Total estradiol, rather than testosterone levels, predicts osteoporosis in aging men

Estradiol prediz melhor osteoporose que testosterona total em homens idosos

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ABSTRACT

Objective: To study and establish sex hormone cutoff levels for osteoporosis risk in men over 50 years old. **Methods:** Case-control study of 216 men > 50 years, 110 with osteoporosis (O) and 106 with normal bone density (C). We measured estradiol (E2), sex hormone binding globulin (SHBG), total testosterone (TT) and albumin. Free testosterone (FT) and bioavailable testosterone (BT) were calculated through Vermeulen's formula. **Results:** There was no difference in TT between groups. Relative risks of osteoporosis were 1.89 for E2 < 37 pg/mL (p = 0.02); 1.91 for SHBG > 55 nmol/L (p = 0.019); 2.5 for FT < 7 ng/dL (p = 0.015); 2.7 for BT < 180 ng/dL (p = 0.0003). **Conclusions:** In men over 50 years old, TT was not indicative of osteoporosis risk while E2 < 37 ng/mL was. SHBG > 55 nmol/L, FT < 7 ng/dL and BT < 180 ng/dL can represent additional indications for osteoporosis screening in men over 50 years old. Arq Bras Endocrinol Metab. 2009;53(8):1020-5

Keywords

Male osteoporosis; estradiol; testosterone; free estradiol; free testosterone; SHBG

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RESUMO

Objetivo: Estudar e estabelecer pontos de corte dos hormônios sexuais para risco de osteoporose em homens após os 50 anos de idade. **Métodos:** Estudo caso-controle de 216 homens > 50 anos, 110 com osteoporose e 106 com densidade óssea normal. Foram dosados: estradiol (E2), globulina ligadora de hormônios sexuais (SHBG), testosterona total (TT) e albumina. Foram calculadas: testosterona livre (TLC) e testosterona biodisponível (TB) pela fórmula de Vermeulen. **Resultados:** Não houve diferença na TT entre os grupos. Os riscos relativos de osteoporose foram de 1,89 para E2 < 37 pg/mL (p = 0,02); 1,91 para SHBG > 55 nmol/L (p = 0,019); 2,5 para TLC < 7 ng/dL (p = 0,015) e 2,7 para TB < 180 ng/dL (p = 0,0003). **Conclusões:** Em homens acima de 50 anos, TT não indicou risco de osteoporose, mas E2 < 37 pg/mL sim. SHBG > 55 nmol/L, TLC < 7 ng/dL e TB < 180 ng/dL podem representar indicações adicionais para pesquisa de osteoporose em homens acima de 50 anos. Arq Bras Endocrinol Metab. 2009;53(8):1020-5

Descritores

Osteoporose masculina; estradiol; testosterona; estradiol livre; testosterona livre; SHBG.

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INTRODUCTION

Male osteoporosis has become recognized as an important clinical and public health problem (1). It has been estimated that, in the United States, 1-2 million men have osteoporosis, and another 8-13 million have osteopenia (2), using World Health Organization (WHO) current guidelines: bone mineral density (BMD) below -2.5 standard deviations (SD) of average peak young adult BMD for osteoporosis, and below -1 SD but above -2,5 SD for osteopenia. These parameters are based on female standards (3); whether the same cutoffs apply to male osteoporosis deserves discussion (4). Male osteoporosis may be secondary to a number of pathologies, major causes

being alcohol abuse, glucocorticoid excess and hypogonadism (2,4).

Hormonal changes are important factors for osteoporosis development in aging men. Total testosterone (TT) levels drop while sex hormone binding globulin (SHBG) rise, and as a consequence, free (FT) and bioavailable testosterone (BT, the sum of free and albumin-bound) levels drop even more. The lower the testosterone levels, the lower the aromatization to estradiol (E2). Recently, estrogen role in male bone homeostasis has been demonstrated through congenital estrogen deficiency description: estrogen resistance due to inactivating mutation in the estrogen alpha receptor gene (5,6) and aromatase (the enzyme that catalyzes androgens conversion into estrogens) deficiency (7-10). In both cases, lack of estrogen activity was associated with osteoporosis or severe osteopenia, demonstrated by low BMD at lumbar and femoral sites (3). Estrogen alpha receptor gene knockout studies in mice, as well as aromatase inhibitors use, proved that severe estrogen deficiency leads to bone mass reduction (11).

However, the relative importance of testosterone *versus* estrogen levels for bone health in men is not clear (12). If estrogen is directly responsible for bone mass, even when testosterone levels fall with aging up to hypogonadal levels, men with genetically determined high aromatase levels or estrogen receptor activity could be protected from osteoporosis. The opposite could also be true: men with normal testosterone levels but lower conversion to E2 or estrogen receptor activity could be at higher osteoporosis risk. The aim of this study was to compare E2, testosterone, SHBG levels and their calculated products (FT, BT, free E2 or E2/SHBG) for male osteoporosis prediction, through a case control study of osteoporotic men over 50 years compared to age and colour-matched control with normal BMD.

METHODS

All subjects took part in the Male Osteoporosis Detection Program, conducted by the Instituto Nacional de Traumatologia e Ortopedia (Into) and sponsored by the Health Ministry. Through this program, men over 50 years from the city of Rio de Janeiro (RJ) were invited, by media announcements, to perform a free dual energy X-ray absorptiometry (DXA) scan with spine and hip BMD measurements. The criterion used for osteoporosis diagnosis was BMD -2.5 SD below average young adult peak in the spine or in the hip.

A subgroup of this population, 216 men, aged 50 to 84 years, was invited to the hormonal study: 110 were osteoporotic (group 1/Case) and 106 were age and colour-matched normal BMD control (group 2/Control). Colour was auto-referred. This sample has been previously studied in relation to prevalence, clinical (13) and laboratorial hypogonadism diagnosis (14). Hormonal evaluation was performed at the Andrology Sector of Hospital da Lagoa, Health Ministry, Rio de Janeiro. A written informed consent was obtained, following the project approved by the Ethics Committee. Anamnesis and physical exam were performed at the first visit, and a blood collection for E2, TT, SHBG and albumin was scheduled to another day. FT and BT were calculated with Vermeulen formula, through the website http://www.issam.ch/freetesto.htm, using the dosages of TT, SHBG and albumin. If calculated FT (CFT) was < 6.5 ng/dL, a second blood collection was scheduled, with a minimum interval of one month from the first one. Late onset hypogonadism was diagnosed when clinical symptoms were associated to two CFT dosages < 6.5 ng/dL.

TT was measured by chemiluminescence, using an automated Advia Centaur® kit (Bayer Diagnostics), with an analytical sensitivity of 100 ng/dL (reference value in men ranges from 241 to 827 ng/dL); SHBG was also determined using a chemiluminescence kit, Immulite 1000® (Siemens), with an analytical sensitivity of 0.2 nmol/L (reference value in men ranges from 13 to 71 nmol/L). E2 was measured by a chemiluminescence kit (Biolab Merieux), with an analytical sensitivity of 9 pg/mL (reference value in men < 62 pg/mL). E2/SHBG was calculated as an index of free E2 levels. Albumin was determined using a colorimetric spectrophotometry kit (Targa BT Plus; Wiener Lab.), reference value ranges from 3.5 to 5. 5 g/dL.

Data were entered and validated using Excel for Windows XP (Microsoft® Office Professional 2007) and analyzed in GraphPad Prism®, version 4.00 for Windows (GraphPad Software; San Diego, California USA, www.graphpad.com). Distributions of continuous variables were described using means and SD when the distribution was close to normal or otherwise medians and first and third quartiles. Chi-squared test was used to compare frequencies of outcomes of interest between categorical variables, with a significant statistical level set at $p < 0.05$.

RESULTS

Late onset hypogonadism was diagnosed in 28/110 osteoporotic men (25%) and in 13/106 men with normal BMD (12.2%, previously published results) (13). There was no difference in TT levels between osteoporotic (499 ± 15 ng/dL) and age and colour matched normal BMD men (538 ± 17 ng/dL, p = 0.0881).

E2 levels were lower in osteoporotic subjects (36.69 ± 1.59 pg/mL) compared to normal BMD men (42.26 ± 2.26 pg/mL; p = 0.04) (Figure 1). Osteoporosis risk was significant from E2 < 37 pg/mL on (OR = 1.89; 95%CI = 1.06-3.38; p = 0.02); for men with E2 < 36 pg/mL, the OR was 2.03 (95%CI = 1.14-3.63; p = 0.01) (Table 1).

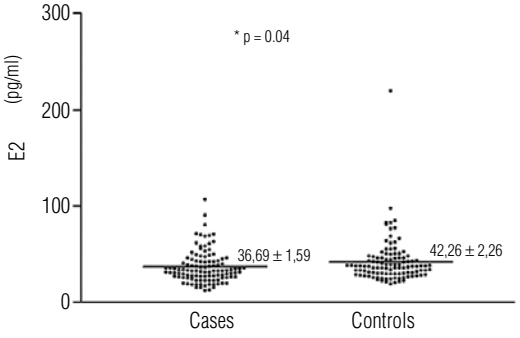

Figure 1. Estradiol (E2) values in osteoporotic men (Case) compared to men with normal bone mineral density (Control).

Table 1. Cutoff points from which a higher risk of osteoporosis were observed

Values	Cutoffs	OR (95%CI)	p-value
CFT (ng/dL)	< 7	2.5 (1.36-4.71)	0.015
BT (ng/dL)	< 180	2.7 (1.52-5.0)	0.0003
SHBG (nmol/L)	> 55	1.91 (1.07-3.41)	0.019
E2 (pg/dL)	< 37	1.89 (1.06-3.38)	0.02
	< 36	2.03 (1.14-3.63)	0.01

CFT: calculated free testosterone; BT: bioavailable testosterone; SHBG: sex hormone binding globulin; E2: estradiol.

Mean SHBG levels were 64.52 ± 3.5 in osteoporotic *versus* 52.72 ± 1.9 nmol/L (p = 0.0045) (Figure 2) in control men. E2/SHBG relation was significantly different between groups: 0.66 ± 0.03 for osteoporotic *versus* 0.88 ± 0.04 for normal BMD control (p = 0.0002) (Figure 3) even after excluding hypogonadal men (p = 0.0003).

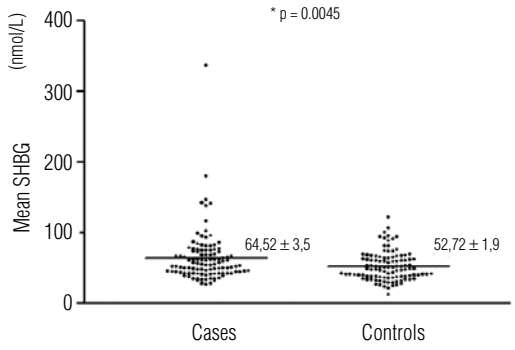

Figure 2. SHBG values in osteoporotic men (Case) compared to men with normal bone mineral density (Control).

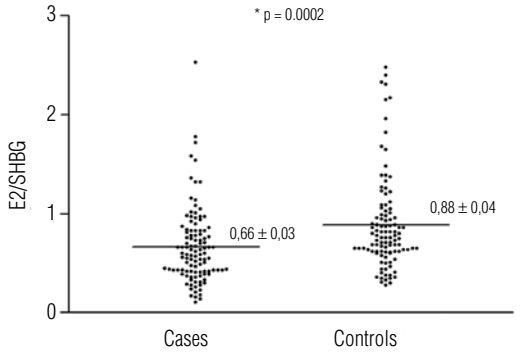

Figure 3. Estradiol/SHBG values in osteoporotic men (Case) compared to men with normal bone mineral density (Control).

CFT, BT and SHBG were also significantly different between groups: mean CFT was 7.07 ± 0.21 in osteoporotic *versus* 8.26 ± 0.23 ng/dL in normal BMD men (p = 0.0002); mean BT values were 173.7 ± 5.5 *versus* 206.8 ± 6.0 ng/dL (p < 0.0001), respectively (Figures 4A and B). Even after excluding hypogonadal men from both groups, osteoporotic men remained with significantly lower CFT (p = 0.015) and BT (p = 0.007) values and higher SGHB levels (p = 0.02) compared with normal BMD men.

Subjects who had at the first dosage CFT values < 7 ng/dL or BT < 180 ng/dL or SHBG > 55 nmol/L had a relative risk for osteoporosis of 2.5 (1.36-4.71; p = 0.015); 2.7 (1.52-5.0; p = 0.0003) and 1.91 (1.07-3.41; p = 0.019), respectively (Table 1).

DISCUSSION

Hypogonadal men have significantly lower bone density, in particular at cancellous bone sites like the spine, than age-matched control (15), and late onset male hypogonadism may be present in 50% of elderly men with

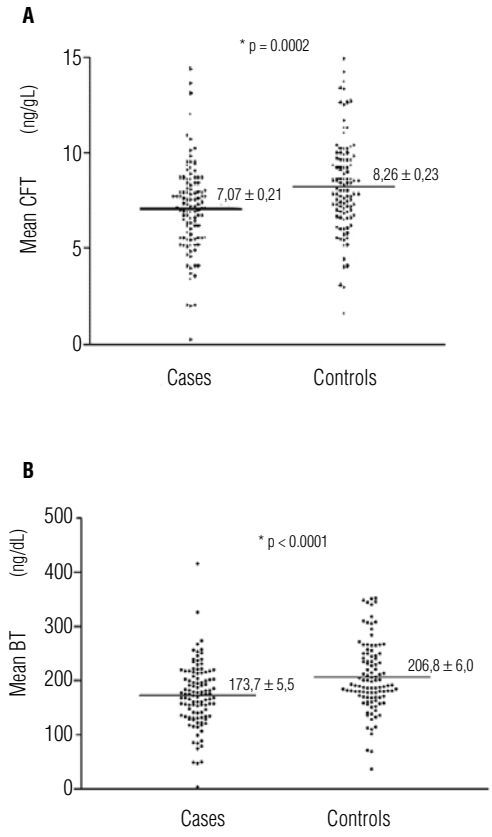

Figure 4. Testosterone values in osteoporotic men (Case) and in men with normal bone mineral density (Control): (A) CFT and (B) BT.

hip fractures (16). The bone loss mechanism in androgen-deficient men is similar to that induced by gonadal insufficiency in women: an imbalance in favor of bone resorption induces net bone loss, especially at cancellous bone sites with large remodeling surfaces (15).

We found no difference in TT levels between osteoporotic and normal bone density men, which is consistent with other studies in which plasma TT levels were not related to BMD in different sites (17). In the MINOS study (18), 760 men, 40-85 years old, were analyzed to correlate testosterone and E2 levels with estimates of bone mechanical properties derived from areal BMD measured by DXA and TT was not associated with any bone variable. Amin and cols. (19), in a study with 405 elderly men from the Framingham cohort (mean age 75.7 years, range 68 to 96 years), found that 71 (17.5%) were hypogonadal and that BMD at any site did not differ in hypogonadal compared with eugonadal men. Hypogonadism diagnosis was based in TT levels which may be misleading, as men with "normal" TT may have lower FT and BT. Thus, TT is a poor marker for aging male gonadal function (14), as well as for osteoporosis risk.

However, TT may be a fracture risk marker. In the Dubbo Osteoporosis Epidemiology Study, 609 Australian men > 60 years were followed for 5.8 years, after baseline serum TT, E2 and SHBG levels assessment. Fracture risk was significantly increased in men with reduced TT and E2 levels. After further adjustment for major risk factors, lower testosterone was still associated with increased fracture risk, particularly with hip and nonvertebral fractures (20). An explanation for this divergence can be found in a recent publication from MrOs Study, in which 1,245 Swedish community-dwelling men over 65 years had their frailty status assessed through criteria such as weakness, slowness, low activity, exhaustion, and shrinking/sarcopenia 4.1 years after enrollment and correlated to sex hormone levels. Low BT levels were independently associated with frailty, which can predispose to falls and consequent fractures (21). Testosterone action may predominate in muscle and E2 in bone, each one complementing the other for normal motor function. MrOS Study was designed to investigate if testosterone levels are associated with BMD and/or prevalent fractures in elderly men (22). A previous publication reported that low E2 levels could predict increased fractures risk independent of testosterone; on the contrary, men with low testosterone but normal E2 levels did not show an increased fractures risk. In multivariate analysis, free E2 was an independent fracture predictor in those elderly men (12).

Serum E2 was also shown to correlate better than testosterone in relation to BMD and its decrease in aging men (12). Different authors (12,23) reported that bone loss exceeded formation when E2 levels were 31 pg/mL or below in aging men. Cellular sex steroids action on bone tissue can be exerted through androgen and estrogen receptors (ER) (24), which belong to nuclear receptor superfamily. Two estrogen receptors, ERα and ERβ have been cloned and are expressed in bone (25-27). Movérare and cols. (24), in a study with orchidectomized wild-type and ER-inactivated mice treated with 5α-dihydrotestosterone (a nonaromatizable androgen), 17β E2, or vehicle, concluded that both ERα and androgen receptor (AR) but not ERβ activation preserved trabecular bone amount. ERα activation resulted both in preserved thickness and trabecular number. In contrast, AR activation exclusively preserved trabecular number, whereas thickness was unaffected. 17β E2 effects could not be mediated by AR. They concluded that ERα, but not AR or ERβ activation resulted in preserved thickness, volumetric density and mechanical strength of cortical bone (24).

Bioavailable E2, i.e., E2 not bound to SHBG, has been shown to decrease with age and not to be influenced by the body mass index (BMI), such as TT or total E2. To measure bioavailable E2, serum has to be equilibrated with tritiated hormones, and then SHBG and SHBG-bound steroids must be precipitated by adding an equal volume of a saturated aqueous solution of ammonium sulfate. Following the precipitation, the sample has to be centrifuged and the tritiated hormone in the supernatant (not SHBG-bound) is quantified and related to the total amount of tritiated hormone. The percentage is applied to provide bioavailable E2 concentrations (28). As tritiated E2 is not easily available, performing this measurement is not a usual practice. E2/SHBG relation could retrieve similar results, within normal laboratorial procedure. We found low E2/SHBG relation to be highly associated to osteoporosis, as many other authors. Gennari and cols. (23), in a study of 200 elderly men, observed that those with total and bioavailable E2 below the median showed higher rates of bone loss at the lumbar spine and femoral neck. Another epidemiological study (29), found an association between higher levels of total and bioavailable E2 and lower fracture prevalence in older men. Estrogens in elderly men originate mainly from androgens peripheral conversion by aromatase (12), whose expression and activity can vary widely among subjects. This variation can be genetically determined (30). Eriksson and cols. (31) demonstrated that CYP19 gene single nucleotide polymorphism (SNP) rs2470152, which codes for aromatase, is strongly associated with serum E2 and estrone levels in men. Men with aromatase deficiency, who have normal androgen but undetectable estrogen levels, have low bone mass and areal density and respond to estrogen therapy with significant BMD increase (7,32,33).

In this study, CFT and BT were significantly lower and SHBG significantly higher in osteoporotic men compared to those with normal BMD. MrOs Study also showed that FT levels below the median were positive independent predictors of lower BMD and more prevalent fractures in elderly men (22). A study of 3,014 Swedish men also established a stronger fracture risk for free than for total androgen and estrogen levels (34): older men with high serum SHBG had an increased risk of fractures, with a hazard ratio of 1.32 per 1 SD increase. In a study of Lormeau and cols. (35) with 105 subjects, 65 of them suffering from idiopathic or secondary osteoporosis (mean age 53.9 years) and 40 age-matched control, SHBG were significantly higher in osteoporotic patients than control, even after secondary osteoporosis (due to alcoholism or hypogonadism) exclusion. Also, SHBG was negatively correlated to femoral neck and lumbar spine BMD, to serum C-telopeptide of type I collagen (CTX), a bone remodeling marker used to evaluate resorption (35). SHBG was significantly associated with fractures; the odds ratio of having a fracture was 2.04 (95%CI: 1.2-3.4; $p < 0.01$) for each increase of 1 SD in the patient's SHBG level (35). SHBG levels are increased in a number of conditions like hyperthyroidism, severe malnutrition, hepatic cirrhosis or aging and may be, more importantly, genetically determined. Legrand and cols. (36) also refer a positive correlation between bone remodeling markers CTX, deoxypyridinoline (D-Pyr), and bone-specific alkaline phosphatase (bSAP) and serum SHBG (36). They suggest that the higher the SHBG, the lower the BT and E2 levels, and the higher are bone remodeling and resorption. Cummings and cols. (37), in a prospective study with postmenopausal women aged 65 years or older, observed that SHBG ≥ 34.7 nmol/L was associated to a relative risk of 2.0 for hip fractures and 2.3 for vertebral fractures. In this study, SHBG > 55 nmol/L was associated with a higher male osteoporosis risk.

In conclusion, TT levels were similar between osteoporotic and normal BMD aging men. Total E2, E2/SHBG, CFT, BT were significantly lower and SHBG were significantly higher in osteoporotic compared to normal BMD men. Cutoff values associated with higher osteoporosis risk in men over 50 years old were: E2 < 37 ng/dL; SHBG > 55 nmol/L; CFT < 7 ng/dL; BT < 180 ng/dL.

In an aging man's laboratorial exam, one or more of these values may represent an indication for osteoporosis screening. Limitations of the present study include the existence of possible confounders for plasma sex steroids values, such as obesity, that were not studied. Nevertheless, these confounders would not impair the predictive levels described.

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