen and testosterone levels (4). These data confirm that low estrogen and testosterone are related to increased bone turnover in postmenopausal women, which is a risk factor for fractures (30, 31). The data suggest that the relation between BMD and estrogen and testosterone is in part mediated by the level of bone turnover. Indeed, we found OC to be negatively related to BMD in the spine and FN.

The pluripotent effects of testosterone and estrogen on bone and muscle open up new avenues for further research into interventions to increase muscle mass and strength. One option would be the development of selective androgen receptor modulators, some of which have been shown to have potent anabolic effects on bone and muscle (10, 32).

This study was subjected to some limitations. First, it was a cross-sectional population-based study, and longitudinal studies will be necessary to confirm our conclusions. Secondly, the women in this study were aged between 55 and 85 years. No data were available on women aged 85 years and over. Thirdly, no measured values of free testosterone and free estrogen were available, but only free testosterone calculated and free estrogen. Fourthly, 25-hydroxyvitamin D (25(OH)D) was not measured. There are studies that show that women with a hip fracture history, with extremely low 25(OH)D levels had reduced lower extremity muscle function (33) and another study showed that higher $1.25(OH)_2D_3$ concentrations are associated with a lower fall risk in older women (34).

We conclude that in postmenopausal women, age-related loss of LBM, MES (but not MGS), and BMD is partly dependent on the presence of endogenous bioavailable testosterone and estrogen. In addition to the well-known effects on bone, bioavailable testosterone and estrogen seem to play a role in the loss of lean mass and muscle strength that occur after menopause, although the cross-sectional nature of the study precludes a definitive conclusion.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

TACMG is the principle investigator and the main author of the manuscript. PPG and GJD are the supervisors of the principle investigator and are, as well as JPJES, responsible for the medical and scientific content of the manuscript. BW is responsible for the statistical analyses of the data. All authors have read and approved the manuscript and agreed with publication of their names.

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