High aromatase activity in hypogonadal men is associated with higher spine bone mineral density, increased truncal fat and reduced lean mass

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Abstract

Objective: Because the aromatase enzyme catalyzes the conversion of testosterone to estradiol (E₂), the activity of this enzyme could be important in the musculoskeletal health of men with low testosterone. The objective of the present study is to determine the influence of aromatase activity on the bone mineral density (BMD) and body composition of patients with hypogonadism.

Design: Cross-sectional study.

Methods: The baseline data of 90 patients between 40 and 74 years old who participated in a genetic study of response to testosterone therapy in men with low testosterone (i.e., <300 ng/dl) were analyzed. BMD and body composition were measured by dual-energy X-ray absorptiometry. Serum testosterone was measured by automated immunoassay, E₂ was measured by ultrasensitive enzyme immunoassay, and sex hormone-binding globulin was measured by enzyme immunoassay.

Results: Men in the highest tertile of E₂ to testosterone ratio (E₂:T) had the highest spine BMD ($P \leq 0.037$), highest truncal fat ($P = 0.046$), and lowest truncal lean body mass ($P = 0.045$). A similar pattern was observed in the upper extremities; that is, fat mass significantly increased ($P = 0.047$), whereas lean mass significantly decreased ($P = 0.034$) with increasing E₂:T tertiles.

Conclusion: The present findings suggest that in men with hypogonadism, aromatase activity could be an important determinant of musculoskeletal health. Men with high aromatase activity are able to maintain a higher BMD despite low circulating testosterone, but they have lower lean and higher truncal fat mass as compared to those with lower aromatase activity.

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Introduction

For years, bone loss associated with male hypogonadism was primarily attributed to a lack of testosterone. However, over the past two decades, estrogen has emerged as the main regulator of the adult male skeleton (1, 2, 3). In males, the main source of estrogen comes from the conversion of testosterone to estradiol (E₂) by the enzyme aromatase, which indicates the crucial role of this enzyme

in defining sex hormone levels in men. Although it is expected that low testosterone levels will also result in relative estrogen deficiency, inter-individual differences in aromatase activity and expression can profoundly impact sex hormone levels and, as a result, bone health (4). Given that the rate of conversion of testosterone to E₂ is dependent on aromatase activity (5, 6), it is possible that

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certain patients with low circulating testosterone actually have reasonable E_2 levels that are high enough to maintain adequate bone mineral density (BMD). Conversely, it is also possible that some patients may have higher or normal circulating levels of testosterone but very low E_2 levels because of low aromatase activity, which in turn may lead to a compromised BMD. For instance, patients with aromatase deficiency from an inactivating mutation of the aromatase gene have normal levels of testosterone but very low or undetectable E_2 levels, and this can result in osteopenia or osteoporosis (7, 8, 9, 10). Although testosterone administration has not been associated with improvement in BMD, estrogen treatment has been shown to result in significant increase in BMD, which confirms the vital role of testosterone conversion to E_2 on the male skeleton (7, 8, 9, 10). In the same way that sex hormone levels influence lean and fat mass composition and distribution among men in general, hypogonadal men could experience variations in body composition depending on their aromatase activity (10, 11). A male with high aromatase activity may be able to maintain an adequate bone mass but may suffer from low muscle mass, and vice versa. Thus, aromatase activity in patients with limited testosterone production may determine the particular phenotype of a hypogonadal man.

Although there have been several studies on hypogonadism and its effects on BMD and body composition (12, 13, 14, 15, 16), to our knowledge, there is little information on the influence of aromatase activity or of the conversion of testosterone to E_2 on BMD and body composition in these patients. This process could be critical for the maintenance of musculoskeletal health in patients with limited testosterone production. Thus, the objective of the present study was to determine the influence of aromatase activity on the BMD and body composition of patients with low testosterone as defined by a total testosterone of <300 ng/dl (17).

Subjects and methods

Study design and study population

The present study is a cross-sectional analysis of baseline data from subjects who volunteered to participate in a study that investigated the effect of genetics on testosterone therapy in male veterans with low total testosterone, which was defined by the Endocrine Society as a total testosterone of <300 ng/dl (17). That study was conducted at the New Mexico VA Health Care System in accordance with the guidelines in the Helsinki Declaration for the

ethical treatment of human subjects. The protocol was approved by the University of New Mexico Institutional Review Board. Participants were recruited from patients who were attending the Endocrine, Urology, and Primary Care Clinics of the New Mexico VA Health Care System. This was accomplished either through flyers or letters to physicians about patients who could qualify for the study. Written informed consent was obtained from each subject. Inclusion criteria included: males between 40 and 75 years of age with no medical problems that would prevent them from finishing the study. Those who were being treated with bone-acting drugs (e.g., bisphosphonates, glucocorticoids, sex-steroid compounds, selective estrogen receptor modulators, androgen deprivation therapy, or anticonvulsants) and those on finasteride were excluded from participation. Furthermore, those who had osteoporosis, a history of fragility fractures, or a disease known to affect bone metabolism, such as hyperparathyroidism, chronic liver disease, uncontrolled or untreated hyperthyroidism, or significant renal impairment, were excluded. Those with a history of prostate cancer, breast cancer, or untreated sleep apnea also met the criteria for exclusion.

BMI

Body weight was measured by a standard weighing scale, and height was obtained by a stadiometer. BMI was calculated as body weight in kilogram divided by the square of the height in meters (m^2) and was expressed as kg/m^2 .

Bone mineral density

BMD was measured using dual-energy X-ray absorptiometry (DXA) (Hologic-Discovery, Hologic, Inc., Bedford, MA, USA). The coefficient of variation (CV) for BMD at our center is 1.1% for the lumbar spine and 1.2% for the proximal femur.

Body composition

Total body mass, lean body mass (mineral-free and fat-free), fat mass, and truncal fat were measured by whole-body DXA (Enhanced Whole Body Software version 11.2; Hologic, Inc.) as previously described (18). The percentages of whole and regional fat mass (% fat) were obtained from the estimated readings given by the machine for each region of interest. The percentage of total and regional lean mass (% lean) was calculated as lean mass/total or regional mass. We used percentages of fat and lean body mass to correct for body size in the study

population. The CV for lean mass and fat mass in our laboratory is 1.5% (18).

Biochemical measurements

Blood samples were collected at baseline; serum samples were extracted and stored at -80°C until analysis, except for testosterone level screenings. Baseline serum testosterone represents the average of two determinations taken 30 min apart between 0800 and 1100 h and was measured by automated immunoassay (Vitros, Ortho Clinical Diagnostics, Rochester, NY, USA). The following were measured by ELISA kits: sex hormone-binding globulin (SHBG) (Alpco Diagnostics, Salem, NH, USA) and E_2 (ultrasensitive ELISA kit, Alpco Diagnostics). The assay sensitivity for E_2 using the ELISA kit was 1 pg/ml. For E_2 values that were less than the assay sensitivity ($n=8$), we used the lowest actual value detected by the assay as the sample value. The CV for these assays in our laboratory are $<10\%$. The free E_2 index (FEI) was calculated as the molar ratio of total E_2 to SHBG (pmol/nmol) (19). The free androgen index (FAI) and the E_2 to testosterone ratio ($\text{E}_2:\text{T}$) were calculated by using the formula previously described by Sowers *et al.* (20): $\text{FAI} = 100 \times \text{T} \text{ (ng/dl)} / 28.84 \times \text{SHBG} \text{ (nM)}$; $\text{E}_2:\text{T} = 10 \times \text{E}_2 / \text{testosterone}$, both of which are unit free.

ELISA kits were used to measure C-terminal telopeptide of type I collagen (CTX) (Crosslaps; Immunodiagnostic System, Inc., Gaithersburg, MD, USA), a marker of bone resorption, and osteocalcin (Metra OC; Quidel Corporation, San Diego, CA, USA), a marker of bone formation. The CV for the above assays in our laboratory are $<10\%$.

Statistical analysis

Results are expressed as means $\pm$ s.d. A P value of <0.05 was considered statistically significant. Simple correlations were analyzed by Pearson correlation analysis. $\text{E}_2:\text{T}$ ratios were grouped into tertiles in order to examine their potential non-linear effects. Group comparisons were analyzed using ANOVA for continuous variables, whereas categorical variables were compared using χ^2 analysis. ANOVA is a valid statistical method for the large sample used here. BMD differences among the different $\text{E}_2:\text{T}$ tertiles were further analyzed by an ANCOVA adjusted for age. *Post hoc* pairwise comparisons were performed using Tukey's honest significant difference method, which protects against multiple comparisons. The data were managed using Excel 2010 (Microsoft) and were analyzed using SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA).

Results

The present study population consisted of 90 male veterans between 40 and 74 years old who had average morning testosterone levels of <300 ng/dl, as observed in two measurements taken 30 min apart. Although the primary criteria for enrollment was an average morning testosterone level of <300 ng/dl, all of the subjects recruited in the study had at least one complaint that could be related to testosterone lack; thus, they could be classified as hypogonadal (17). The baseline characteristics of the participants are as shown in Table 1. The average BMI was in the obese range (31.5 ± 6.1 kg/m²). Forty-two percent of the participants had a history of past smoking, whereas 24% were current smokers. The sex hormone levels of the study population are shown in Table 1. There was no baseline $\text{E}_2:\text{T}$ data for three of the subjects; thus, the following analyses were based on the 87 patients with corresponding $\text{E}_2:\text{T}$, bone density, body composition, and other biochemical data.

Because we used $\text{E}_2:\text{T}$ as an index of aromatase activity, we divided our subjects into tertiles of $\text{E}_2:\text{T}$. Table 2 shows the age, BMD, and markers of bone turnover according to the tertiles of $\text{E}_2:\text{T}$. There were no differences in the mean age of the participants across the tertiles. Subjects in the highest tertile of $\text{E}_2:\text{T}$ had the highest lumbar spine BMD as compared to the lower tertiles. However, there were no

Table 1 Baseline clinical and laboratory values of the study population. Values are means $\pm$ s.d.

Age (years)	59.7 $\pm$ 8.8 (range: 40–74)
40 to <50	$n=14$
50 to <60	$n=21$
60 to <70	$n=47$
≥ 70	$n=8$
BMI (kg/m ²)	31.5 $\pm$ 6.1 (range: 21.8–48.6)
Smoking history	
Past	38/90 (42%)
Current	23/90 (24%)
Alcohol consumption	39/90 (43%)
Total testosterone (nmol/l (ng/dl))	7.22 $\pm$ 2.23 (208.1 $\pm$ 64.3) (Ref: 9.09–55.27 nmol/l or 262–1593 ng/dl) ^a
Total estradiol (pmol/l (pg/ml))	45.11 $\pm$ 92.20 (11.7 $\pm$ 27.0) (Ref: 0–183.54 pmol/l or 0–50 pg/ml) ^a
SHBG (nmol/l)	81.7 $\pm$ 97.1
FEI (pmol/nmol)	2.0 $\pm$ 6.9
FAI	24.7 $\pm$ 22.6
$\text{E}_2:\text{T}$	0.56 $\pm$ 0.88

SHBG, sex hormone-binding globulin; FEI, free estradiol index; FAI, free androgen index; $\text{E}_2:\text{T}$, estradiol to testosterone ratio.

^aReference ranges for testosterone and estradiol at the New Mexico VA Health Care System Clinical Laboratory, Albuquerque, NM, USA.

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Table 2 Age, bone mineral density, and markers of bone turnover according to tertiles of E₂:T. Values are means $\pm$ s.d.

	First (n=29)	Second (n=29)	Third (n=29)	P value
Age (years)	60.8 $\pm$ 9.0	57.7 $\pm$ 7.4	60.6 $\pm$ 8.8	0.30
BMD (g/cm ²)				
Spine	1.074 $\pm$ 0.119*	1.121 $\pm$ 0.137	1.197 $\pm$ 0.193	0.037
Total femur	1.079 $\pm$ 0.127	1.040 $\pm$ 0.126	1.109 $\pm$ 0.119	0.19
Femoral neck	0.823 $\pm$ 0.129	0.770 $\pm$ 0.078	0.818 $\pm$ 0.082	0.15
Trochanter	0.763 $\pm$ 0.107	0.735 $\pm$ 0.087	0.806 $\pm$ 0.118	0.08
Intertrochanter	1.293 $\pm$ 0.142	1.235 $\pm$ 0.154	1.317 $\pm$ 0.131	0.14
Biochemical markers of bone turnover				
Osteocalcin (μ g/l)	7.08 $\pm$ 6.21	7.05 $\pm$ 2.93	4.73 $\pm$ 3.22	0.17
CTX (ng/l)	320 $\pm$ 170	290 $\pm$ 180	240 $\pm$ 140	0.33

E₂:T, estradiol to testosterone ratio; BMD, bone mineral density; CTX, C-terminal telopeptide of type 1 collagen. *P<0.05 tertile 1 vs tertile 3.

significant differences in BMD in different regions of the femur according to the tertiles of E₂:T. There were no significant differences in serum CTX or osteocalcin across the tertiles among the 63 patients who had serum bone marker data.

Table 3 shows the BMI and body composition parameters according to the tertiles of E₂:T. There were significant differences in BMI across the different tertiles of E₂:T; men in the highest E₂:T had the highest BMI relative to the lower tertiles. A trend for increasing total % fat and truncal % fat with increasing tertiles of E₂:T was observed. This trend was significant for truncal % fat; *post hoc* analysis showed the difference between the first and third tertiles to be significant. On the other hand, a trend for decreasing total % lean and truncal % lean was observed with increasing E₂:T tertiles. This trend was significant for truncal % lean; *post hoc* analysis showed the difference between the first and third tertiles to be significant. Analyses of the appendicular body composition showed that in the upper extremities combined, there was a

significant increase in % fat and a significant decrease in % lean with increasing E₂:T tertiles; *post hoc* analysis showed the differences between the first and third tertiles for both parameters to be significant. On the lower extremities, there was a nonsignificant trend for increasing % fat and decreasing % lean with increasing tertiles of E₂:T.

Finally, analyses of the hormonal profiles of our patients showed that patients in the third, or highest, tertile of E₂:T had significantly higher total E₂ and FEI as compared to the lower two tertiles (Table 4). There were no significant differences in E₂ and FEI levels between the first and second tertiles. There were no significant differences in total testosterone, FAI, or SHBG among the tertiles. E₂:T was significantly higher in the third tertile as compared to the lower two tertiles, whereas there was no difference in E₂:T between the first and second tertiles. There was no correlation between any of the hormonal variables and BMD or body composition parameters or between E₂:T and other hypogonadal symptoms or age (data not shown).

Table 3 BMI and body composition according to tertiles of E₂:T. Values are means $\pm$ s.d.

	First (n=29)	Second (n=29)	Third (n=29)	P value
BMI (kg/cm ²)	29.8 $\pm$ 3.5*	32.0 $\pm$ 6.0	33.1 $\pm$ 4.7	0.042
Total and truncal body fat composition				
Total % fat	29.6 $\pm$ 4.9	31.5 $\pm$ 6.5	33.1 $\pm$ 5.4	0.068
Total % lean	67.5 $\pm$ 4.6	65.8 $\pm$ 6.2	64.2 $\pm$ 5.5	0.070
Trunk % fat	30.5 $\pm$ 5.7*	33.3 $\pm$ 8.2	35.1 $\pm$ 6.1	0.046
Trunk % lean	68.1 $\pm$ 5.6*	65.4 $\pm$ 8.1	63.7 $\pm$ 6.0	0.045
Appendicular body composition				
UE % fat	29.7 $\pm$ 5.4*	31.4 $\pm$ 6.3	33.6 $\pm$ 5.8	0.047
UE % lean	67.0 $\pm$ 5.4*	65.1 $\pm$ 6.0	63.1 $\pm$ 5.7	0.034
LE % fat	28.7 $\pm$ 6.0	29.3 $\pm$ 5.7	31.0 $\pm$ 5.8	0.32
LE % lean	68.0 $\pm$ 5.8	67.2 $\pm$ 5.3	65.6 $\pm$ 5.5	0.26

E₂:T, estradiol to testosterone ratio; UE, upper extremities; LE, lower extremities. *P<0.05 tertile 1 vs tertile 3.

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Table 4 Hormonal profile according to tertiles of E₂:T. Values are means $\pm$ s.d.

	First (n=29)	Second (n=29)	Third (n=29)	P value
Estradiol, (pmol/l (pg/ml))	6.83 $\pm$ 4.19* (1.56 $\pm$ 1.14)	16.44 $\pm$ 7.59 [†] (5.04 $\pm$ 2.07)	112.07 $\pm$ 138.68 (30.27 $\pm$ 37.78)	<0.001
Testosterone (nmol/l (ng/dl))	7.5 $\pm$ 2.3 (216.65 $\pm$ 66.43)	7.14 $\pm$ 2.13 (205.59 $\pm$ 61.58)	7.15 $\pm$ 2.29 (206.41 $\pm$ 66.17)	0.77
FEI (pmol/nmol)	0.19 $\pm$ 0.26*	0.64 $\pm$ 0.68 [†]	5.64 $\pm$ 11.94	0.01
FAI	22.80 $\pm$ 20.76	23.61 $\pm$ 21.85	28.27 $\pm$ 26.21	0.70
SHBG (nmol/l)	67.63 $\pm$ 77.34	103.31 $\pm$ 123.91	77.32 $\pm$ 86.86	0.37
E ₂ :T	0.069 $\pm$ 0.04*	0.25 $\pm$ 0.07 [†]	1.37 $\pm$ 1.12	<0.001

E₂:T, estradiol to testosterone ratio; FEI, free estradiol index; FAI, free androgen index; SHBG, sex hormone-binding globulin. *P<0.05 tertile 1 vs tertile 3.

[†]P<0.05 tertile 2 vs tertile 3.

Discussion

Total and free, or bioavailable, testosterone levels in men have been shown to decrease with advancing age, even after adjusting for potential confounding factors, such as comorbid conditions, medications, timing of samples, and technical issues related to the methods for hormone assays (21, 22, 23, 24). After age 40, circulating levels of testosterone gradually decline at a rate of 1.6% per year for total testosterone and 2–3% per year for bioavailable testosterone (23). Because there is an age-related increase in SHBG, the magnitude of the decrease in bioavailable testosterone in men is much greater than the decrease in total testosterone levels is (12). This reduction in testosterone production in men is associated with age-related declines in muscle and bone mass, which lead to sarcopenia and osteoporosis respectively and result in an increased incidence of frailty-related falls and fractures (13, 25). In fact, testosterone deficiency has been reported in more than half of elderly men with a history of hip fracture (14, 26).

The main, if not only, source of estrogen in men comes from the conversion of androgens to estrogens, and it is mediated by the enzyme aromatase. This explains why patients with aromatase deficiency resulting from inactivating mutation of the aromatase gene show undetectable levels of E₂ and, as a result, low BMD (9). Among male patients who are not aromatase deficient, aside from influencing bone maturation and peak bone mass acquisition, aromatase activity may also influence body composition, even at an early age (27, 28). In a study among young males aged 6–19 years old, Vandewalle *et al.* (27, 28) reported that circulating E₂ was positively associated with skeletal maturation and areal and volumetric BMD. In addition, lean mass in these patients was also found to positively correlate with higher testosterone levels (27). Collectively the findings in that study suggest that in non-aromatase-deficient males, the influence of aromatase on male musculoskeletal health starts very early

in life and extends to old age, and it likely impacts those who develop hypogonadism (4, 29).

Sex steroids by themselves have been shown to modulate aromatase activity. Vottero *et al.* (30) reported an increase in aromatase expression in the peripheral blood leukocytes of men who were given testosterone. By inference, it is likewise conceivable that a testosterone lack may influence aromatase activity in an opposite direction and could result in relatively low levels of E₂ as well. Nevertheless, although it is presumed that hypogonadism also results in relative estrogen deficiency, given the inter-individual differences in aromatase activity, it is possible that E₂ levels may be adequate for the skeletal health in a certain subset of hypogonadal men because of their high aromatase activity. Conversely, it is also possible that some men may have normal circulating testosterone levels but relative estrogen deficiency because of low aromatase activity, which results in markedly low BMD.

The present results showed that among the group of men with low testosterone, those that had increased conversion of testosterone to E₂ (high E₂:T from increased aromatase activity) had higher BMD of the spine. Gennari *et al.* (31) also found that elderly nonosteoporotic men had a higher E₂:T ratio as compared to men with osteoporosis. But that study was performed in a group of men with average testosterone levels in the normal range. The present study was confined only to men with low testosterone who were, for the most part, assumed to be in a negative calcium balance. Moreover, unlike with the spine BMD, we did not detect any differences in BMD at any femoral site across the tertiles. Cortical and trabecular bone are both influenced by E₂ levels, as has been reported in several studies (32, 33). However, Khosla (32) highlights a difference between the influence of circulating bioavailable E₂ on the trabecular and its influence on the cortical bone. In a study of 314 men between the ages of 22 and 91 years old, he found a cutoff of 30 pmol/l of bioavailable E₂ below which volumetric BMD in the femoral neck linearly

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decreased with decreasing bioavailable E_2 . This relationship was less pronounced in the spine volumetric BMD, which decreased over the spectrum of decreasing bioavailable E_2 above or below the cutoff, although the decline in BMD was steeper when bioavailable E_2 was <30 pmol/l. Because the main components of the lumbar spine and the femur are trabecular and cortical bone respectively, it is possible that the difference we observed in the present study resulted from the relatively higher susceptibility of the trabecular to fluctuations in E_2 levels as compared to the cortical bone. In the present study, the lack of bioavailable E_2 measurements limits our ability to determine if this critical threshold for bioavailable E_2 corresponds to a particular cutoff for $E_2:T$, where a relationship between $E_2:T$ and BMD exists most especially in the femur. Nevertheless, our findings suggest that irrespective of testosterone level, it is possible for men to maintain BMD in the spine, provided they have inherently high aromatase activity.

It is well accepted that androgen deficiency in men is associated with both an increase in adiposity and a loss of lean body mass (15, 16). In a study of experimentally induced gonadal hormone deficiency among men, Finkelstein *et al.* (29) unequivocally confirmed that androgens regulate lean body mass. In addition, these investigators also demonstrated that experimental estrogen deficiency in men leads to an increase in total body fat. These findings suggest that although an increase in lean mass from testosterone therapy can be attributed to the androgenic effect of testosterone, a reduction in adiposity or, more broadly, a regulation of fat mass in men is mediated by the conversion of testosterone to E_2 . Contrary to the results of Finkelstein *et al.*, the present study showed that a higher E_2 level or a high $E_2:T$ was associated with increase in truncal and total body fat in men with low testosterone. This finding is more likely a reflection of the increased E_2 production by an expanded adipose tissue volume among patients in the third tertile rather than E_2 causing an increase in fat mass.

In the present study, men in the highest tertile of $E_2:T$ had the lowest lean body mass, whereas those in the lowest tertile had the highest lean body mass. Theoretically, in aging men with limited testosterone production, testosterone levels should be lower in those subjects with increased aromatase activity relative to those who have lower aromatase activity. However, in our subjects, there was no significant difference in testosterone levels across the tertiles. Considering the higher lean mass among the patients in the lower tertiles of $E_2:T$ in the absence of high testosterone levels, and vice versa, our findings suggest

that $E_2:T$ likely determines the overall androgenic or estrogenic environment and not the absolute testosterone or E_2 levels. In the Finkelstein study (29), lean mass, muscle area, and strength are all reduced in men with experimental androgen deficiency. In those patients, the reduction in lean mass was similarly prevented (or even increased) with the administration of testosterone regardless of whether an aromatase inhibitor was added or not. However, this was an artificial setting, wherein endogenous sex hormone production was totally or partially eliminated, as opposed to the usual aging male in the present study whose sex hormone production was still available, albeit at a lower rate. Although it cannot be demonstrated in the present study, the absence of an association between body composition in both legs and aromatase activity could be the result of the influence of activity on the lower extremities.

A study by Gates *et al.* (11) in a group of men between 39 and 79 years of age found a positive correlation between $E_2:T$ and all measures of body composition. Although the present findings are not entirely similar to the findings of those authors, the disparity could be related to differences in the population under investigation. Whereas their subjects had normal mean testosterone levels, the present subjects had testosterone levels that were by study design below the normal range; the levels in the present study were merely half of the reported testosterone levels in their study.

The present study has several limitations. First, given the cross-sectional design of the study, we could not establish a causal mechanism for the association between aromatase activity and body composition. Moreover, we did not find any significant differences in body composition and aromatase activity in certain sites, probably because of the limited sample size. Second, the use of DXA as a means of assessing truncal fat may not accurately reflect visceral fat, considering that it also measures abdominal wall subcutaneous fat. Third, $E_2:T$ ratio at this time is only a surrogate but not a validated marker of aromatization, and it has no established normal reference values. Fourth, the type of assay we used for both E_2 (ultrasensitive ELISA) and testosterone (automated immunoassay) are not the gold standards for these sex steroids (which is liquid chromatography-mass spectrometry in both cases), and they lack the sensitivity to detect low levels of both hormones. Finally, we have no data from molecular studies to determine if the $E_2:T$ ratio actually correlated with aromatase activity/expression in our patients.

Nevertheless, our results have clinical implications that may impact the treatment of men with

hypogonadism. Prior studies have suggested a skeletal threshold for serum E_2 in men (34, 35, 36, 37, 38). In a review by Rochira *et al.* (37), the authors identified a threshold between 15 and 25 pg/ml, whereas Khosla *et al.* (38) suggested a threshold between 20 and 25 pg/ml using immunoassays and 16 pg/ml using mass spectroscopy. Given the possible inter-individual differences in aromatase activity, it remains uncertain if the E_2 level that is required for skeletal health maintenance was reached in some of the patients, despite the normalization of testosterone levels with treatment. Alternatively, it is also possible that in some patients, the majority of the administered testosterone was converted to E_2 , which could have resulted in estrogenic side effects (i.e., gynecomastia). Although testosterone alone may be adequate to correct the symptoms and side effects of hypogonadism in most patients, the addition of aromatase inhibitors (although they have not been approved for this purpose) to testosterone may be appropriate (theoretically) in some patients with inherent increased aromatase activity for improving/maintaining adequate muscle mass and thereby for avoiding sarcopenia.

In summary, the present findings revealed two important influences of aromatase activity on the musculo-skeletal system in men with hypogonadism. We demonstrated that hypogonadal men with the highest aromatase activity were able to maintain spine BMD regardless of their testosterone levels, but they had the lowest % lean and the highest % fat mass. Because testosterone is the substrate of aromatase enzyme, testosterone deficiency is supposed to lead to relative estrogen deficiency. However, considering the inter-individual differences in the rate of conversion of testosterone to E_2 , men with low testosterone do not necessarily have insufficient estrogen levels. Additionally, the present study also suggests that absolute sex hormone levels may not be that important in determining BMD or body composition; instead, the ratio between the estrogenic and androgenic sex hormones could be more relevant. Although other studies have alluded to the importance of aromatase activity in bone health and body composition of men in general (10, 11, 31), the present study was conducted primarily in men with hypogonadism. Considering the limited sample size, a study in a larger population of hypogonadal men is needed to confirm our findings.

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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