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Guest Editors: Pawel Szulc, Jean Marc Kaufman, and Eric S. Orwoll

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Editorial Board

Contents

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Editorial

Osteoporosis in Men

Pawel Szulc,[1] Jean Marc Kaufman,[2] and Eric S. Orwoll[3]

[1] *INSERM UMR 1033, Hôpital Edouard Herriot, University of Lyon, Pavillon F, Place d'Arsonval, 69437 Lyon, France*
[2] *Department of Endocrinology, University Hospital Ghent, B-9000 Gent, Belgium*
[3] *Department of Medicine, Endocrinology, Diabetology & Clinical Nutrition, Oregon Health & Science University, Portland, OR 97239, USA*

Correspondence should be addressed to Pawel Szulc, pawel.szulc@inserm.fr

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Osteoporotic fractures (fragility fractures) are more frequent in postmenopausal women than in older men [1]. However, osteoporosis in men is one of the major and the most neglected public health problems for several reasons.

First, morbidity, mortality, and loss of independence after major fragility fracture are greater in men than women [2, 3]. The interpretation of data concerning mortality after fragility fracture should be, however, cautious. As life expectancy is lower in men than women, it is not appropriate to compare mortality after hip fracture between men and women of the same age. Therefore, it is important that, for a given age, the additional increase in mortality after a hip fracture is greater in men than women [4]. In addition, the fraction of the potential time of life lost after a hip fracture (taking into account the sex-specific life expectancy) is greater in men than women [5]. Thus, these studies show that the mortality after a hip fracture is really higher in men than in women.

Secondly, the number of osteoporotic fractures in men increases rapidly. The overall number of fractures is on the rise because the elderly population increases due to the lengthening of the lifespan. However, several studies have shown that the age-specific incidence of major osteoporotic fractures in postmenopausal women, especially hip fracture, has been slightly, but consistently, decreasing for the last 15 years [6–8]. Such trends have been observed in North America, Australia, and several European countries. By contrast, in these countries, the age-specific incidence of hip fracture in men decreased less than in women or even remained stable [8–10]. In addition, in some European countries and in Japan, age-specific hip fracture incidence continues to increase in both sexes [11, 12]. Therefore, over the next decades, the number of osteoporotic fractures is expected to increase faster in men than in women. Consequently, fragility fractures in men will constitute higher percentage of all the osteoporotic fractures than they do now.

Thirdly, the identification of men at high risk of fracture is not satisfactory. Increasing age, history of fragility fracture, and low bone mineral density (BMD) measured by dual energy X-ray absorptiometry (DXA) are important risk factors of fracture. Nevertheless, only 20% of men who sustain a hip fracture or major osteoporotic fracture have osteoporosis diagnosed by DXA using the sex-specific T-score < -2.5, half as many compared with women from the same cohort [13–15].

The diagnostic criteria of osteoporosis in men are a matter of controversy. The International Society for Clinical Densitometry (ISCD) recommends sex-specific T-score < -2.5 [16]. By contrast, International Osteoporosis (IOF) recommends the threshold corresponding to T-score $= -2.5$ in premenopausal women [17]. It corresponds to a T-score of approximately -2.75 compared with peak BMD in young men. The justification for using a female-based threshold is that the risk of fracture is similar in both sexes for the same absolute BMD, not for the same sex-specific T-score. If we accept the threshold recommended by ISCD, men with lower risk of fracture will be treated and we will have to treat more men to avoid one fracture. If we accept the threshold recommended by IOF, fewer men will be treated and fewer fractures will be avoided. Does it mean that the threshold of ISCD should be preferred? Not necessarily. Aside from the individual fracture risk, there is also variation from country

to country in the willingness to pay for therapy and in the choice of the treatment criterion. If the sex-specific T-score = −2.5 identifies men at a lower risk of fracture than women, the health authorities may refuse the reimbursement of any antiosteoporotic treatment in men. If the IOF threshold is recommended in these countries, it will be easier to obtain the reimbursement for the osteoporosis treatment in men. Fewer men will be treated, but at least, it will be possible to reduce the risk of fracture in those who have the most severe osteoporosis and the highest risk of fracture.

An additional problem is that other bone parameters do not provide much improvement in the prediction of fracture in men. Classical biochemical bone turnover markers (BTMs) are not predictive of fracture in the multivariable models adjusted for BMD [18, 19]. Ultrasound parameters predict fractures in men similarly to BMD, but their joint use does not improve fracture prediction compared with BMD alone [20]. Quantitative computed tomography (QCT) predicts hip fracture, but not better than DXA alone [21]. Young healthy men with prevalent fractures had lower cortical bone volume assessed by peripheral QCT than men without fracture [22]. However, these analyses were not adjusted for BMD measured by DXA. High-resolution peripheral quantitative computed tomography (HR-pQCT) allows assessment of bone microarchitecture at the distal radius and tibia. Men with vertebral fractures had thinner cortex and lower cortical volumetric BMD assessed by HR-pQCT compared with men without vertebral fracture, even after adjustment for BMD measured by DXA [23]. However, these cross-sectional data have to be confirmed in the prospective studies.

Several studies have assessed other approaches that aimed to improve fracture prediction in men. The FRAX algorithm is a significant landmark in the assessment of the individual risk of fracture [24]. It takes into account several risk factors, which determine bone fragility, for example, history of fracture, parental history of hip fracture, corticotherapy, and so forth. Another algorithm is the Garvan nomogram [25]. It takes into account history of fractures and that of falls. Falls, especially multiple falls, seem to be associated with a substantial increase in the risk of peripheral fracture in the elderly men [26]. However, both FRAX and the Garvan nomogram have been introduced only recently and few studies assessed their utility in men [27, 28].

Limited data suggest an association between bone size and risk of fracture. Low bone width was associated with higher risk of fracture independently of BMD [29]. History of fracture was associated with lower cross-sectional area (CSA) measured by peripheral QCT [23]. However, no method of measurement of bone width or CSA could be recommended currently for the clinical practice. Several studies showed that longer femoral neck axis and wider neck-shaft angle were associated with higher risk of hip fracture, mainly cervical fracture [30, 31]. However, these associations were weak and not consistent between the investigated groups.

More and more studies suggest utility of the finite element analysis (FEA) for fracture prediction in men. The load-to-strength ratio remained significantly associated with the risk of hip fracture after adjustment for BMD [32]. More recently, vertebral compressive strength improved vertebral fracture risk assessment in comparison with DXA-measured BMD [33]. However, FEA is not available in the clinical practice.

Serum and urinary levels of C-terminal telopeptide of type I collagen did not predict fracture after adjustment for BMD in men. By contrast, measurement of serum levels of native, nonisomerized form of CTX-I (α-CTX-I) and of beta-isomerized form (β-CTX-I) showed that higher α-CTX-I/β-CTX-I ratio is associated with higher risk of fracture in men [34]. However, it is not clear if the increased α-CTX-I/β-CTX-I ratio reflects higher bone turnover rate or an intrinsic defect of posttranslational modifications of bone collagen. The use of these measures has not been shown to improve the prediction of fracture or the management of care in men with osteoporosis.

In most of the cohorts, decreased levels of total or bioavailable 17β-estradiol were associated with higher risk of fragility fracture in men [35–37]. However the analyses were not systematically adjusted for BMD. Decreased serum level of 25-hydroxycholecalciferol was associated with higher risk of hip fracture in American men aged >65 and with higher risk of clinical fracture in Swedish men aged >65 [38, 39]. However, this association was markedly attenuated after adjustment for hip BMD [38]. Low serum level of insulin-like growth factor I (IGF-I) was associated with a higher risk of ostoporotic fracture in men, also after adjustment for BMD [40]. The potential mechanism underlying this association is not clear. This observation is, however, interesting because serum IGF-I concentration was not correlated with BMD in older men [41, 42]. Finally, increased level of fibroblast growth factor 23 was associated with higher risk of nonspine fracture in the elderly men, even after adjustment for confounders including BMD and parathyroid hormone concentration [43]. However, the exact mechanism underlying this association has not been elucidated.

Thus, FRAX appears to improve the assessment of the fracture risk in both sexes; however, it needs to be verified in a higher number of cohorts of men. By contrast, other available studies do not provide reliable methods permitting to identify with a satisfactory probability older men at high risk of fracture.

Fourthly, fewer studies concern the antiosteoporotic treatment in older men compared with the studies carried out in postmenopausal women. Most studies assessed only changes in BMD measured by DXA and in the BTM levels induced by antiosteoporotic treatment [44–48]. Then, the antifracture efficacy of the investigated medications in men is inferred indirectly from these bridging studies by comparing their results with the data obtained previously in postmenopausal women. By contrast, few studies assessed the antifracture efficacy of the antiosteoporotic therapies in men [48–52]. Moreover, some of these studies were not powered to this type of analysis. Some of these studies were observational surveys, not randomized pharmaceutical trials. The effect of the antiosteoporotic medications on the risk of fracture in men was assessed specifically only in few studies.

The antifracture efficacy of denosumab and toremifene has been investigated in men receiving androgen-deprivation therapy for prostate cancer [53–55]. In older osteoporotic men, zoledronic acid decreased significantly the incidence of vertebral fractures [56].

Fifthly, even men with an increase in fracture risk are rarely treated. In men, the parameter that indicates higher risk of fracture most consistently in epidemiological studies is prevalent fragility fracture [26, 57]. A history of osteoporotic fracture is associated with a two- to fourfold higher risk of another osteoporotic fracture. This is a very similar situation to that found in postmenopausal women. However, about 50 percent of women who sustained fragility fracture benefit from bone densitometry and/or antiosteoporotic treatment [58–60]. By contrast, less than 10 percent of men who sustained an osteoporotic fracture will have bone densitometry and/or osteoporotic treatment [58–60]. In some studies, only 1 out of 20 men having sustained a hip fracture (and were hospitalized for this fracture) benefited from bone densitometry and/or antiosteoporotic treatment [60]. Even men receiving chronic glucocorticoid therapy or androgen deprivation therapy are not systematically screened and, whenever appropriate, treated for osteoporosis [61, 62].

In conclusion, on one hand, osteoporosis in older men is a major public health problem. The number of fragility fractures in men is rapidly increasing, the consequences of these fractures are more severe in men than in women, and the identification of men at high risk of fracture is suboptimal. On the other hand, osteoporosis in older men should be considered a neglected public health problem. There are fewer data on the appropriate measures to assess fracture risk, less information on the antifracture efficacy of the available medications in men than in women, and only few men with the evidently increased risk of fracture obtain adequate treatment.

Pawel Szulc
Jean Marc Kaufman
Eric S. Orwoll

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Research Article

Sex- and Age-Related Differences in Bone Microarchitecture in Men Relative to Women Assessed by High-Resolution Peripheral Quantitative Computed Tomography

Shreyasee Amin[1,2] and Sundeep Khosla[3]

[1] *Division of Rheumatology, Department of Internal Medicine, College of Medicine, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA*
[2] *Division of Endocrinology, Diabetes, Metabolism and Nutrition, Department of Internal Medicine, College of Medicine, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA*
[3] *Division of Epidemiology, Department of Health Sciences Research, College of Medicine, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA*

Correspondence should be addressed to Shreyasee Amin, amin.shreyasee@mayo.edu

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The trabecular and cortical compartments of bone each contributes to bone strength. Until recently, assessment of trabecular and cortical microstructure has required a bone biopsy. Now, trabecular and cortical microstructure of peripheral bone sites can be determined noninvasively using high-resolution peripheral quantitative computed tomography (HR-pQCT). Studies that have used HR-pQCT to evaluate cohorts of both men and women have provided novel insights into the changes in bone microarchitecture that occur with age between the sexes, which may help to explain the lower fracture incidence in older men relative to women. This review will highlight observations from these studies on both the sex- and age-related differences in trabecular and cortical microstructure that may underlie the differences in bone strength, and thereby fracture risk, between men and women.

1. Introduction

High-resolution peripheral quantitative computed tomography (HR-pQCT) is a novel imaging modality that noninvasively assesses trabecular and cortical bone microstructure at the distal radius and tibia [1, 2]. Although areal bone mineral density (aBMD), as assessed by dual-energy X-ray absorptiometry (DXA), remains an important predictor of fractures, DXA technology is nevertheless limited in its inability to measure the trabecular and cortical bone compartments separately, each of which contributes to bone strength [3]. The ability to assess these compartments may help improve fracture prediction in men and women. Until recently, assessment of trabecular and cortical microstructure has required a bone biopsy. With the development and subsequent validation of HR-pQCT [1, 4–7], trabecular and cortical microstructure of peripheral bone sites can now be determined noninvasively and is thus more amenable for clinical study. Indeed, there are a growing number of studies that have now used HR-pQCT to evaluate relatively large cohorts of men and women. The findings from these studies have provided important advances to our understanding of the age-related changes in bone microarchitecture that occur, which may in turn help to explain the lower fracture incidence observed in aging men relative to aging women.

2. High-Resolution Peripheral Quantitative Computed Tomography

2.1. HR-pQCT Imaging and Processing. Bone histomorphometry from iliac crest bone biopsies provided invaluable two-dimensional information on bone microarchitecture [9], but the use of bone biopsies in clinical studies was

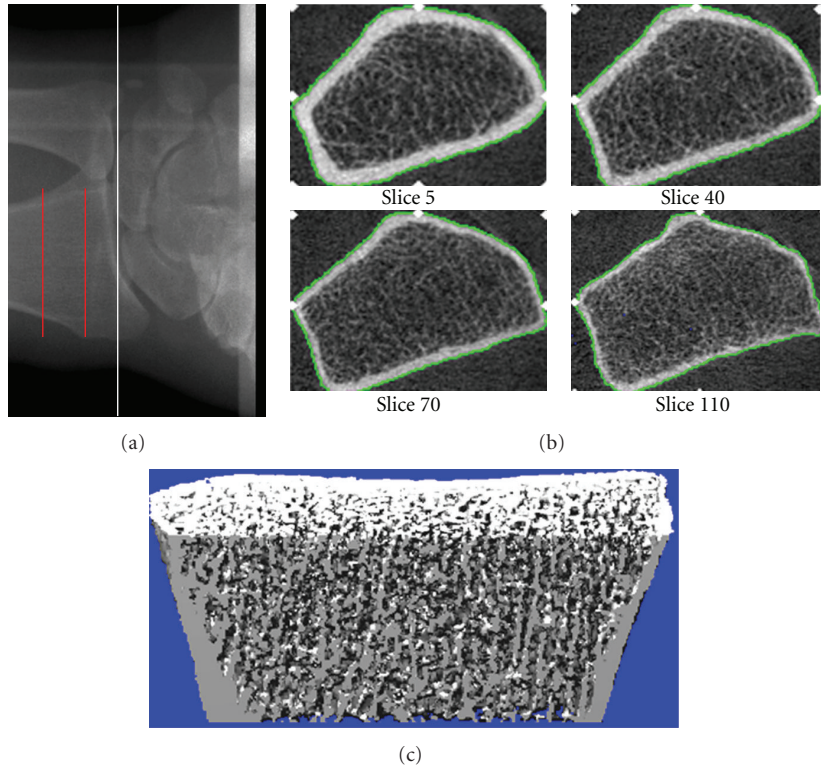

Figure 1: (a) Radiograph showing the site of imaging by HR-pQCT at the distal radius. The white line indicated the proximal level of the joint space, and the red lines indicate the section of bone over which images are acquired. (b) Representative cross-sectional images from the stack of CT slices. (c) Representative 3D image. Images were obtained using the initial prototype of the HR-pQCT scanner (Figure is reprinted with permission from Khosla et al. [8] ©2006 American Society for Bone and Mineral Research).

limited due to its invasive nature and the inability to assess a section of bone more than once. Furthermore, the three-dimensional (3D) aspect of bone microarchitecture could not be taken into account to fully understand its biomechanical properties. Imaging modalities were thus developed that could assess, noninvasively, the 3D bone microarchitecture in vivo [1, 2] and this work led to the now commercially available 3D HR-pQCT scanner (Xtreme CT, Scanco Medical AG, Switzerland; voxel size 82 μm) that can image the distal radius and distal tibia of human subjects. The imaging protocol provides not only an assessment of both trabecular and cortical bone microstructure parameters, which will be described in more detail below, but also total volumetric BMD (vBMD, g/cm^3) of the distal radius or tibia as well as separate measures for trabecular and cortical vBMD. In recent software updates, an assessment of cortical porosity has become available [10] as well as an assessment of bone strength at the radius and tibia determined through microfinite element analysis (IPL v1.12, Scanco Medical AG) although similar parameters can be derived from the images independently from the built-in software [11]. Several additional trabecular and cortical microstructure parameters are provided through the commercial software; however, these novel parameters have not been as well studied as those described below.

Imaging of the distal radius or tibia by HR-pQCT involves a similar protocol with the acquisition of a 3D stack of 110 high-resolution CT slices at either the distal end of the radius (Figure 1(a)) or tibia, with an isotropic voxel size and slice thickness of 82 μm. The processing and analyses of these images has been validated [4–7]. Figures 1(b) and 1(c) show representative cross-sectional scan slices and 3D images, respectively, that can be obtained from HR-pQCT imaging.

2.2. Assessment of Trabecular Parameters by HR-pQCT. Some trabecular microstructure parameters assessed using HR-pQCT are measured directly while others are derived. The trabecular vBMD is determined directly, and then the trabecular bone volume/total volume (BV/TV) is derived, assuming a mineral density of fully mineralized bone of 1.2 g hydroxyapatite/cm^3. Recognizing that individual trabeculae would not be resolved at their correct thickness (~100 μm) due to partial volume effects, a thickness-independent structure extraction is employed whereby 3D ridges (the center points of the trabeculae) are detected in the gray-level images [5]. Trabecular number (TbN, 1/mm) is then taken as the inverse of the mean spacing of the ridges [6]. Then, in analogy with standard histomorphometry [12], trabecular thickness (TbTh, mm) is calculated using the formula TbTh = BV/TV ÷ TbN, and trabecular spacing or separation (TbSp, mm) is calculated using the formula TbSp = (1 − BV/TV) ÷ TbN. Validation studies show excellent correlation ($R \geq 0.96$, $P < 0.0001$) for these parameters when

compared with the gold-standard ex vivo microcomputed tomography (μCT) technique [13].

2.3. Assessment of Cortical Parameters by HRp-QCT. The cortex is segmented from the gray scale image with a Gaussian filter and threshold [6]. Cortical vBMD (g/cm^3) bone cross-sectional area (CSA, mm^2) and cortical cross-sectional area (Ct CSA, mm^2) are measured directly, and the periosteal circumference calculated from the contour. Cortical thickness (CtTh, mm) is then derived using the formula CtTh = Ct CSA ÷ circumference. Excellent correlation has been shown for CtTh measurements with HR-pQCT versus μCT [7].

3. Bone Microarchitecture by HR-pQCT in Men

Recent studies that have assessed both men and women using HR-pQCT have led to important insights into the sex- and age-related differences in bone microarchitecture. Greater bone strength in men than women is, in part, related to larger bone size of men. However, differences between the sexes with respect to both the trabecular and cortical compartments of bone may also contribute to their differences in bone strength and fracture risk.

3.1. Differences in Bone Microarchitecture in Young Men versus Young Women. Khosla et al. [8] first reported on the bone microarchitecture by HR-pQCT in both men and women. Using the initial prototype of the HR-pQCT scanner, the distal radius was imaged in 278 men and 324 women (ages of 21–97 years), who represented an age-stratified, random sample of the Rochester, MN community adults [8]. Relative to young women age 20–29 years of age ($n = 17$), young men of a similar age range ($n = 19$) had higher BV/TV and greater TbTh but had similar TbN and TbSp [8]. Young men also had greater bone CSA, but similar CtTh, when compared with young women [8].

Sode et al. [14] imaged both the distal radius and tibia in 146 healthy volunteers (53 men and 93 women), aged 20–78 years. They found that young men (age 20–29 years) had greater BV/TV and TbTh at both the distal radius and tibia when compared with young women of the same age range [14]. TbN and TbSp were not significantly different between the sexes at the radius but were at the tibia [14]. Findings were thus similar to what was observed by Khosla et al. at the radius. The tibia was not imaged in the study by Khosla et al., but findings by Sode et al. suggested that the sex-related differences in bone microarchitecture may not be the same between the radius and tibia sites. Sode et al. also noted substantial variations in trabecular parameters within a cross-section of either the distal radius or tibia and identified sex-specific subregional variations in trabecular microstructure [14].

In a population-based study from Canada involving 202 men and 442 women (ages 20–99 years) where both the distal radius and tibia were measured, MacDonald et al. [15] reported that young men (age 20–29 years, $N = 28$) had greater bone size, BV/TV and TbTh at the distal radius when compared with young women of the same age range ($N = 58$), observations that were again similar to reports at the same site by Khosla et al. and Sode et al. In contrast, they observed that young men also had greater TbN and CtTh than women [15]. At the distal tibia, young men again had greater bone size, BV/TV, TbN and CtTh than women [15]. TbTh at the tibia was also slightly higher in men but did not reach statistical significance [15].

Interestingly, in a study involving almost 300 adolescent boys and girls between the ages of 15–20 years who had their distal tibia imaged by HR-pQCT, sex-related differences were more apparent by the age of 17–20 years, with boys in this age range having greater bone size, BV/TV and CtTh relative to girls [16]. However in this study, TbTh was not significantly different between the sexes at any age, whereas TbN was higher, while TbSp was lower, in boys than girls at all ages [16]. However, a cross-sectional study by Wang et al., involving almost 130 boys and girls ages 5–18 years, found that BV/TV and TbTh increased more in boys than girls over puberty at both the radius and tibia sites, while TbN remained similar for both boys and girls [17]. Similarly, in a cross-sectional study by Kirmani et al. assessing the distal radius of 140 healthy boys and girls ages 6–21 years, BV/TV and TbTh also increased more in boys than girls over puberty [11]. TbN transiently increased in boys more than girls across age but then became similar again in both sexes at more mature ages [11].

Collectively, these studies confirm that there are clear bone microarchitectural differences apparent between young men and women, particularly bone size, BV/TV and TbTh, with these differences developing over puberty and appearing to be established as early as late adolescence. These studies also suggest that sex-related differences for some parameters of bone microstructure may be site-specific. Nevertheless, there was a lack of consistency among studies on findings for some parameters, but they may reflect imaging resolution issues or power limitations.

3.2. Age-Related Differences in Bone Microarchitecture in Men. To date, studies reporting on the age-related differences in bone microarchitecture in men and women have been cross-sectional in nature. The determination of true age-related changes in bone microarchitecture will require longitudinal studies. Nevertheless, these cross-sectional studies do provide key information on differences in the trabecular and cortical compartments of bone between young and old and thus impart the most practical insights on structural changes that may be related to aging in men and women.

In the study by Khosla et al., cross-sectional decreases in BV/TV at the radius between the ages of 20 and 90 years were reported to be similar in men and women [8]. However, in contrast to women, where TbN decreased and TbSp increased with age, there were little age-related changes in these parameters in men [8]. On the other hand, TbTh appeared to decrease to a greater extent in men than women ($P < 0.01$) [8] (Figure 2). These findings suggested that the mechanism for loss in BV/TV with aging in men and women may be different, with loss of trabeculae being the main factor in women but trabecular thinning being the

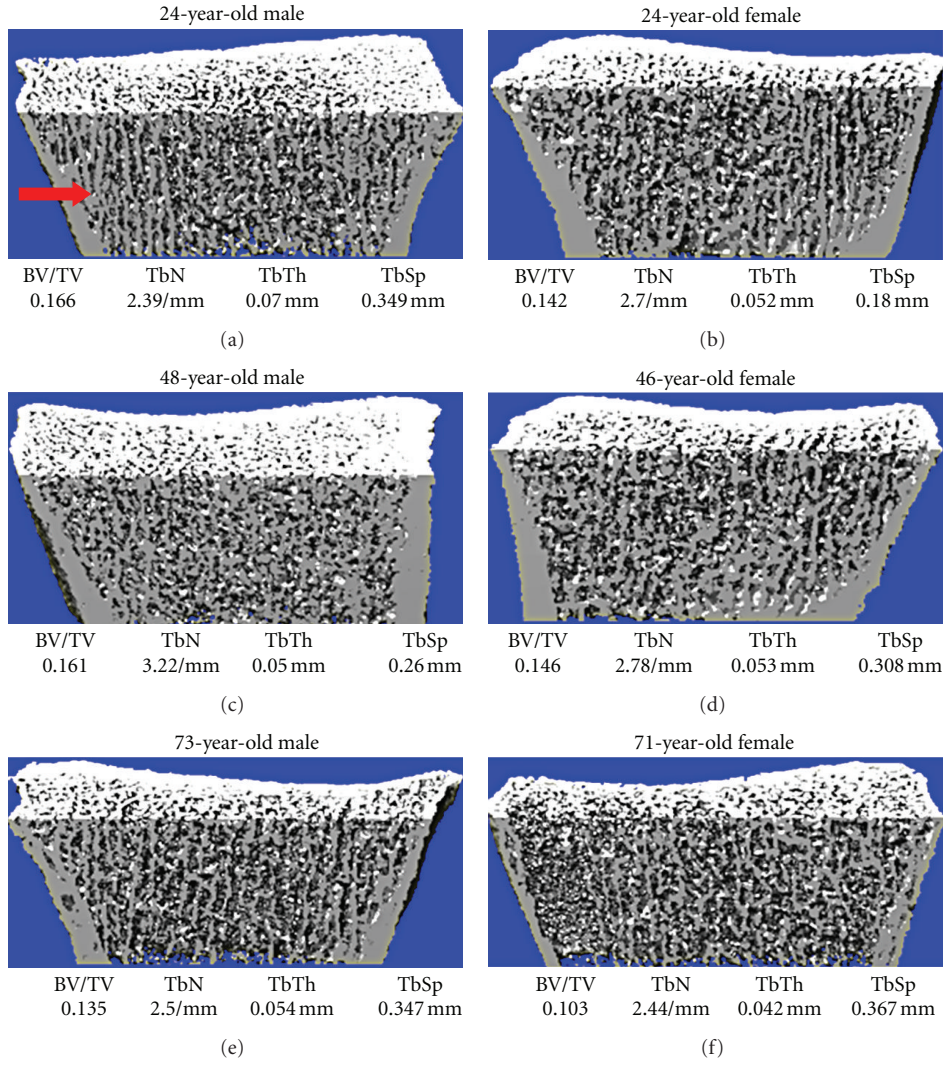

Figure 2: Representative reconstructions of high-resolution peripheral quantitative computed tomography images from young, middle-aged, and elderly men (left) and women (right). Values for trabecular microstructural variables for each subject are also provided. The arrow indicates the prominent plate-like trabeculae in the young man, which are less prominent or absent in the other images. Images were obtained using the initial prototype of the HR-pQCT scanner. (Figure is reprinted with permission from Khosla et al. [8] ©2006 American Society for Bone and Mineral Research).

mechanism for men. As decreases in TbN have been shown to have greater impact on impairing bone strength compared with decreases in TbTh [18], it was suggested that these findings could explain the lower life-long fracture risk in men relative to women. MacDonald et al. observed similar findings as Khosla et al. at the distal radius [15]. However, at the distal tibia, MacDonald et al. found that TbTh, TbN, and TbSp changed to a similar extent in both men and women with age [15]. The distal tibia was not assessed in the study by Khosla et al. Patterns of trabecular loss may thus vary by skeletal site. Sode et al. reported that age-related differences in men and women were more prominent at particular subregions of each bone [14]. On the other hand, Dalzell et al. failed to see any significant association between trabecular microstructure parameters and age, at either the distal radius or tibia, in their study involving 58 men and 74 women (ages 20–79 years) from the UK [19].

Khosla et al. reported that, in both men and women, bone CSA increased with aging, but while the percentage change was similar between men and women, older men continued to have greater bone size than older women [8]. In contrast, CtTh decreased to a lesser extent in men than women (−38% versus −52%, resp.; $P = 0.001$) [8]. The greater bone size and thicker cortex thus confers a greater biomechanical advantage for bones of elderly men compared with elderly women [8]. In contrast to the trabecular compartment, where parameters showed cross-sectional age-related declines before and after age 50 years, the cortical parameters tended to remain stable until after age 50 years when they then started to decline [8]. Dalzell et al. also

identified that cortical thickness declined faster in women than men after age 50 [19].

MacDonald et al. observed that cortical porosity increased to a lesser extent in men than women with advancing age [15]. However, cortical porosity appeared to be greater in men than women at all age ranges except at the eighth decade [15]. In a study involving 57 men and 94 women (age range 20–78 years), Burghardt et al. [20] reported similar observations, with men having greater cortical porosity than women, especially before age 30. Differences became attenuated in those over the age of 50 years due to a 3-fold increase in cortical porosity in women from age 20 to 60 years, with men having an increase in cortical porosity of only 60% over the same age range [20].

Based on observations from these cross-sectional studies, men appear to have more trabecular thinning than dropout with increasing age, while women have both trabecular thinning and dropout. Furthermore, men have greater bone size than women across age and suffer less cortical thinning than women with aging. Although men have greater cortical porosity than women, it increases more in women than men with age. Overall, these apparent age-related changes in trabecular and cortical microstructure in men would thus seem to confer less of an adverse effect on bone strength and, thereby, explain the lower fracture risk, in aging men when compared with aging women.

4. Bone Microarchitecture by HR-pQCT and Fracture Risk in Men

To date, only the Structure of the Aging Men's Bones (STRAMBO) study has evaluated the relation between prevalent fractures in men with bone microarchitecture parameters from HR-pQCT [21, 22]. In a subset of men from the STRAMBO cohort, 185 men (mean age, 71 years) with a prevalent fracture and 185 men without fracture had both the distal radius and tibia imaged by HR-pQCT and had assessments of bone strength by finite element analysis [21]. At both bone sites, CtTh, TbN, and TbSp were significantly worse in cases than controls, as was the aBMD and vBMD [21]. Bone strength parameters of stiffness and failure load also were 8-9% lower in fracture cases relative to controls ($P < 0.01$) [21]. In a larger cohort from STRAMBO, involving 920 men over the age of 50 years who had HRpQCT measurements at the distal radius and tibia, 98 men had a prevalent vertebral fracture, and 100 men had a prevalent peripheral fracture [22]. Again, almost all bone microarchitecture parameters from HRpQCT scans were worse in men with either vertebral or peripheral fractures [22]. Following adjustment for aBMD, there were no associations observed between bone microarchitecture parameters and peripheral fractures, whereas cortical vBMD and Ct Th at both the distal radius and tibia remained lower in men with vertebral fractures [22]. Whether the bone microarchitecture parameters based on the radius or tibia will be able to predict overall or site-specific fragility fractures in men prospectively remains to be determined.

Imaging studies using HR-pQCT of bone microarchitecture among children at various stages of puberty also have provided further understanding on the pathogenesis of increased fractures in adolescence among both boys and girls although findings are again limited by their cross-sectional nature. In the study by Kirmani et al. [11], the proportion of load borne by cortical bone and the ratio of cortical to trabecular bone volume at the radius decreased transiently during mid-to-late puberty in both sexes, with apparent cortical porosity peaking during this time [11]. These findings appeared to mirror the incidence of distal forearm fractures seen in boys and girls of this age group [11]. Regional deficits in cortical bone were thus suggested to underlie the adolescent peak in forearm fractures [11], a hypothesis that was also suggested by Wang et al. [17].

5. Conclusion

Although studies in men imaged by HR-pQCT are limited, current findings have provided novel insights into the sex- and age-related differences in trabecular and cortical microstructure that underlie the differences in bone strength and fracture risk observed in men relative to women. Nevertheless, the cross-sectional nature of studies to date does limit the ability to make definitive conclusions, and longitudinal studies remain the ideal in order to best determine age-related changes in bone microarchitecture and their relation to fracture risk in both men and women.

Conflict of Interests

There are no conflict of interests with respect to this work. Dr. Amin serves on a scientific advisory board for Merck & Co, Inc.

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Review Article

Biochemical Bone Turnover Markers and Osteoporosis in Older Men: Where Are We?

Pawel Szulc

INSERM UMR 1033, Université de Lyon, Hôpital Edouard Herriot, Pavillon F, Place d'Arsonval, 69437 Lyon, France

Correspondence should be addressed to Pawel Szulc, pawel.szulc@inserm.fr

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In men aged less than 60, the association of serum and urinary levels of biochemical bone turnover markers (BTMs) and bone mineral density (BMD) is weak or not significant. After this age, higher BTM levels are correlated weakly, but significantly, with lower BMD and faster bone loss. Limited data from the cohort studies suggest that BTM measurement does not improve the prediction of fragility fractures in older men in comparison with age, BMD, history of falls and fragility fractures. Testosterone replacement therapy (TRT) decreases bone resorption. During TRT, bone formation markers slightly increase (direct effect on osteoblasts), then decrease (slowdown of bone turnover). Bisphosphonates (alendronate, risedronate, ibandronate, zoledronate) induce a rapid decrease in bone resorption followed by a milder decrease in bone formation. In men receiving antiresorptive therapy for prostate cancer, zoledronate, denosumab and toremifene decrease significantly levels of bone resorption and bone formation markers. Teriparatide induced a rapid increase in serum concentrations of bone formation markers followed by an increase in bone resorption. We need more studies on the utility of BTM measurement for the improvement of the persistence and adherence to the anti-osteoporotic treatment in men.

1. Introduction

Biochemical markers of bone turnover (BTMs) have been investigated in several male cohorts for the last 15 years. These studies provided data on age-related changes in bone turnover rate in men and on potential determinants of bone turnover rate in men, such as hormones, lifestyle factors, diseases, or medications. However, data on practical use of BTM in the clinical management of osteoporosis in men are limited and rather disappointing.

There are two groups of biochemical bone turnover markers (BTMs), markers of bone formation and markers of bone resorption (Table 1). OC, PICP, and PINP are released during the synthesis of OC and type I collagen which are constituents of bone matrix. CTX-I, NTX-I, PDP, and PYD are products of catabolism of type I collagen [1]. Bone AP and TRACP5b are enzymes reflecting the metabolic activity, respectively, of osteoblasts and osteoclasts. Recently, it has been proposed that PINP and serum CTX-I become the referent markers of bone formation and resorption, respectively [2].

2. Association of BTM Levels with Bone Mineral Density and Bone Loss in Men

The association between BTM levels and areal bone mineral density (aBMD) measured by dual energy X-ray absorptiometry (DXA) was assessed in cross-sectional studies. Before the age of 60, the association was nonsignificant [3]. It may reflect two processes. In young adult men, the consolidation, that is, formation of peak aBMD after the growth arrest, is associated with a slowdown of bone turnover. However, one BTM level cannot reflect different trends which vary according to the skeletal site. In middle-aged men, apparent stability of aBMD is a trade-off between periosteal apposition and endosteal bone loss. As these processes are slow, aBMD may depend more on the peak aBMD acquired previously than on the current bone turnover rate.

After the age of 60, BTM levels were correlated moderately negatively with aBMD [3, 4]. The difference in average aBMD between the lowest and the highest BTM quartiles varied from 3 to 12%. Prospective data on the association between BTM levels and bone loss in men are scanty and,

Table 1: Biochemical bone turnover markers.

Bone formation
Osteocalcin (*OC*)
Bone alkaline phosphatase (*bone ALP*)
N-terminal propeptides of type I procollagen (*PINP*)
C-terminal propeptides of type I procollagen (*PICP*)
Bone resorption
C-terminal cross-linking telopeptides of type I collagen (*CTX-I*),
N-terminal cross-linking telopeptides of type I collagen (*NTX-I*)
C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinases (*CTX-MMP, ICTP*)
Helical peptide 620–633 of the a1 chain
Deoxypyridinoline (*DPD*)
Pyridinoline (*PYD*)
Isoform 5b of tartrate resistant acid phosphatase (*TRACP5b*)

most often, negative. Higher BTM levels were associated with faster bone loss at some skeletal sites (e.g., hip, distal forearm), but not all (e.g., spine) [5–7].

Several factors can contribute to these results. Bone loss is slower in men than n women, especially before the age of 70. Thus, a long-term follow-up is necessary to obtain an accurate estimate of bone loss. The rate of bone loss may vary according to the skeletal site. In particular, lumbar spine is not a reliable skeletal site for estimation of bone loss in men because of frequent osteoarthritis. The assessment of bone loss (which is in fact the endosteal bone loss) is biased by the concomitant periosteal apposition. This process is present in both older men and women; however, it may have a greater impact in men who have lower endosteal bone loss and greater periosteal apposition than in women. Finally, bone turnover is influenced by many factors and a single measurement of BTM may not correspond to the average bone turnover rate over a long period of time.

3. Association between BTM Levels and the Risk of Fracture in Men

Several prospective cohort and case-control studies showed that elevated BTM levels predict fractures in postmenopausal women after adjustment for age, aBMD, and history of fracture. This association was found mainly in follow-ups lasting <5 years, mainly for bone resorption markers, and mainly for major osteoporotic fracture (mainly hip fracture) [1, 7]. Fewer studies concerned the prediction of fractures by BTM in men. The cohort studies (MrOS, MINOS) showed that, in older men, higher BTM levels did not predict fractures in models adjusted for age and aBMD [5, 6]. Two studies suggested that BTMs predict fractures in men. However, the association between low gammacarboxylation of osteocalcin (OC) and fracture risk was not adjusted for aBMD [8]. In the Dubbo cohort, only C-terminal cross-linking telopeptide of type I collagen generated by metalloproteinase (ICTP), but not other BTM, was predictive of fracture [9]. In addition, some methodological limitations should be recognized (lack of standardization of blood collection, suboptimal ascertainment of incident fractures). In a more recent study in the MrOS cohort, higher levels of urine alpha CTX isomer/Cr and greater alpha/beta CTX isomer ratio were associated with a higher risk of clinical vertebral fracture [10].

The reason for the difference between men and women is not clear. The higher bone turnover results in the deterioration of bone strength through several mechanisms: faster bone loss, increased formation of stress risers, impaired trabecular connectivity, higher fraction of bone which is only partly mineralized, and greater fraction of protein matrix which has undergone posttranslational modifications only partly. However, the "elevated" BTM levels (e.g., the highest sex-specific quartile) correspond to higher absolute BTM levels and faster bone turnover in women than men. Thus, the loss of bone strength due to faster bone turnover may be less in men than in women. In addition, in men, this smaller decline in bone strength occurs in bones which are larger and stronger. Thus, the loss of bone strength due to the faster bone turnover may have a smaller impact on the overall fracture risk in men than in women.

4. Limitations and Potential Interest of BTM in the Clinical Practice

Analytical and preanalytical variability have a strong effect on the BTM levels. The analytical variability depends on the BTM, the measurement method, and the technician's expertise.

The preanalytical variability comprises many factors. Circadian rhythm has a strong impact on the BTM variability, mainly serum bone resorption markers (CTX-I) [11]. Bone turnover is influenced by the vitamin D and calcium status, mainly in the elderly. BTM levels are increased especially in the institutionalized and home-bound vitamin D-deficient elderly who have secondary hyperparathyroidism associated with markedly increased BTM levels [12]. The seasonal variation of sunlight exposure leads to lower vitamin D level in winter. Consequently, levels of parathyroid hormone and BTM are higher in winter, mainly in the elderly.

Furthermore, BTM levels are influenced by a recent fracture, especially during the first 4 months [13]. GnRH agonists (used in the treatment of prostate cancer) inhibit the androgen secretion and decrease the 17β-œstradiol level leading thereby to a higher bone turnover and bone loss.

In some clinical situations, BTM levels may help to establish diagnosis. For instance, BTMs are elevated in hyperthyroidism, primary hyperparathyroidism, or Paget's disease [1]. By contrast, BTMs are decreased in hypothyroidism, hypoparathyroidism, or hypopituitarism. BTM levels are usually increased in patients with bone metastases and their levels are correlated with the spread of bone metastases [14]. A particular situation is multiple myeloma characterized by low OC concentration and markedly increased bone resorption.

Endogenous and exogenous corticosteroids inhibit bone formation [15]. The decrease in OC level is rapid and

followed by a delayed and milder decrease in PICP and PINP. Bone resorption can increase transiently. Inhaled corticosteroids decrease OC levels in a dose- and drug-dependent manner without significant effect on other BTMs [16].

5. Effect of Antiosteoporotic Treatment on BTM in Men

5.1. Testosterone Replacement Therapy (TRT). In most, but not all, studies, TRT decreased bone resorption marker levels [17–21]. However, decrease in the urinary excretion of bone resorption markers per mg urinary creatinine is partly related to the increase in muscle mass, and this result should be interpreted cautiously. Bone formation markers (especially osteocalcin and N-terminal type I collagen propeptide (P1NP)) increase slightly during the first weeks of TRT [18, 20]. This increase may reflect the direct stimulatory effect of TRT on osteoblasts in preexisting bone remodeling units. Later on, bone formation markers decrease, what reflects the general slowdown in bone turnover [20, 21]. However, this trend has not been found consistently [19].

Overall, data on the effect of TRT on BTM levels are discordant. The effect of TRT on bone turnover in hypogonadal men depends on factors such as the initial hormonal status (severity and duration of hypogonadism, hypogonadism developed in childhood or in adult age, and isolated hypogonadism or multihormonal deficit), baseline bone turnover rate, intramuscular or transdermal route of TRT administration, and normalization of testosterone level during treatment and treatment duration [22]. Testosterone increases the collagen synthesis in other tissues (e.g., muscles), which is confirmed by an increase in the serum level of N-terminal propeptide of type III collagen [23]. Thus, the potential contribution of muscle collagen to the circulating PINP and PICP levels is possible. Methodological limitations of certain studies should be recognized (small heterogeneous groups, lack of control group, large dropout, various doses of testosterone analyzed jointly, and impossibility to assess adherence to treatment).

5.2. Antiresorptive Treatment. Bisphosphonates are potent inhibitors of bone resorption. The studies in men concern mainly alendronate, risedronate, ibandronate, and zoledronate. After a given type of bisphosphonate, changes in BTM levels were similar in various groups of men with primary and secondary osteoporosis. Alendronate (10 mg daily *per os*) decreased bone resorption marker levels by about 50% after 3 months of treatment, what was followed by a milder (30 to 40%) decrease in the serum levels of bone formation markers [24–26]. Similar trends were observed in men with primary or secondary osteoporosis related to hypogonadism or corticotherapy.

Risedronate (2.5 or 5 mg daily or 35 mg weekly *per os*) decreased bone resorption markers by 40 to 50% after 3 months of treatment, what was followed by a milder (25–30%) decrease in serum levels of bone formation markers. Similar trends were observed in osteoporotic men aged 30 and over, older Japanese men with untreated osteoporosis, men on corticosteroid therapy, elderly men after stroke and elderly men with Parkinson's disease [27–31].

In 14 patients with Klinefelter's syndrome, intravenous ibandronate (2 mg every 3 months) decreased urinary levels of bone resorption markers by 30 to 70% after one month [32]. This decrease was followed by a milder decrease (25–35%) in bone formation markers. In men with low aBMD (STRONG study), once-monthly oral ibandronate (150 mg) induced a 40 to 50% decrease in serum concentration of C-terminal telopeptide of type I collagen (CTX-I) accompanied by a 20–30% decrease in bone alkaline phosphatase (BAP) activity [33].

BTMs were assessed in several studies carried out in men treated with zoledronate (5 mg *i.v.* once yearly in men with primary osteoporosis or hypogonadism, 4 mg *i.v.* once yearly in HIV-infected men with low aBMD, single dose *i.v.* 4 mg in osteopenic cancer survivors) [34–36]. Zoledronate induced a profound decrease (>60%) in bone resorption markers within the first week of treatment followed by a milder (30–40%) decrease in bone formation markers.

Finally, antiresorptive medications were assessed in men receiving androgen-deprivation therapy for prostate cancer. In this group, zoledronate, denosumab (monoclonal anti-RANKL antibody), and toremifene (selective estrogen receptor modulator) decreased significantly levels of bone resorption and bone formation markers [37–39].

5.3. Treatment with Bone Formation-Stimulating Agents. Recombinant human parathyroid hormone (1–34) (rhPTH-(1–34)) induced a rapid increase in serum concentrations of bone formation markers, especially P1NP, followed by an increase in bone resorption marker levels [40, 41]. After 6 to 9 months of treatment, BTM attained the maximum (50 to 250% above baseline), then slightly decreased, but remained elevated. By contrast, during combined treatment (alendronate and rhPTH-(1–34)) started 6 months after the beginning of the anti-resorptive treatment, BTM levels were lower and the increase in aBMD at the spine and femoral neck was smaller than after rhPTH-(1–34) alone [41].

6. Summary, Conclusions, and Perspectives

Overall, in older men, higher BTM levels are associated with lower aBMD and faster bone loss. Thus, in older men, accelerated bone turnover is probably a major determinant of bone loss and osteoporosis. There are two major directions for the further studies on BTM in men. From the scientific point of view, development of markers reflecting qualitative properties of bone would improve our understanding of the mechanisms of bone fragility in older men. From the practical point of view, BTMs do not improve the identification of men at high risk of accelerated bone loss or fragility fracture. In older men, changes in BTM levels induced by anti-osteoporotic treatment are similar to those found in postmenopausal women. However, we need more studies on the association between changes in bone turnover rate and the decrease in the risk of fragility fracture. In particular, we need studies on the utility of BTM measurement for the improvement of the persistence and adherence to the anti-osteoporotic treatment in men.

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Research Article

Age-Related Changes in Bone Remodelling and Structure in Men: Histomorphometric Studies

Juliet Compston

Department of Medicine, Cambridge University Hospitals NHS Foundation Trust, Cambridge CB2 0QQ, UK

Correspondence should be addressed to Juliet Compston, jec1001@cam.ac.uk

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Histomorphometric studies of the age-related changes in bone remodelling and structure in men are relatively sparse and mainly limited to the iliac crest. The available data indicate that loss of trabecular bone is predominantly due to decreased formation at the level of individual bone remodelling units and that an increase in remodelling rate does not play a major role. The main structural consequence of the changes in bone remodelling is trabecular thinning. In cortical bone, an age-related reduction in cortical width and increase in porosity have been demonstrated at several skeletal sites. However, the alterations in bone remodelling responsible for these changes remain to be established.

1. Introduction

Loss of bone with ageing is a universal phenomenon affecting both men and women and is associated with reduced bone strength and increased fracture risk. Whereas the changes in bone remodelling and structure underlying bone loss have been relatively well studied in women, there have been few studies in men. This paper reviews the bone histomorphometric data available in men and discusses the similarities and differences in cellular and structural mechanisms of bone loss in men and women.

2. Cellular and Structural Mechanisms of Bone Loss

In trabecular bone, bone loss may occur as a result of increased remodelling rate and/or a negative remodelling balance at the level of individual bone remodelling unit of basic multicellular unit (BMU) (Figure 1). The remodelling balance is determined by the amount of bone resorbed and that subsequently formed; hence a negative balance may be the result of increased resorption, reduced bone formation, or a combination of the two. The structural changes occurring during bone loss are determined by the underlying alterations in bone remodelling. An increase in remodelling rate, particularly if resorption within individual BMUs is also increased, will favour trabecular penetration and loss of connectivity whereas remodelling imbalance due to decreased formation in the BMU will predominantly be associated with trabecular thinning and preservation of trabecular microarchitecture. However, as trabecular thinning progresses a point will be reached when a resorption cavity of normal depth can result in trabecular penetration.

Cortical bone loss results from changes in both endocortical and intracortical remodelling. In addition, cortical thickness is influenced by the rate of periosteal apposition. As in cancellous bone, these structural alterations are determined by changes in remodelling rate and balance.

3. Strengths and Limitations of Bone Histomorphometry

In humans, histomorphometric studies of age-related changes in bone are mostly restricted to iliac crest bone. Bone loss during ageing varies according to skeletal site, and thus the iliac crest may not always be representative of the rest of the skeleton. Furthermore, there is a large measurement variance associated with bone histomorphometry. However, bone histomorphometry remains the only technique currently

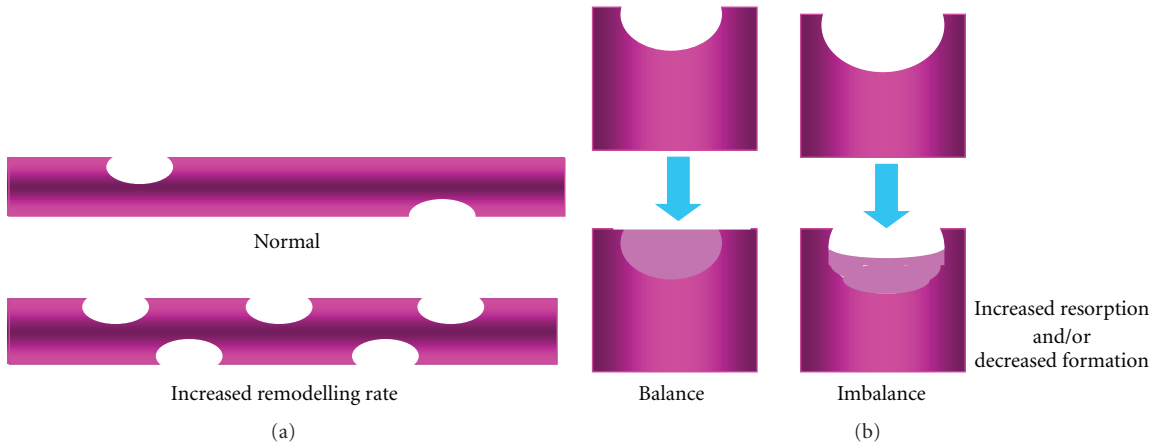

Figure 1: Mechanisms of trabecular bone loss. (a) Increased remodelling rate, (b) negative remodelling imbalance.

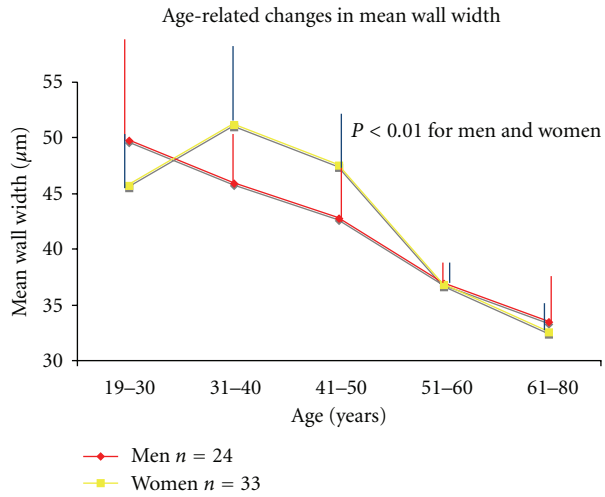

Figure 2: Age-related changes in mean wall width in healthy men and women (see [1]).

available that enables detailed assessment of remodelling rate, remodelling balance, and cellular activity.

4. Age-Related Changes in Trabecular Bone Remodelling and Structure in Men

Histomorphometric studies of age-related changes in men have all been cross-sectional and have contained relatively small numbers of individuals. In iliac crest bone a decrease in trabecular bone volume with age in men has been reported, generally becoming detectable around the fifth to sixth decade of life [2–5]. In some studies, the rate of bone loss was similar to that observed in women, but in two larger studies trabecular bone loss was significantly greater in women than in men [2, 3].

In the vast majority of studies in men tetracycline labelling was not given, and hence changes in remodelling rate could not be directly assessed. However, in one study of 24 men across a wide age range in which tetracycline was given prior to biopsy, no clear age-related increase in remodelling rate was seen, in contrast to that observed in women [1]. Consistent with this observation osteoid surface, which reflects tissue level bone formation, does not show the same age-related increase in men as seen in women [4]. In contrast, there is consistent evidence that mean wall width, which reflects the amount of bone formed within individual BMUs, decreases with age both in men and women (Figure 2) [1, 6]. Changes with ageing in the depth of resorption cavities are more difficult accurately to assess, and in men, no change was reported in one study whilst in another, a decrease in men, but not in women was reported although in women, a transient increase was noted around the time of the menopause [7, 8].

The available evidence thus indicates that, at least in iliac crest bone, trabecular bone loss in men is predominantly due to a negative balance within BMUs, which results mainly from a decrease in the amount of bone formed and that increased remodelling rate does not make a substantial

contribution to bone loss. These conclusions are supported by the demonstration that trabecular thinning is greater and loss of trabeculae less prominent with ageing in men than in women [5, 9]. Thus in a study of 49 men, a significant age-related decrease in mean trabecular width was demonstrated with nonsignificant trends towards decreased trabecular number and increased trabecular separation. In women, however, there was a nonsignificant decrease in trabecular width and a significant decrease in trabecular number and increase in trabecular separation [9]. The results of more recent studies using high-resolution peripheral quantitative computed tomography (HRpQCT) suggest that these sex-specific patterns of bone loss are also seen in other parts of the skeleton [10].

5. Changes in Cortical Bone Remodelling and Structure in Men

Histomorphometric data on age-related changes in cortical bone in men are sparse. In addition, skeletal heterogeneity of changes is likely to be particularly evident as a result of differences in the weight-bearing properties at different sites, resulting in site-specific differences in the onset and rate of age-related bone loss [11]. Even within a single site, significant variations in cortical bone structure may be observed, as recently reported in the femoral neck [12].

Notwithstanding this variability, a decrease in cortical width during ageing in men has been reported at a number of skeletal sites [13–15]. Whether there are differences between men and women in the magnitude of age-related cortical thinning is uncertain; however, greater periosteal apposition and less endocortical resorption in men may result in lower rates of thinning. Most studies have shown an age-related increase in cortical porosity in both men and women in the iliac crest and other skeletal sites including the vertebrae, metacarpal, rib, and femur [3, 14, 16]. This is due at least in part to an increase in the size of individual pores, as assessed by Haversian canal diameter, although an increase in the number of pores may also contribute.

Age-related changes in cortical bone remodelling have not been well documented. In a cross-sectional study of 41 women and 23 men Brockstedt et al. [14] reported a decrease in mean wall width in iliac crest bone in women, but not in men. Since osteonal diameter and Haversian canal diameter increased with age in both sexes, the authors concluded that in men, an increase in resorption depth accounted for the negative remodelling imbalance. Similar findings were reported by Broulik et al. [17], but as direct measurements of resorption depth were not made in either study, this conclusion remains speculative. In the rib, mean wall width does not change with age in either sex [18]. Age-related changes in remodelling rate in cortical bone in men have not been reported; however, Brockstedt et al. [14] reported a significantly higher activation frequency in postmenopausal than premenopausal women, supporting an increase in remodelling rate during and after the menopause.

Overall, the available data support qualitatively similar age-related changes in cortical bone in men and women, with a decrease in cortical width and increase in cortical porosity. However, there may be differences between the sexes in the magnitude of these changes; furthermore, the underlying alterations in bone remodelling remain to be clearly defined.

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Review Article

Therapy of Osteoporosis in Men with Teriparatide

Natalie E. Cusano, Aline G. Costa, Barbara C. Silva, and John P. Bilezikian

Metabolic Bone Diseases Unit, Division of Endocrinology, Department of Medicine, College of Physicians and Surgeons, Columbia University in the City of New York, New York, NY 10032, USA

Correspondence should be addressed to John P. Bilezikian, jpb2@columbia.edu

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Osteoanabolic therapy is an attractive therapeutic option for men with osteoporosis because it directly stimulates bone formation, an action not shared by any antiresorptive drug. Teriparatide (recombinant human PTH(1-34)) and PTH(1-84) are available in many countries but PTH(1-84) is not available in the United States. Only teriparatide is approved for the treatment of osteoporosis in men. It is also indicated in glucocorticoid-induced osteoporosis. Teriparatide is associated with major gains in bone density at the lumbar spine and, to a lesser extent, in the hip regions. Vertebral and nonvertebral fractures are reduced in postmenopausal women treated with teriparatide. Fracture reduction data in men are less secure because the number of study subjects is small and the studies have not been powered to document this endpoint. Nevertheless, observational data in men suggest a reduction in vertebral fractures with teriparatide. Attempts to show further beneficial effects of teriparatide in combination with antiresorptive agents have not been demonstrated yet to be superior to monotherapy with teriparatide alone. The duration of therapy with teriparatide is limited to 2 years. Thereafter, it is necessary to treat with an antiresorptive drug to maintain, and perhaps increase, densitometric gains. Teriparatide is well tolerated with a good safety profile.

1. Introduction

PTH(1-84) and its foreshortened variant, teriparatide (PTH(1-34)), represent the only available osteoanabolic therapies for osteoporosis. In contrast to antiresorptive therapies, which are the mainstay of osteoporotic treatment, these agents directly stimulate bone formation, and as a result, improve not only bone mass but also skeletal microstructure, including trabecular connectivity and cortical thickness.

Although postmenopausal women are the dominant cohort at risk for osteoporosis, men are not spared. Current figures show that men represent approximately 25% of all osteoporotic individuals [1]. Morbidity and mortality figures after a hip fracture in men are disproportionately higher than in women [2], perhaps because men are older when they develop their osteoporotic fractures and have, therefore, more comorbid conditions. Because men have not been the center of attention in this disease, the data supporting the use of teriparatide are not as secure as in postmenopausal women, a point that is also true for data related to the use of antiresorptive therapy in osteoporotic men. In this report, we summarize the available data on the use of osteoanabolic therapy in male osteoporosis.

2. Anabolic Activity of Parathyroid Hormone

Parathyroid hormone (PTH) has the interesting property of harboring both catabolic and anabolic proclivities in bone. The prototypical disease that illustrates best the catabolic disposition of PTH is primary hyperparathyroidism. It is of interest that the property to resorb bone in primary hyperparathyroidism is seen most in the cortical skeleton with cancellous bone being relatively spared [3]. In fact, microarchitectural studies of cancellous bone in primary hyperparathyroidism suggest that even under conditions of chronic excessive exposure to PTH, microstructure is maintained, if not enhanced [4]. This clue to the anabolic potential of PTH is realized by low-dose, intermittent administration of teriparatide or PTH(1-84). The mechanisms by which PTH induces an anabolic effect on bone are likely to be multifactorial, including a number of pathways such as

Wnt (via stimulating Wnt 10b [5], and inhibiting sclerostin [6]), Runx2, and insulin-like growth factor (IGF-I). The net effect of low-dose, intermittent PTH exposure is an initial recruitment of osteoblast progenitor cells and direct stimulation of mature osteoblasts [7].

In both men and women, PTH increases bone mineral density (BMD) in the lumbar spine, a site rich in cancellous bone (Figure 1). Increases in the hip region are more modest and PTH therapy reduces BMD at the distal 1/3 radius, a cortical site. The early effect of PTH is an initial rapid increase in bone formation markers subsequently followed by an increase in bone resorption markers (Figure 2). These changes in bone formation markers are accompanied by histomorphometric observations that confirm an effect of PTH to increase processes associated with bone formation without any early evidence for bone resorption (Figure 3). This effect is reminiscent of bone metabolism in growing children in whom bone *modeling* is dominant. Thereafter, teriparatide leads to an increase in bone resorption giving rise to the more typical characteristics of bone metabolism in adults, namely, bone *remodeling*. Approximately 30% of the overall effect of PTH is thought to be due to the early effect on bone modeling with the majority being the subsequent action of PTH to stimulate bone remodeling. The period of time when PTH stimulates bone formation directly, before bone remodeling is stimulated, is explained by the concept of the "anabolic window" [8] (Figure 4). Even after bone turnover is stimulated, there is more bone formation than bone resorption ongoing, thus maintaining the anabolic window at least for a finite period of time.

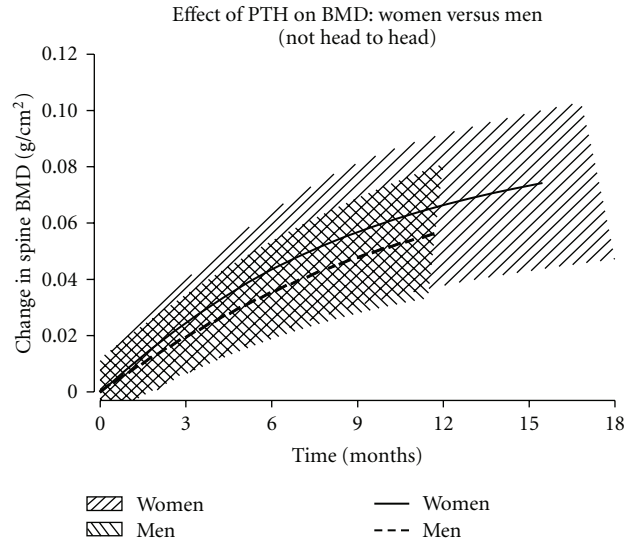

Figure 1: Changes in lumbar spine bone mineral density with teriparatide 20 μg daily. Although the figure does not show a head-to-head comparison, the increase in bone mineral density in men over the 11 months of the trial by Orwoll et al. tracks closely along the trajectory in bone mineral density women over the same period of time in the trial of Neer et al. (from Satterwhite et al. [26]).

3. Indications for Teriparatide Use

Teriparatide (PTH(1-34)) was approved by the United States Food and Drug Administration (FDA) in 2002 for the treatment of osteoporosis in men and postmenopausal women at high risk of fracture and in 2009 for the treatment of glucocorticoid-induced osteoporosis in men and women at high risk for fracture. Hodsman et al. [9] proposed criteria to establish the condition of "high risk," including preexisting osteoporotic fractures, very low bone density (T-score ≤ -3.5), and/or an unsatisfactory response to antiresorptive therapy. An unsatisfactory response to antiresorptive therapy, or drug failure, could be argued in the context of an incident fracture during treatment or gastrointestinal or other intolerance to bisphosphonates.

4. Teriparatide in Male Osteoporosis

Although both teriparatide and PTH(1-84) are available widely for the treatment of postmenopausal osteoporosis, only teriparatide has been studied and is available in men. The results of clinical trials with teriparatide in men, while more limited and less conclusive than those in women, show nevertheless results that are similar to larger studies in postmenopausal women. Kurland et al. [10] performed the first randomized trial evaluating the use of PTH(1-34) in men with idiopathic osteoporosis. The double-blind, placebo-controlled trial included 13 controls and 10 men treated with teriparatide 400 IU daily (approximately equivalent to 25 μg daily) for 18 months. There was a 13.5% increase in BMD at the lumbar spine in the PTH-treated group compared with controls ($P < 0.001$). Femoral neck BMD in the treatment group increased, however, more slowly and to a lesser extent (2.9% at 18 months; $P < 0.05$), and there was no significant change in the 1/3 distal radius site. Markers of bone turnover increased rapidly in the teriparatide-treated cohort, with bone formation markers rising and peaking earlier than bone resorption markers. These data demonstrate that the concept of a PTH-induced anabolic window is valid for men as well as women.

In a larger clinical trial that was also randomized, double-blinded and placebo-controlled, Orwoll et al. [11] studied 437 men with idiopathic or hypogonadal osteoporosis. Men were assigned to placebo (147 men), teriparatide 20 μg daily (151 men), or teriparatide 40 μg daily (139 men). The trial was terminated prematurely, after only 11 months because of the rat osteosarcoma toxicity results (see Section 6). Lumbar spine and femoral neck BMD increased by 5.9% and 1.5% respectively ($P < 0.05$ in the 20 μg group versus placebo). There were even larger increases in BMD at the 40 μg group, but adverse events were more frequently encountered.

Individuals in the study by Orwoll et al. were followed for up to 30 months after teriparatide was discontinued as part of a safety study [12]. Radiographs were available for comparison between baseline and 18 months after treatment discontinuation in 279 of the 437 men. The risk of new vertebral fractures in men treated with teriparatide (20 and 40 μg groups were combined) was reduced by 51% versus placebo ($P = 0.07$). Absolute risk reduction was 6%, similar to the fracture reduction data in women [13]. When only

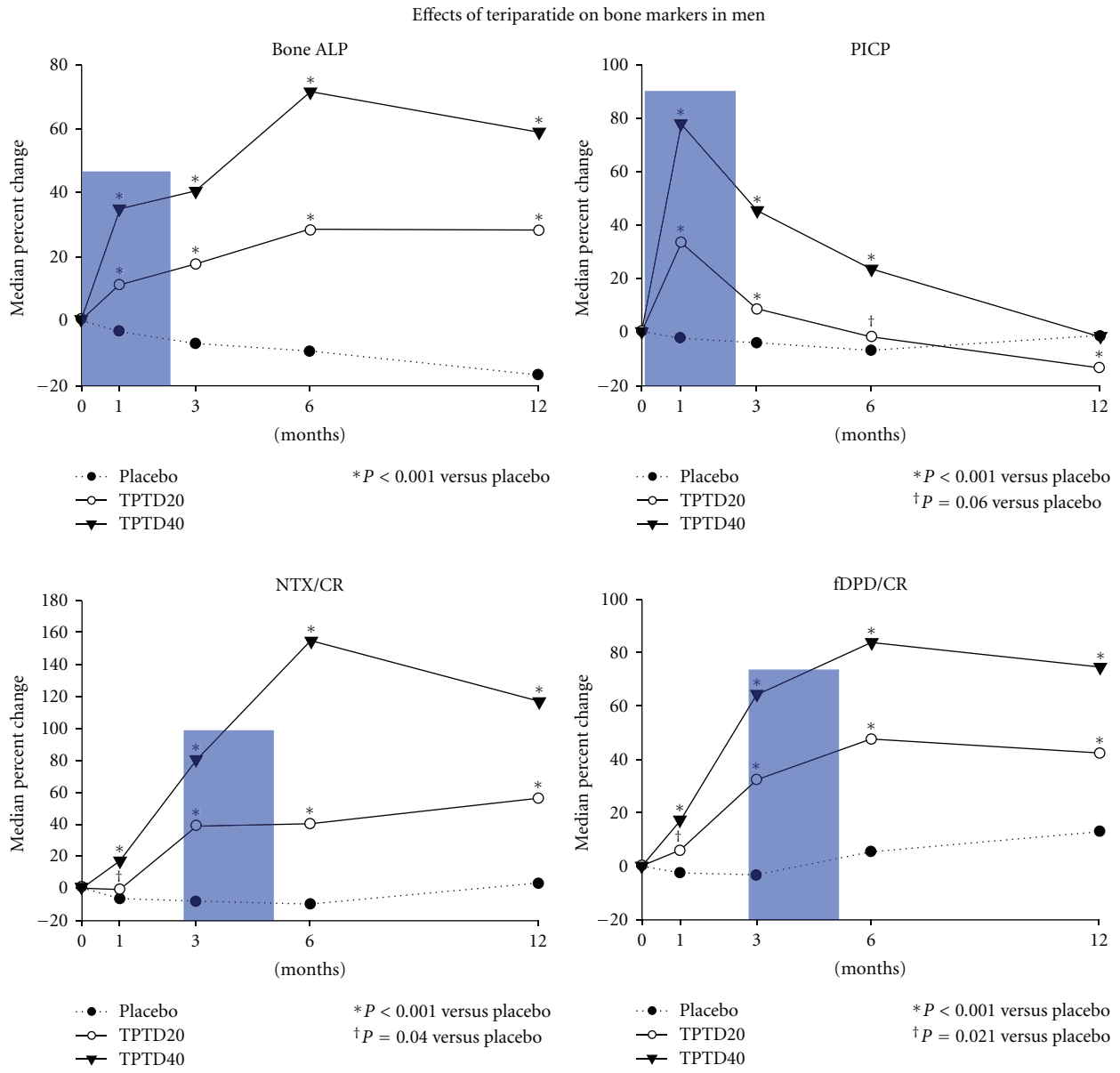

Figure 2: Changes in bone turnover markers after teriparatide administration to men with osteoporosis. Bone formation markers increase before bone resorption markers after men are exposed to teriparatide. Bone ALP, bone alkaline phosphatase; PICP, procollagen I carboxy-terminal; NTX/CR, urinary N-telopeptide/creatinine ratio; fDPD/CR, free deoxypyridinoline/creatinine ratio; TPTD20, teriparatide 20 μg; TPTD40, teriparatide 40 μg. From Orwoll et al. [11].

moderate or severe vertebral fractures were assessed, the difference from the placebo group reached significance (6.8% versus 1.1%; $P < 0.01$). In the 114 men who had a preexisting vertebral fracture at baseline, the absolute risk reduction for new vertebral fractures was 13.1%. In the follow-up period, other osteoporosis therapies were used, complicating the analysis and raising the possibility that subsequent treatment might have affected the fracture outcome data. More men in the placebo group received other treatment than those who received teriparatide (36% versus 25%; $P = 0.03$), suggesting that this confounding point might not be an issue. However, it is possible, if not likely, that those receiving further treatment after the clinical trial period had more severe osteoporosis. The greater use of osteoporosis therapy after the clinical trial was terminated was in the placebo group and thus, does not negate this confounder.

Glucocorticoid-induced osteoporosis (GIO) is the most common secondary cause of osteoporosis. Its major histomorphometric and dynamic element is reduced bone formation, a feature that may make GIO particularly well suited to an osteoanabolic approach. Saag et al. [14] compared alendronate and teriparatide in a head-to-head, randomized, double-blind, double-dummy trial of 83 men and 345 women with glucocorticoid-induced osteoporosis. All subjects had received 5 mg of daily prednisone, or the equivalent, for at least 3 months. In addition, enrollment

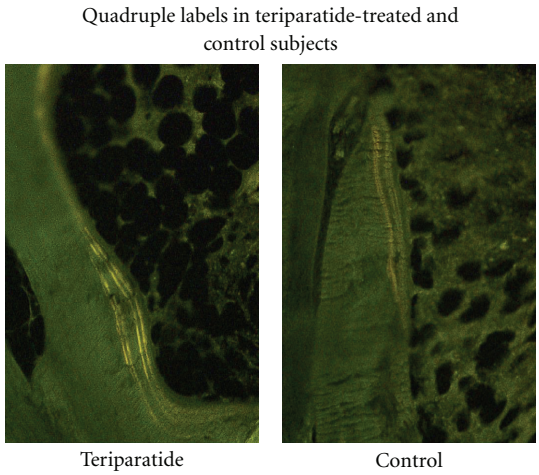

Figure 3: Early effects of teriparatide to increase bone modeling. Subjects were labeled before and 1 month after exposure to teriparatide. The control subjects did not receive treatment. The two sets of labels in the teriparatide-treated subject clearly demonstrate a marked increase in bone formation after teriparatide exposure. From Lindsay et al. [27].

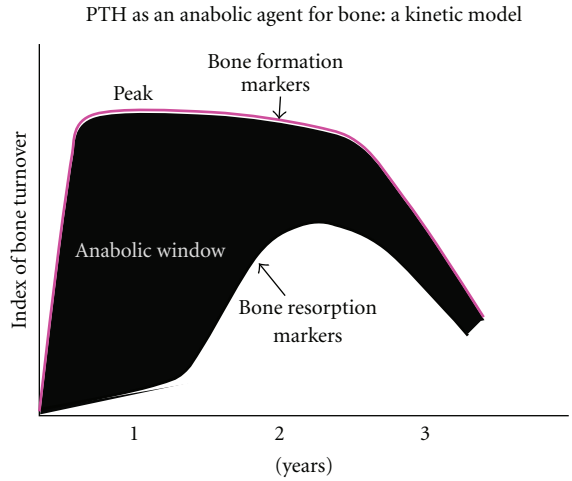

Figure 4: The anabolic window. The figure demonstrates the concept that bone formation is first stimulated by teriparatide or PTH(1-84) followed by an increase in bone resorption (adapted from [8]).

criteria included baseline lumbar spine or total hip T-scores ≤ -2.0 or ≤ -1.0 with at least 1 fragility fracture during glucocorticoid treatment. An equal number of subjects ($n = 214$) received either daily teriparatide 20 μg or daily alendronate 10 mg. The change in lumbar spine BMD (the primary end point) was greater in the teriparatide group, as early as 6 months after therapy was started, and by 18 months, the teriparatide group (+7.2%) was significantly greater ($P < 0.001$) than the alendronate group (+3.4%). When the men were analyzed separately from the women, the differences between teriparatide and alendronate were virtually identical to the combined data for men and women (7.3% versus 3.7%; $P = 0.03$) [15]. Total hip BMD in men increased significantly from baseline in the teriparatide group only. Although this study was not powered to detect a difference in fractures, they were captured as a reporting item. Notably, there were significantly fewer new vertebral fractures in the teriparatide group than in the alendronate group (0.6% versus 6.1%; $P < 0.01$). In the men, there were 4 new vertebral fractures in the alendronate group and none in the teriparatide group ($P = 0.05$).

This study was extended for an additional 18 months, with findings that continued to show major differences between those who were treated with teriparatide or alendronate. After 36 months, increases in lumbar spine and femoral neck BMD from baseline were significantly ($P < 0.001$) greater in the teriparatide group than in the alendronate group (11.0% versus 5.3% (lumbar spine); 6.3% versus 3.4% (femoral neck)) [16]. The major difference in fracture incidence was also maintained after 36 months when the teriparatide and alendronate groups were compared (1.7% versus 7.7%; $P = 0.007$).

5. Combination or Sequential Treatment with Teriparatide and Antiresorptive Therapy

5.1. Antiresorptive Therapy Prior to Teriparatide. Therapy with teriparatide typically follows a course of bisphosphonate or other antiresorptive therapy. In Europe, virtually all subjects who receive teriparatide or PTH(1-84) therapy have been previously treated with a bisphosphonate. Sequential therapy with an antiresorptive followed by teriparatide has only been studied in women, with the results suggesting that the potency of the antiresorptive drug to reduce bone turnover tends to determine whether or not there will be a delay in responsiveness to teriparatide. For example, bone markers in prior alendronate-treated patients increase later and peak at lower levels than in patients previously treated with raloxifene [17]. In a head-to-head comparison between risedronate and alendronate, the response to teriparatide was more rapid and greater in subjects previously treated with risedronate, but the results were not explained by the smaller effect of risedronate than alendronate to reduce bone turnover markers. These observations, made only in postmenopausal women, have not led to a general recommendation to wait for a period of time after bisphosphonate therapy before teriparatide is started. Eventually and rather soon after teriparatide therapy is started, bone turnover markers and BMD will begin to increase.

5.2. Combination Therapy. Finkelstein et al. [18] evaluated the simultaneous use of bisphosphonate and teriparatide in 83 osteoporotic men who were randomized to receive alendronate 10 mg (28 men), teriparatide 40 μg (27 men), or both (28 men) on a daily basis. Alendronate was given for 30 months, with teriparatide started at month 6 and continued for 24 months. The amount of daily teriparatide was twice the FDA-approved 20 μg dose. After 30 months, BMD at the lumbar spine increased to a greater extent in men treated with teriparatide alone than in the other groups (18.1% teriparatide versus 14.8% combination, or versus 7.9%

alendronate; $P < 0.001$). Femoral neck BMD also increased to a greater extent in the group treated with teriparatide alone (9.7% teriparatide versus 6.2% combination, or versus 3.2% alendronate; $P = 0.001$). Increases in vertebral trabecular BMD as determined by quantitative computed tomography (QCT) were markedly higher with teriparatide alone (48% teriparatide versus 17% combination, or versus 3% alendronate; $P < 0.001$). Bone turnover markers in the combination group were similar to those seen in the group treated with alendronate alone, namely, a rapid decrease in markers of both formation and resorption [19]. This lack of an effect of teriparatide in combination with alendronate to increase bone turnover markers confirms data in women who were evaluated with a protocol that differed only slightly (shorter time period; PTH(1-84) instead of teriparatide) [20]. Deal et al. [21] found that in women, the combination of teriparatide with raloxifene, a less potent antiresorptive agent, may enhance the bone-forming effects of teriparatide. In the study by Deal et al., bone formation markers increased with combination therapy to an extent similar to teriparatide alone while bone resorption markers did not increase to the same extent as teriparatide alone, suggesting that the anabolic window was greater with this approach to combination therapy. More recently, Cosman et al. [22] found that the combination of a single dose of zoledronic acid with daily teriparatide increased BMD after 6 months at the spine and hip to a greater degree than either drug alone. However, the major differences after 6 months were much less apparent at the end of the 12-month study.

5.3. Antiresorptive Therapy Following Teriparatide. Kurland et al. [10] were the first to show in men that when teriparatide is not followed by an antiresorptive agent, lumbar spine and hip bone density falls precipitously. In those whose teriparatide therapy is followed by alendronate, gains in lumbar spine and hip BMD are maintained [23]. Although not as clearly definitive, Kaufman et al. [12] also showed that lumbar spine and hip BMD tended to decline in men previously treated with teriparatide who received no subsequent treatment for osteoporosis. These results are similar to those observed in women [13]. Despite the lack of fracture outcome data, the results of these studies establish the importance of maintenance treatment with an antiresorptive following the recommended 2-year course of teriparatide therapy.

5.4. Teriparatide Retreatment. Subjects who completed the 30 month trial of Finkelstein et al. [18] comparing the effects of alendronate, teriparatide, or both on BMD and bone turnover were monitored for 12 months after therapy was discontinued and then randomized again to treatment with alendronate, teriparatide, or both for an additional 12 months [24]. Only the data for the group receiving teriparatide alone for 2 years, followed by 1 year of no therapy, then 1 year of teriparatide retreatment ($n = 21$) were presented. Not surprisingly, bone turnover markers and BMD fell when teriparatide was stopped. Teriparatide retreatment for 12 months resulted in an increase in lumbar spine BMD of 5.2%, compared with 12.5% during the first 12 months of treatment ($P < 0.001$). Bone turnover markers also increased more during the first 12 months of teriparatide treatment as compared to the 12 month retreatment period. The authors interpreted the data to reflect an attenuated response to teriparatide retreatment. The possibility remains that gains may have been greater if treatment had been continued beyond the first retreatment year.

6. Safety of PTH

Teriparatide is well tolerated in men and women. The FDA-approved treatment regimen is 20 μg daily for up to 24 months. Clinical trials have shown a very small risk of hypercalcemia at the 20 μg dose [11, 13]. In the postapproval period, the risk of hypercalcemia appears to be even lower than previously thought. Hypercalcemia is even less likely to occur if calcium supplementation is reduced by 500 mg/day when teriparatide is initiated. Teriparatide does not appear to significantly increase urinary calcium excretion [11, 12].

In the animal toxicity studies, male and female rats treated with teriparatide or PTH(1-84) at doses that were 3–60-times the equivalent dose to human subjects for 75 years of human equivalent time develop osteosarcoma [25]. This rat toxicity has not been seen in monkeys. With almost 9 years of clinical experience, the number of reported cases of osteosarcoma in patients is even less than expectations based upon epidemiological data of osteosarcoma in human subjects not treated with PTH. With no more than 3 cases reported among approximately 1.5 million subjects who have received teriparatide or PTH(1-84) worldwide, this toxicity does not appear to be a human one.

There are contraindications to the use of teriparatide, such as primary hyperparathyroidism. It should not be used in children with open epiphyses, in subjects at risk for osteosarcoma (Paget's disease of bone; previous external ionizing skeletal irradiation), or in subjects with a previous history of osteosarcoma. An unexplained elevation in the alkaline phosphatase is also a relative contraindication to teriparatide use.

7. Conclusions

Teriparatide, the only available osteoanabolic agent for men, is indicated for the treatment of osteoporosis when fracture risk is high. It is also indicated for the treatment of men with glucocorticoid-induced osteoporosis. The data for men in terms of increases in bone density and changes in bone turnover markers track virtually identically with the more extensive data that are available for postmenopausal women. Reduction in fracture incidence, although not conclusive, also appear to mirror the more extensive and conclusive data in women. Teriparatide is well tolerated for the recommended 2-year treatment period, and it should be followed by an antiresorptive drug to maintain increases in bone density. Combination therapy with antiresorptives has not been shown to be superior to monotherapy with teriparatide alone. Ongoing research, however, may offer

new insights into effective approaches to combination or sequential osteoanabolic and antiresorptive therapy.

Disclosure

Dr. J. P. Bilezikian is a consultant for Eli Lilly, NPS Pharmaceuticals, Merck, Novartis, Amgen, and receives research support from NPS Pharmaceuticals and GSK. Drs. N. E. Cusano, A. G. Costa, and B. C. Silva report no conflicts of interest.

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Review Article

RANKL-Targeted Therapies: The Next Frontier in the Treatment of Male Osteoporosis

Alicia K. Morgans and Matthew R. Smith

Division of Hematology/Oncology, Massachusetts General Hospital Cancer Center, POB 221, Boston, MA 02114, USA

Correspondence should be addressed to Alicia K. Morgans, aliciak_morgans@dfci.harvard.edu

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Male osteoporosis is an increasingly recognized problem in aging men. A common cause of male osteoporosis is hypogonadism. Thousands of men with prostate cancer are treated with androgen deprivation therapy, a treatment that dramatically reduces serum testosterone and causes severe hypogonadism. Men treated with androgen deprivation therapy experience a decline in bone mineral density and have an increased rate of fracture. This paper describes prostate cancer survivors as a model of hypogonadal osteoporosis and discusses the use of RANKL-targeted therapies in osteoporosis. Denosumab, the only RANKL-targeted therapy currently available, increases bone mineral density and decreases fracture rate in men with prostate cancer. Denosumab is also associated with delayed time to first skeletal-related event and an increase in bone metastasis-free survival in these men. It is reasonable to investigate the use of RANKL-targeted therapy in male osteoporosis in the general population.

1. Overview

Male osteoporosis is an important issue in men's health. More than 2 million American men have osteoporosis and approximately 7 million more at risk of developing the disease. It results in an increased risk of fracture due to a disrupted bone microenvironment with associated decreased bone mineral density (BMD) and increased bone fragility.

The impact of osteoporosis on otherwise healthy men is substantial. As men age, they generally lose BMD at a rate of 1% per year [1]. Fractures occur in one of eight men over 50 years old, with approximately 30% of all hip fractures occurring in men [2, 3]. Mortality one year after hip fracture for men has been estimated around 31–35%, a striking number when compared with 17–22% one-year mortality for women of the same age [4]. In-hospital mortality alone after a fraction is twice as high for men than for women [5, 6].

Osteoporosis has historically been classified as either primary or secondary. Primary osteoporosis is age-related or idiopathic osteoporosis that is not clearly due to another cause. Secondary osteoporosis results from various causes, the most common of which in men are glucocorticoid excess, heavy alcohol use, and hypogonadism. Although it can also result from other disorders, including hyperparathyroidism, hyperthyroidism, and malabsorptive disorders, more than 50% of male osteoporosis can be attributed to these three causes [7]. One of the more closely studied populations with secondary osteoporosis due to hypogonadism is prostate cancer survivors who have been treated with androgen deprivation therapy (ADT). This review will focus on this unique population as a model of hypogonadal osteoporosis to discuss the use of RANKL-targeted therapies in male osteoporosis.

2. Prostate Cancer

In the United States, prostate cancer is the most common malignancy in men and the second leading cause of cancer death. There were over 217,730 new diagnoses of prostate cancer in 2010, and this number continues to rise [8]. In 2010, it is estimated that there were approximately 32,050 deaths due to metastatic prostate cancer in the US [8]. Despite this, survival among all patients is 95% at 5 years, meaning a substantial portion of the prostate cancer population are survivors of their disease for lengthy periods of time [8].

3. Androgen Deprivation Therapy

Androgen deprivation therapy (ADT), by either bilateral orchiectomies or chronic administration of a gonadotropin releasing hormone (GnRH) agonist or antagonist, is the mainstay of therapy for metastatic prostate cancer. The intended therapeutic effect of ADT is severe hypogonadism. ADT decreases serum levels of total testosterone by more than 95% to below 20 ng/dL in most men.

ADT use has been associated with a survival advantage in several subgroups of prostate cancer patients. For men with high-risk locally advanced disease, the use of ADT in addition to definitive external beam radiation therapy results in improved disease-free and overall survival as compared to radiation therapy alone [10]. Additionally, there is evidence that the use of ADT after radical prostatectomy in men with positive lymph nodes likely improves overall survival [11]. ADT can be used alone or in combination with salvage radiation in men with a rising PSA after definitive radiation or prostatectomy although there is not yet sufficient evidence to prove a survival benefit [10].

4. Bone Loss and Fractures during ADT

In addition to causing numerous other metabolic side effects, ADT use is associated with a decline in BMD [12–16]. A reduction in BMD can be seen within six to nine months of initiating therapy, and BMD of the hip and spine continue to decrease by approximately 2-3% per year [5, 12–14].

The incidence of fractures in men receiving ADT is also elevated, approaching 20% after 5 years of therapy [15]. Several large population-based studies demonstrated a 21–45% relative increase in fracture risk among men being treated with ADT when compared to men without such treatment [15–17]. A recent analysis of SEER and Medicare data including over 50,000 men found a 19.4% rate of fracture in men receiving ADT as opposed to a rate of 12.6% in those who were not ($P < 0.001$) [15]. Similarly, an analysis of 3,887 Medicare records from men with nonmetastatic prostate cancer found ADT use associated with a relative risk of fracture of 1.21 when compared to men who were not on ADT (95% CI, 1.14–1.29, $P < 0.01$) [16].

The mechanism of ADT-related bone loss is likely partially due to increased bone turnover [14]. Markers of osteoclast and osteoblast activity, such as osteocalcin, are increased in men receiving ADT and tend to plateau around 6 months after initiating treatment [14]. There is also evidence that alterations in skeletal sensitivity to parathyroid hormone may cause increased bone turnover [18].

The effects of estrogen on bone also likely contribute to ADT-associated bone loss. ADT causes testosterone levels to plummet, which also results in low levels of serum estradiol due to the peripheral conversion of testosterone to estrogen. Estrogen signaling through estrogen receptors on osteoblasts and osteoclasts contributes to the regulation of bone remodeling in men [19]. Additionally, levels of estradiol in healthy older men correlate with spinal bone mineral density and are inversely associated with vertebral fracture risk [20–22].

Recently there has been interest in using RANKL-targeted therapy to reduce the incidence of osteoporosis and fracture in men with nonmetastatic prostate cancer receiving ADT. Like men in the general population with hypogonadal osteoporosis, the cause of osteoporosis in these men is low levels of testosterone. Because there is not yet primary data with RANKL-targeted therapy in men with osteoporosis in the general population, it is reasonable to consider extrapolating this data to men with other forms of hypogonadal osteoporosis for hypothesis generation and future investigation.

5. RANK-L Targeted Therapy

Bone exists in state of continuous remodeling, striking a delicate balance between osteoclast resorption and osteoblast formation of new bone. The receptor activator of nuclear factor-κB ligand (RANKL) system plays a critical role in this balance. RANKL is a member of the tumor necrosis factor (TNF) superfamily of proteins that is expressed by osteoclast precursors, marrow stromal cells, and activated T-cells, among others. It acts on its receptor, RANK, which is expressed by osteoclasts and their precursors, to stimulate osteoclast activation, differentiation, migration, and survival via downstream signaling through the nuclear factor kappa B (NFκB) signaling pathway [23]. This process ultimately results in increased osteoclast resorption of bone. A second TNF superfamily member, osteoprotegerin (OPG), functions as the brakes in the system by counteracting the resorptive effects of the RANKL/RANK interaction. OPG, which is produced by osteoblasts as well as many other tissues, is a soluble decoy receptor of RANKL, binding RANKL and inhibiting its interaction with RANK [23]. The quantity of OPG in relation to RANK-L is believed to be the mechanism by which bone achieves a balance between resorption and formation [24].

Manipulation of the RANKL system has been a target of pharmaceutical development, and denosumab is currently the only RANKL targeted therapy available. Denosumab is a fully human monoclonal antibody directed at RANKL. It has a half life of more than 30 days and does not accumulate in bone like bisphosphonates [25]. The drug works by mimicking the effects of OPG, binding RANKL, and resulting in a reduction in osteoclast formation and action.

Clinical trials with denosumab have demonstrated efficacy in fracture prevention and increased BMD in postmenopausal women [26, 27]. A fracture prevention trial included 7868 postmenopausal women with osteoporosis who were randomized to receive placebo or twice yearly denosumab. The denosumab group had significantly fewer new vertebral fractures, nonvertebral fractures, and hip fractures than the placebo group during the 36-month study (relative decreased risk of vertebral fractures 68%, of nonvertebral fractures 20%, and of hip fractures 40%) [28]. Based on this study, denosumab has been approved by the Food and Drug Administration to treat postmenopausal women with osteoporosis.

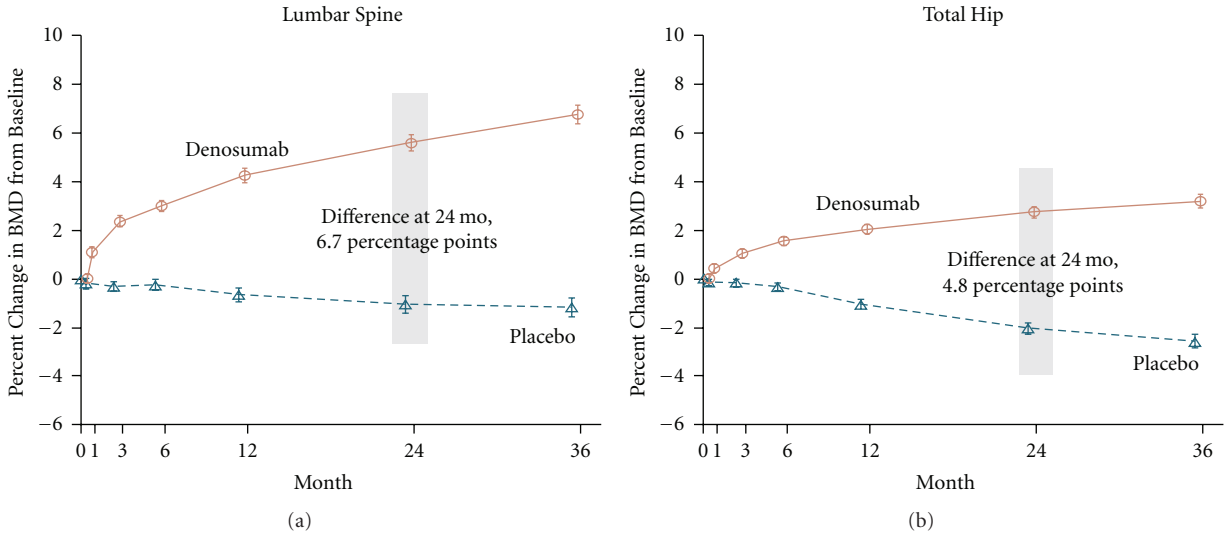

Figure 1: Mean BMD percent changes from baseline in lumbar spine and total hip sites. Results are reported as least-square means of BMD at the lumbar spine and total hip. All values are significantly greater in the denosumab group than the placebo group ($P \leq 0.001$) [9].

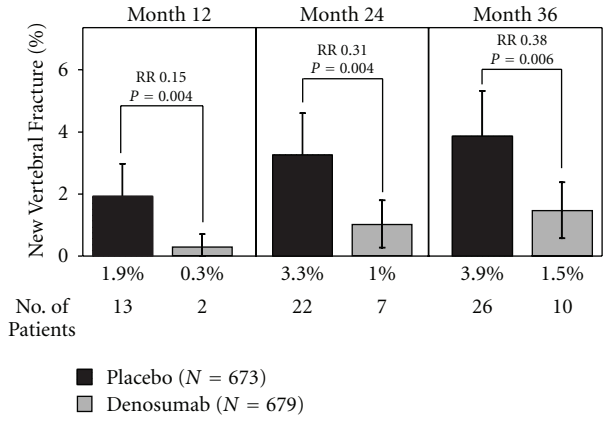

Figure 2: Incidence of new vertebral fractures during study period in denosumab and placebo groups. Relative risk calculated for vertebral fracture in 679 patients in the denosumab group versus 673 patients in the placebo group were 0.15 (12 months), 0.31 (24 months), and 0.38 (36 months) [9].

Denosumab has also been shown to increase BMD in women with breast cancer who are being treated with aromatase inhibitors [29]. Aromatase inhibitors stop estrogen production in peripheral tissues and cause a decline in BMD in women using them. A recent study of women being treated for breast cancer with aromatase inhibitors has demonstrated that denosumab significantly increased BMD as compared to placebo at the lumbar spine (BMD increased by 5.5% and 7.6% at 12 and 24 months, respectively ($P < 0.0001$ at both time points)).

6. Denosumab to Prevent Fractures during ADT for Prostate Cancer

A phase 3, multicenter, double-blind, randomized controlled trial evaluated the effect of denosumab on osteoporosis and fracture rate in men treated with ADT [9]. The trial included men with nonmetastatic, hormone-sensitive prostate cancer who were being treated with GnRH agonists. They were randomized to receive denosumab or placebo once every 6 months with an evaluation of bone mineral density at 24 and 36 months. Similarly to prior studies with bisphosphonates and SERMs, the primary endpoint was the change in lumbar spine BMD. However, this trial also reported the incidence of new vertebral fractures and the incidence of fractures at any site as more clinically meaningful secondary endpoints.

The trial demonstrated that denosumab improved BMD and decreased the rate of clinical fractures in men who were treated with ADT [9] (Figures 1 and 2). At 24 months, patients who were randomized to denosumab had an increase in BMD of the lumbar spine of 5.6% as compared with a decrease in BMD of 1.0% in the placebo group (<0.001). Significant differences in BMD were evident in some patients as soon as one month after treatment. At 36 months, there was a significant difference in the incidence of vertebral fractures, with an incidence of 3.9% in the placebo group versus 1.5% in the denosumab group (relative risk 0.38, $P = 0.006$).

Subgroup analyses found an improvement in BMD with denosumab at all skeletal sites and in all subgroups assessed (Figure 3) [30]. The most pronounced improvement in BMD occurred in men with the highest markers of bone turnover (serum C-telopeptide and tartrate-resistant alkaline phosphatase). Adverse events were not significantly different between the two groups.

Denosumab has been recently approved in Europe for the treatment of men receiving ADT for fracture prevention based on this study. Approval of the drug in the United States for men with nonmetastatic prostate cancer is pending.

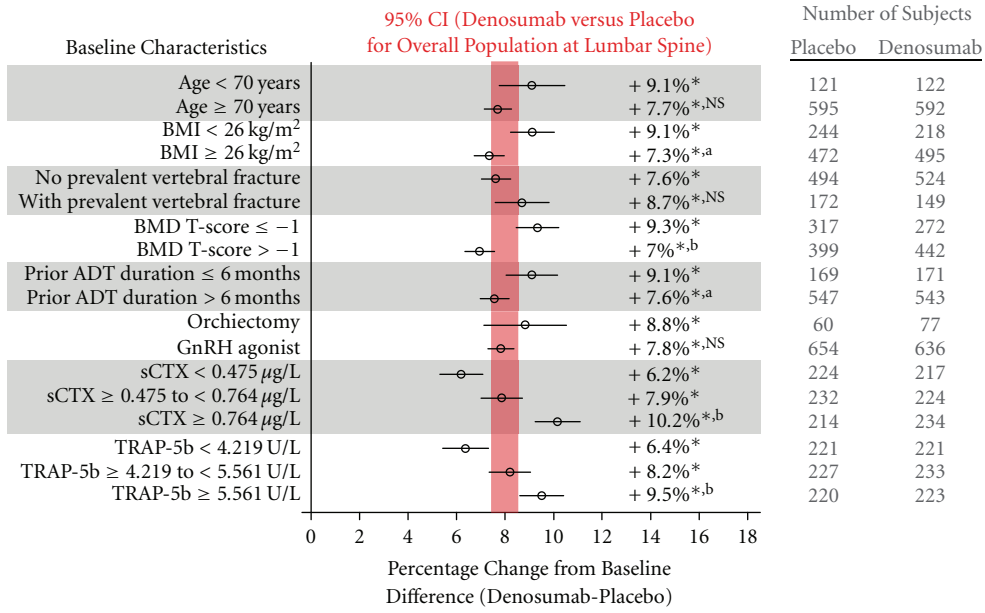

Figure 3: Forest plot of percentage change in BMD from baseline of denosumab versus placebo at lumbar spine. Vertical bar outlines the percentage difference in BMD between denosumab and placebo at lumbar spine at 36 months [30].

7. Denosumab to Prevent Skeletal Related Events in Metastatic Prostate Cancer

Men with prostate cancer commonly develop complications of disease related to bony metastases. Because these complications are not infrequent, and because they have a drastic impact on patients' quality of life, trials of bone-targeted therapies frequently include them as study endpoints. These bone-related complications are referred to as skeletal related events (SREs) and are specifically defined as pathologic fracture, need for radiation therapy or surgery to bone, or spinal cord compression.

Denosumab was evaluated for fracture prevention in men with metastatic castration-resistant prostate cancer (CRPC) in a phase III study with time to first SRE as the primary endpoint. CRPC is an advanced disease state marked with progression despite treatment with GnRH agonists and castrate levels of serum testosterone. This population frequently has multiple sites of metastatic prostate cancer involvement in the bone, and fracture is a common adverse event.

This study randomized 1,904 men with at least one site of bony metastasis to receive monthly zoledronic acid or denosumab [31]. The primary endpoint was time to first skeletal related event, and the primary objective was the demonstration of noninferiority of denosumab when compared to zoledronic acid. Denosumab significantly delayed the time to first SRE when compared with zoledronic acid (time to first SRE 20.7 months with denosumab versus 17.1 months with zoledronic acid; HR 0.82; $P = 0.008$ for superiority) [31]. Adverse events, including incidence of osteonecrosis of the jaw, were not significantly different between the two groups.

As of early 2011, denosumab has been FDA approved to prevent SREs in patients with solid tumors and bone metastases, including men with prostate cancer.

8. Denosumab to Prevent Bone Metastases

Almost all men with fatal prostate cancer eventually develop metastatic disease to bone [32]. Impairing signaling in the bone microenvironment through RANKL-targeted therapies has not previously been explored for metastasis prevention in prostate cancer. A recently completed phase III trial evaluated the efficacy of denosumab in the prevention of bone metastases in men with nonmetastatic CRPC [33]. The study randomized 1432 men who were at high risk to develop bony metastases to receive denosumab (120 mg monthly) or placebo. The primary endpoint was bone metastasis-free survival, and secondary endpoints included time to first bone metastasis and overall survival.

Denosumab significantly increased bone metastasis-free survival (time to first occurrence of bone metastasis or on-study death from any cause) when compared to placebo (median bone metastasis-free survival 29.5 months and 25.2 months for denosumab and placebo, resp.) [33]. This was a 15% decrease in risk of developing a bone metastasis for patients treated with denosumab. Although there was no significant difference in overall survival between the groups, denosumab increased the time to first bone metastasis (median time to first bone metastasis 33.2 months with denosumab versus 29.5 months with placebo).

9. Conclusions

Osteoporosis in men is a common and important health problem. Men with prostate cancer are at particular risk for

osteoporosis and fractures based on older age and androgen deprivation therapy. ADT decreases bone mineral density and increases fracture risk. In men receiving ADT for prostate cancer, denosumab (60 mg every 6 months) significantly increases bone mineral density and decreases incidence of vertebral fractures. In men with castration-resistant prostate cancer and bone metastases, denosumab (120 mg monthly) is superior to zoledronic acid to prevent skeletal related events. Denosumab in the same dose and schedule also significantly increases bone metastasis-free survival in men with castration-resistant nonmetastatic prostate cancer.

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Research Article

Femoral Neck Shaft Angle in Men with Fragility Fractures

S. P. Tuck,[1] D. J. Rawlings,[2] A. C. Scane,[3] I. Pande,[4] G. D. Summers,[5] A. D. Woolf,[6] and R. M. Francis[7]

[1] *Department of Rheumatology, The James Cook University Hospital, Marton Road, Middlesbrough TS4 3BW, UK*
[2] *Regional Medical Physics Department, Freeman Hospital, Newcastle upon Tyne NE7 7DN, UK*
[3] *Hunter Rural Aged Care Assessment Team, Lang Street, Kurri Kurri, NSW 2327, Australia*
[4] *Rheumatology Department, Nottingham University Hospital, Nottingham NG7 2UH, UK*
[5] *Medical Specialities OPD, Royal Derby Hospital, Uttoxeter Road, Derby DE22 3NE, UK*
[6] *Department of Rheumatology, Royal Cornwall Hospital, Truro TR1 3LJ, UK*
[7] *Institute for Ageing and Health, The Medical School, University of Newcastle, Newcastle upon Tyne NE2 4HH, UK*

Correspondence should be addressed to S. P. Tuck, stephen.tuck@stees.nhs.uk

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Introduction. Femoral neck shaft angle (NSA) has been reported to be an independent predictor of hip fracture risk in men. We aimed to assess the role of NSA in UK men. *Methods*. The NSA was measured manually from the DXA scan printout in men with hip (62, 31 femoral neck and 31 trochanteric), symptomatic vertebral (91), and distal forearm (67) fractures and 389 age-matched control subjects. Age, height, weight, and BMD (g/cm^2: lumbar spine, femoral neck, and total femur) measurements were performed. *Results*. There was no significant difference in mean NSA between men with femoral neck and trochanteric hip fractures, so all further analyses of hip fractures utilised the combined data. There was no difference in NSA between those with hip fractures and those without (either using the combined data or analysing trochanteric and femoral neck shaft fractures separately), nor between fracture subjects as a whole and controls. Mean NSA was smaller in those with vertebral fractures (129.2° versus 131°: $P = 0.001$), but larger in those with distal forearm fractures (129.8° versus 128.5°: $P = 0.01$). *Conclusions*. The conflicting results suggest that femoral NSA is not an important determinant of hip fracture risk in UK men.

1. Introduction

Osteoporosis is generally considered to be a condition affecting women, but up to 30% of fragility fractures occur in men [1–3]. The lifetime risk of fracture at the age of 50 years has been estimated to be 20% for men [1, 4]. Bone mineral density (BMD) has long been recognised as an important skeleton determinant of fracture risk, but it is becoming apparent that skeletal geometry also influences the risk. This has been most extensively studied in women at the hip, in terms of hip axis length (HAL), femoral neck axis length (FNAL), neck shaft angle (NSA), and femoral neck width (FNW). The role of all of these factors as independent predictors of hip fracture risk is controversial in both sexes, with studies giving conflicting results [5, 6]. This uncertainty may have arisen partly because of differences in study design, numbers of patients studied, and also because of wide variations in geometric parameters in different countries and races [7, 8]. Given this variation, it may be necessary to generate data specific to the population under consideration. It may also be necessary to generate gender-specific data, as suggested by our previous paper [9], which showed that men had a mean femoral NSA of 130° (SD 3.3, range 121–138°), whilst women had a significantly ($P < 0.0001$) smaller mean femoral NSA of 128° (SD 1.7, range 119–137°). Only one study has examined hip geometry solely in men in England and this failed to show any relationship between HAL and hip fracture [10]. However, it did not measure NSA or femoral neck width, so there is a need for further study of the role of femoral geometry in men.

Men with forearm fractures and vertebral fractures are at increased risk of developing hip fractures [10, 11], which may be due in part to altered skeletal geometry. We have therefore examined femoral neck NSA measurements in

three UK case-control studies of low trauma hip, vertebral, and distal forearm fractures in men [12–14]. These studies have previously demonstrated significantly lower BMD in men sustaining these fractures compared with controls, and between 42% and 83% were osteoporotic on the basis of a T-score ≤ -2.5 using male-specific reference data [12–14]. It is also important to note that there can be differences in geometry between femoral neck and trochanteric hip fractures and for this reason, these fractures types need to be considered separately. The Cornwall Hip Fracture recruited men with hip fractures of the femoral neck and trochanteric regions and so provides an opportunity to study the role of NSA in both fracture types.

2. Methods

2.1. Subjects. The full details of each of the three studies have already been published, but they will be described briefly [12–14]. In all three, low trauma fractures were defined as those occurring spontaneously without trauma or following a fall from standing height or less. Local research ethics committee approval was obtained. All subjects gave their written informed consent.

2.1.1. Case-Control Study of Hip Fractures. Data were collected from the Cornwall Hip Fracture Study of men with low trauma femoral neck hip fractures [12]. One hundred consecutive admissions of men over 50 years with low trauma hip fractures to the Royal Cornwall Hospital in Truro between 1995 and 1997 were recruited. One hundred age-matched controls were recruited concurrently from a large general practice within the catchment area of the hospital. Fracture subjects were recruited during their admission, so it was only possible to perform DXA scans on 62 men with hip fracture (31 with femoral neck, 31 with trochanteric fractures) and 100 control subjects. Of the men with trochanteric fractures, only 16 could have their NSA measured because the rest had bilateral hip fractures, so no hip DXA could be performed.

2.1.2. Case-Control Study of Vertebral Fractures. Men referred to the Bone Clinic in Newcastle upon Tyne with symptomatic low trauma vertebral fractures aged 80 years or less were invited to take part in the study [13]. The spine radiographs were reviewed to confirm the presence of at least a 20% reduction in anterior and/or posterior vertebral height. Control subjects were recruited from the age-sex registers of General Practitioners to match the age of the index case within two years. Those with a previous diagnosis of osteoporosis were excluded. Of the control subjects who agreed to take part (43% of those approached), one was selected at random to serve as the control and underwent the same clinical assessment and investigations as the patients with vertebral deformation. Spinal radiographs were not taken in the control subjects however, because of the relatively high-radiation exposure involved. In total, 91 case-control pairs were recruited.

2.1.3. Case-Control Study of Distal Forearm Fractures. A retrospective case-control study design was chosen and all men aged 40–80 years who had suffered a distal forearm fracture between 1996 and 1998 were identified from the Accident and Emergency Department records of attendance at Derbyshire Royal Infirmary [14]. The case notes and X-ray reports were then examined to confirm the fracture and eligibility. In this way, 147 men were identified of whom 103 responded to questionnaires and 67 agreed to dual energy X-ray absorptiometry (DXA) scanning. A total of 198 age-matched control subjects were selected from a preexisting local database of 692 healthy men without distal forearm fractures, so that two control subjects were matched with each man with fracture taking part in the study.

2.2. Bone Area, Bone Mineral Content, and Bone Mineral Density. In all studies, anthropometric measurements were performed, including height and weight. DXA was used to determine scan area (cm^2), BMC (g), and areal BMD (g/cm^2). The lumbar spine (L1 to L4) and hip (total hip, femoral neck) were measured. Hip measurements were always taken from the left side, unless there was a fracture or joint replacement. DXA scanning was performed using either Hologic QDR 1000 or QDR 2000 equipment (Hologic Instruments, Waltham, Mass, USA) [12–14], but there was no consistent difference in measurements obtained with the two machines [13]. Daily calibration checks were performed using the Hologic spine phantom and had a coefficient of variation of 0.5% throughout the studies. *In vivo* precision for measurement with these systems is 1.0% at the lumbar spine (L1–L4) and 1.5% for the femoral neck.

2.3. Femoral Neck Shaft Angle Measurements. Although the Hologic 1000 machine was a pencil-beam machine demonstrating virtually no magnification error, the Hologic 2000 DXA scanner included a fan beam capability and so created the potential for magnification errors. This precluded the measurement of HAL or FNW. However, we have previously found the effect of possible magnification on NSA to be minimal using a fan beam scanner [9]. Subjects were all positioned on the DXA using the standardised international recommendations as described recently [15]. For completeness, the following is extracted from the article: "The patient is positioned straight on the table (spine is straight on the image), not rotated (spinous processes are centred) and centred in the field (roughly equal soft tissues fields on either side of the spine). The patient has the femur positioned straight on the table (shaft parallel to the edge of the picture), with 15–25° of internal rotation where possible, achieved by the use of a single positioning device, thereby presenting the long axis of the femoral neck perpendicular to the X-ray beam, providing the greatest area and the lowest BMC (and the lowest BMD). This is confirmed on the scan by seeing little or none of the lesser trochanter." Such standardisation of subject position should reduce error in measuring the NSA, although extreme angles of anteversion at the hip were not specifically excluded. The NSA was measured from a Hologic standard DXA scan printout using a method

Table 1: Summary of anthropometric and BMD data from the three case-control studies.

Study	Group	Age (Years)	Height (m)	Weight (kg)	Spine BMD (g/cm^2)	Femoral neck BMD (g/cm^2)	Total hip (g/cm^2)	Percentage with osteoporosis
Forearm	Fracture $n = 67$	60.97	1.727	81.71	0.985	0.748	0.951	42
	Control $n = 198$	60.60	1.731	79.7	1.065***	0.848***	1.026***	10***
Vertebral	Fracture $n = 91$	64	1.691	70.36	0.812	0.709	0.787	56
	Control $n = 91$	64	1.732***	78.12***	1.060***	0.845***	1.009***	3***
Hip	Fracture $n = 62$	78.4	1.712	67.6	0.92	0.61	0.716	83
	Control $n = 100$	75.1	1.706	77.7**	1.08**	0.76**	0.921***	39***

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

adapted from that of Faulkner et al. and Quereshi et al., 2001 [15, 16] and previously published by the authors [9]. All measurements were made by a single observer (the corresponding author). The femoral neck axis was identified on the printout by the DXA analysis software. A line was then drawn manually from the junction between the greater trochanter and the femoral neck down to a point in the middle of the shaft at the bottom of the scan (Figure 1). The junction of these two lines gives the femoral NSA, which was measured with a long-armed protractor with 0.5° intervals (a BIOMET Inc. goniometer). The method described gave an intraobserver error of 0.79%, interobserver error of 1.2%, and precision of ±1.2%. The details of how these errors and precision were derived have been given in our previously published paper [9] and are similar to those given in other papers in this field [5, 6, 8, 16–18].

2.4. Statistical Methods. Statistical analysis was performed using standard statistical software packages (Graphpad Prism) and SPSS for Windows (SPSS Inc. Chicago, Ill). Descriptive statistics were obtained and data were tested for normality using Kolmogorov-Smirnov test for Gaussian distribution. All data were normally distributed. Each of the three case-control studies available had their NSAs measured in men with fractures and control subjects. These were then examined separately to look for any correlations with age, height, weight, and BMD using Pearson correlation coefficients. The groups were then compared via Student's *t*-tests (unpaired) to see if there was any significant difference in NSA between fracture and control subjects (Figure 2). Chi-squared tests were performed to compare proportions. As there were significant differences in height and weight, ANCOVA tests were performed in order to adjust the NSA results for these covariables.

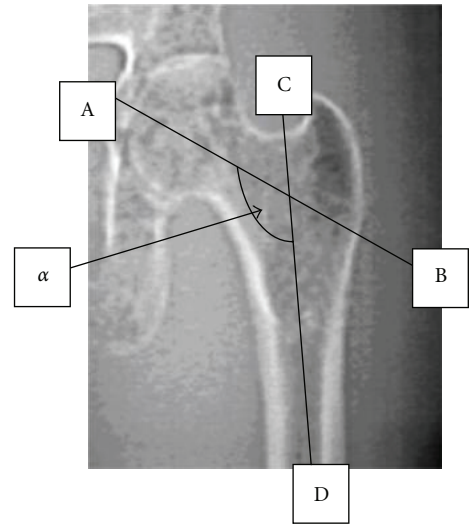

Figure 1: Measurement of the femoral NSA from the DXA scan printouts. The line AB is the hip axis marked on by the scanner's software. A line is then drawn from C to D, in which C is the point at which the greater trochanter joins the femoral neck and D is the midpoint of the shaft at the bottom of the picture. The angle α is the femoral neck shaft angle.

3. Results

Table 1 summarises the anthropometric and BMD data for the three individual studies, all of which have been previously published [12–14]. Only the vertebral fracture study demonstrated any significant height differences between fracture and control subjects, presumably because of height loss associated with vertebral fractures. Weight was significantly lower in the men with hip and vertebral fractures compared with their respective control subjects, but not in the forearm study.

Table 2 shows the correlations found between NSA and age, height, weight, and BMD at the lumbar spine, femoral neck, and total femur for each of the study groups. The only significant correlations identified were inverse relationships with height and lumbar spine BMD amongst control subjects

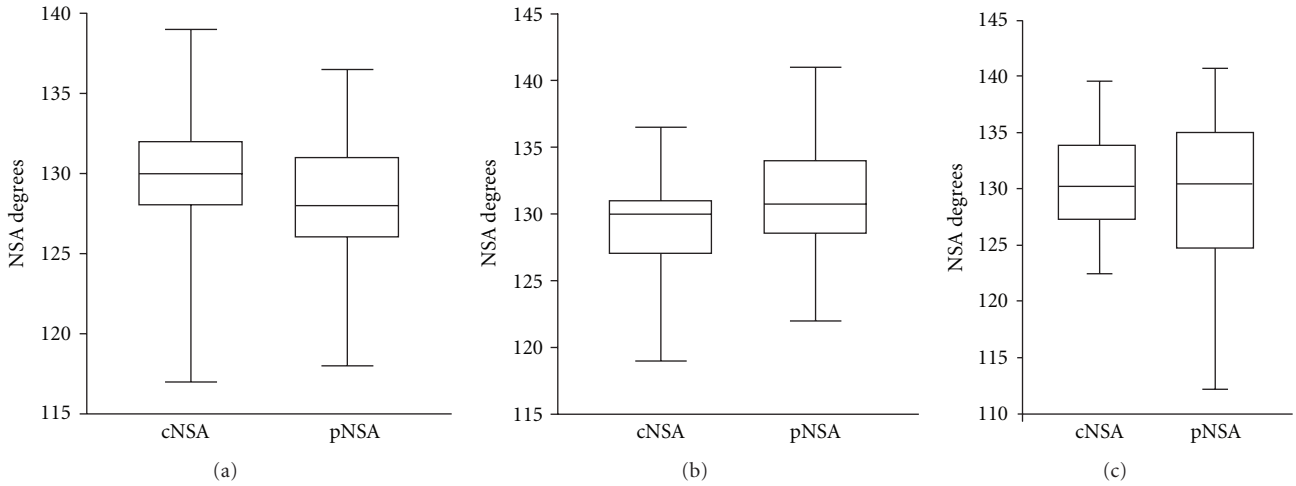

Figure 2: (a) Box and whisker plot of femoral NSA in male patients with distal forearm fractures (pNSA) compared with control subjects (cNSA). (b) Box and whisker plot of femoral NSA in male patients with symptomatic vertebral fractures (pNSA) and control subjects (cNSA). (c) Box and whisker plot of femoral NSA in male patients with hip fractures (pNSA) compared with control subjects (cNSA).

Table 2: Correlations (r) between neck shaft angles and anthropometric and BMD data.

Study	Group	Age	Height	Weight	Spine BMD	Femoral neck BMD	Total hip BMD
Forearm	Fracture $N = 67$	0.04	−0.07	−0.07	0.167	0.097	0.058
	Control $N = 198$	0.03	−0.27***	−0.09	−0.19*	−0.06	−0.09
Vertebral	Fracture $N = 91$	0.06	−0.01	0.19	0.1	0.03	0.02
	Control $N = 91$	0.11	−0.22*	0.006	−0.15	−0.1	−0.09
Hip	Fracture $N = 62$	0.08	−0.17	−0.19	0.09	−0.1	0.06
	Control $N = 100$	0.09	−0.004	−0.03	0.02	0.001	−0.009

* $P < 0.05$, *** $P < 0.001$.

Table 3: Results of the means and ranges of NSAs in each study.

Study group	Control group Mean (SD) and range	Fracture group Mean (SD) and range	Difference of the means 95% CI	P value (unpaired t-test)
Forearm study (67 fracture and 198 control subjects)	129.8 (3.495) 117 to 139	128.5 (3.519) 118 to 136.5	−1.265 (−0.29 to −2.24)	0.01
Vertebral study (91 case-control pairs)	129.2 (3.573) 119 to 136.5	131 (3.536) 122 to 141	1.752 (0.72 to 2.78)	0.001
Hip study (62 fracture and 100 control subjects)	130.7 (3.506) 122.5 to 139	130.1 (5.496) 111 to 143.5	−0.58 (−0.82 to 1.97)	0.42

in the forearm fracture study and with height alone in the control subjects in the vertebral fracture study

The means, ranges, and standard deviations in each control group are all very similar (Table 3). The mean NSA for men with forearm fractures was significantly smaller than that of control subjects, whereas it was significantly larger in the men with vertebral fractures. However, the differences were small in each case and were in opposite directions. The NSA data for hip fracture subjects was first of all analysed by each fracture type to establish whether or not there was any difference between them. The femoral neck fractures had a mean of 129.8°, SD of 6.155, and range of 111° to 143.5° compared with mean 130.6°, SD 5.228, and range 121.5° to 139° for the trochanteric fracture group, with no significant difference between them ($P = 0.67$). Neither was there any significant difference in NSA between the femoral neck fracture group and control subjects ($P = 0.31$), nor the trochanteric and control group ($P = 0.90$). Therefore, all further analyses used the data from both hip fracture groups combined. There was no significant difference seen between the men with hip fractures (combined data) compared with control subjects. Combining all data showed no significant differences in NSA between fracture subjects (mean 130° and SEM ± 0.29) and control subjects (mean 129.9° and SEM ± 0.18): $P = 0.88$. ANCOVA tests were performed to adjust NSA for height and weight as covariables. The results are shown in Tables 4, 5, and 6 and show that doing so results in no significant difference in NSA between fracture groups

Table 4: ANCOVA test results for differences in NSA in the forearm-fracture study after adjusting for weight and height as covariables.

Source	Adjusted means		SS	df	MS	F	P
	Fracture group	Control group					
Height	128.4	125.2	526.86	1	526.86	1.18	0.27
Adjusted error			116516.07	262	444.72		
Adjusted total			117042.94	263			
Weight	128.7	125.1	658.74	1	658.74	1.49	0.22
Adjusted error			116022.87	262	442.84		
Adjusted total			116681.61	263			

Table 5: ANCOVA test results for differences in NSA in the vertebral fracture study after adjusting for weight and height as covariables.

Source	Adjusted means		SS	df	MS	F	P
	Fracture group	Control group					
Height	129.6	127.7	159.98	1	159.98	0.8	0.37
Adjusted error			35706.05	179	199.48		
Adjusted total			35866.03	180			
Weight	128.9	128.4	6.97	1	6.97	0.04	0.84
Adjusted error			34844.67	179	194.66		
Adjusted total			34851.64	180			

Table 6: ANCOVA test results for differences in NSA in the hip fracture study after adjusting for weight and height as covariables.

Source	Adjusted means		SS	df	MS	F	P
	Fracture group	Control group					
Height	124.0	130.7	1695.96	1	1695.96	5.28	0.02
Adjusted error			51422.31	160	321.39		
Adjusted total			53118.28	161			
Weight	127.1	128.7	76.51	1	76.51	0.25	0.617762
Adjusted error			48959.79	160	306		
Adjusted total			49036.3	161			

and control subjects, except when NSA is adjusted for height in the hip fracture group when the difference just makes significance at $P = 0.02$.

4. Discussion

In all three case-control studies, BMD has been found to be significantly lower in the fracture groups than control subjects, with significantly higher proportions osteoporotic. The measurement of NSAs from DXA scan printouts has produced very consistent means, ranges, and standard deviations across the studies. They are also similar to those described in our previous work in men from the Newcastle Thousand Families Study, which gave a femoral NSA of 130° and SD of 3.3 and range of 121–138° [9]. Furthermore, the mean values and ranges are similar to those reported in other studies [5, 6, 8, 16–18]. There were few correlations between NSA and height and BMD; those that were observed could well have been the result of multiple testing. The lack of change with age would suggest that the NSA is fixed over time. A study in Finland also found no relationship between age and NSA, but did confirm that men had larger NSAs than women [19].

No significant difference in NSA could be found between those with hip fractures and control subjects and between the fracture groups and control groups as a whole. Furthermore, the NSA results for the distal forearm fracture and vertebral fracture studies were conflicting, being in opposite directions. When all data were combined, there was no significant difference in NSA between those with and those without fractures. Furthermore, ANCOVA, to adjust for height and weight, resulted in the previous differences between vertebral and forearm fracture subjects and controls disappearing. The only significant difference occurred between hip fracture and control subjects after adjusting for height ($P = 0.02$), but there was no difference after adjusting for weight. This suggests that there is no role for NSA in predisposing to hip fractures in men from the United Kingdom. These results are at variance with other studies. Karlsson et al. (1996) showed that men with hip fractures have a wider pelvis, shorter HAL, wider femoral necks, and larger NSAs than male control subjects [5]. A larger study by Gómez Alonso et al. (2000)

found that one standard deviation increase in NSA or FNW approximately doubled the risk of hip fracture in men, but there was no association with HAL [6]. These contradictions could be due to the wide geographic differences in hip geometry that have been reported [7, 8], and data may need to be specific for race and gender. However, a recent large Chinese study published by Zhang et al., including 4067 men (38 with hip fractures) across an age range from 15 to over 85 years, confirmed our findings [20]. The NSA did not change with age and there was no significant difference in NSA between hip fracture subjects and controls. They did find significantly lower BMD and reduced cross-sectional area [20].

The study has a number of limitations. It is relatively small and it is possible that larger studies could reveal important, but smaller effects of NSA. It was also unable to assess other aspects of structure and geometry, such as FNW which may be important in determining hip fracture risk in addition to low BMD. Such factors may also contribute to the known increased risk of hip fracture following vertebral or forearm fractures. The vertebral fracture study included neither vertebral morphometry nor spinal radiographs of the control subjects and so could not exclude the possibility of asymptomatic fracture and, indeed, was never designed to do so. Approximately, 20–25% of vertebral fractures are clinically diagnosed [21] and therefore the control group may not have been a true control population, which may have altered the results obtained. However, there was a significant difference in height between the vertebral fracture group and control subjects. One particular strength of the hip fracture study is that all the hip fracture subjects had femoral neck fractures. There have been differences in geometry reported between trochanteric and femoral neck hip fractures [18, 19], and so it is important to investigate the possible geometric contributions to these fractures separately.

It is worth noting that the men in the hip fracture study had a larger standard deviation than in the other groups. These men had their DXA scans performed whilst they were in hospital, and it is possible that the recent fracture made it more difficult for them to lie in the ideal scanning position. This could reduce the ability of the study to detect a true difference.

5. Conclusions

A manual method of measuring femoral NSAs from DXA scan printouts has been described. The method has proven to be both reliable and precise. It has given consistent results in terms of means, ranges, and standard deviations in all the studies in which it was used. In our previous work, men were shown to have larger femoral NSAs than women, despite their lower fracture risk [9]. Furthermore, the results of NSA measurements in the forearm, vertebral fracture, and hip fracture studies could find very little evidence to support a role for NSAs even after ANCOVA testing to adjust for height and weight as covariables. This suggests that NSA is not an important determinant of hip fracture risk in English men. Other aspects of geometry and structure may be more important risk factors and need evaluation.

Conflict of Interests

The authors declare no conflict of interests.

Acknowledgments

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Research Article

Lack of Association of Bone Morphogenetic Protein 2 Gene Haplotypes with Bone Mineral Density, Bone Loss, or Risk of Fractures in Men

Satya S. Varanasi,[1,2] Stephen P. Tuck,[1,3] Sarabjit S. Mastana,[4] Elaine Dennison,[5] Cyrus Cooper,[5] Josephine Vila,[1,3] Roger M. Francis,[1] and Harish K. Datta[1]

[1] *Musculoskeletal Research Group, Institute of Cellular Medicine, Newcastle University, Framlington Place, Newcastle upon Tyne NE2 4HH, UK*
[2] *Department of Biology, The University of York, York YO10 5YW, UK*
[3] *Department of Rheumatology, The James Cook University Hospital, Middlesbrough TS4 3BW, UK*
[4] *Human Genetics Laboratory, SSEHS, Loughborough University, Loughborough LE11 3TU, UK*
[5] *MRC Environmental Epidemiology Unit, University of Southampton, Southampton SO16 6YD, UK*

Correspondence should be addressed to Harish K. Datta, h.k.datta@ncl.ac.uk

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Introduction. The association of bone morphogenetic protein 2 (BMP2) with BMD and risk of fracture was suggested by a recent linkage study, but subsequent studies have been contradictory. We report the results of a study of the relationship between BMP2 genotypes and BMD, annual change in BMD, and risk of fracture in male subjects. *Materials and Methods*. We tested three single-nucleotide polymorphisms (SNPs) across the BMP2 gene, including Ser37Ala SNP, in 342 Caucasian Englishmen, comprising 224 control and 118 osteoporotic subjects. *Results*. BMP2 SNP1 (Ser37Ala) genotypes were found to have similar low frequency in control subjects and men with osteoporosis. The major informative polymorphism, BMP2 SNP3 (Arg190Ser), showed no statistically significant association with weight, height, BMD, change in BMD at hip or lumbar spine, and risk of fracture. *Conclusion*. There were no genotypic or haplotypic effects of the BMP2 candidate gene on BMD, change in BMD, or fracture risk identified in this cohort.

1. Introduction

Osteoporosis is primarily a disease of older people and advancing age is one of the main determinants of osteoporosis and fragility fractures. The estimated lifetime risk of hip, spine, or distal forearm fracture for Caucasian man at the age of 50 years is 20% and approximately 30% of all hip fractures occur in men [1]. Heredity is now generally accepted as playing an important part in the pathogenesis of osteoporosis in both men and women. Indeed, after excluding secondary risk factors and age then the other main causal factor for osteoporosis in men is genetic [2]. This view is supported by genetic epidemiological studies on BMD, an important surrogate of fracture risk, which show that peak bone density has a substantial heritable component [3]. Variation in BMD is determined by multiple genes; objective evidence for this is provided by candidate gene SNP studies, as well as quantitative trait locus (QTL) studies in inbred animals and humans [3]. QTL studies have identified multiple chromosomal regions which influence bone mass and are linked to osteoporosis-related phenotypes.

Studies of classical and novel candidate genes have only been able to account for a small proportion of the variance in bone mass. Candidate gene contributions to bone mass also show gender, geographic, and ethnic variability. Therefore, studies aimed at identifying the contribution of specific genes towards BMD and the heritable component to osteoporosis should ideally be gender and ethnic specific. One such candidate gene is bone morphogenetic protein 2 (BMP2), located on chromosome 20p12.3. It is a growth factor that

possesses osteoinductive properties and promotes osteoblast differentiation and bone formation [4–9]. Linkage analysis in extended families with osteoporosis in Iceland, using a phenotype that combines osteoporotic fractures and BMD measurements, showed linkage to chromosome 20p12.3 [4]. Three variants in the BMP2 gene, a missense polymorphism, and two anonymous single nucleotide polymorphisms and the resultant haplotypes have been associated with an osteoporotic phenotype, including osteoporotic fractures as well as low BMD, both before and after the menopause. A number of follow-up studies however have yielded inconsistent results [5–9]. To date no investigation has been performed in an exclusively male cohort and no follow-up study has investigated the association with risk of osteoporosis in men and rate of bone loss. In view of the inconclusive nature of the earlier study and to determine possible gender and geography-related variability, we therefore investigated the association of Ser37Ala and Arg190Ser genotypes/haplotypes with BMD, rate of bone loss, and fracture risk in men from the UK.

2. Materials and Methods

2.1. Participants. Participants comprised 342 male subjects, 224 controls, and 118 subjects with osteoporosis, who were all Caucasian men from England, UK [10–12]. The men with osteoporosis comprised men who had a BMD T score below −2.5 at either the femoral neck or lumbar spine or had a low trauma fracture and a T score below −1.0 at either the femoral neck or lumbar spine. Of the patients 89 also had a history of fractures (46 had distal forearm fracture, 33 symptomatic vertebral, 6 hip, and 4 other fractures). Their fractures had all occurred at least six months prior to their inclusion in the study, and all blood samples were taken before the initiation of therapy. Underlying secondary causes of osteoporosis were excluded by medical history, physical examination, and laboratory investigations. Controls were all volunteers without any history of low trauma fractures [10]. The laboratory investigations included full blood count, ESR, biochemical profile, thyroid function tests, serum testosterone, sex-hormone-binding globulin, gonadotrophins, and serum and urine electrophoresis [12].

In a subset of 145 control subjects, there was a follow-up BMD estimation and annual change in BMD was determined, in order to test the association between BMP2 genotypes and change in BMD. A similar analysis of the annual change in the BMD measurement was not performed in the men with osteoporosis, because of the confounding effect of antiresorptive treatment.

2.2. Biochemical and BMD Measurements. Serum testosterone, sex-hormone-binding globulins, follicular stimulating hormone, and luteinizing hormone were measured by commercially available radioimmunoassay (SAS laboratory, Royal Victoria Infirmary, Newcastle upon Tyne).

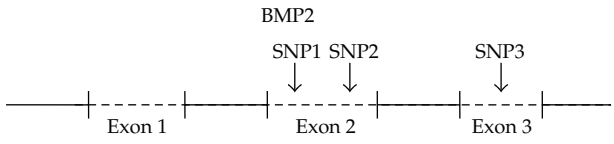
All bone density measurements, for both the osteoporotic and control subjects, were performed by DXA using a Hologic QDR 2000 Bone Densitometer (Hologic, Waltham, Mass). *In vivo* precision for measurement with this system is 1.0% at the lumbar spine (L1–L4) and 1.5% for the femoral neck. BMD results were obtained as an areal density in g/cm^2, but were also given as T and Z scores. The T score is the number of standard deviation units above or below the mean for normal young men, whilst the Z score is the number of standard deviation units above or below the age-related normal men (calculated using the manufacturer's standard normal reference database).

2.3. BMP2 Genotyping. Three BMP2 SNPs previously identified were genotyped [4]. Individual PCR standardisation reactions were set up prior to setting up multiplex PCR for the three BMP2 SNPs. The three BMP2 SNP polymorphisms (SNP1, Ser37Ala; SNP2, Ala94Ser and SNP3, Arg190Ser) studied are shown in the schematic diagram (Figure 1). The multiplex reactions were transferred into 384 well plates and treated with shrimp alkaline phosphatase to remove the excess dNTPs in the reaction mixture. Extension primers designed to detect the three BMP2 SNPs were added to the samples and incubated as a PCR reaction for 2 h. Salt ions were removed from the reaction mixture using a desalting resin and finally resuspended with 16 μL ddH_2O. 15 nL of each sample was spotted onto the chip and analysed on the Sequenom (Maldi-TOF mass spectrometer). The results were collected by Spectroanalyser software and the SNP information, represented by chromatogram peaks at G, T, A, GT, or AT, was sorted by the Typeranalyser software. The SNP information was then exported along with the sample details into MS-Excel file(s) for further statistical analysis.

2.4. Data Analysis. Results are presented as mean ± standard deviation. Data were analyzed using appropriate statistical packages (Microsoft Excel XL, SPSS); the significance of differences in genotype and allele frequency between control subjects and men with vertebral fractures was determined using odds ratio and χ^2 tests. ANOVA and Student's unpaired t-test were used to determine significant differences in BMD and anthropometric measurements between different BMP2 genotypes and between control subjects and osteoporotic men, respectively. Genetic association analyses were carried out using an online HWE calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) and SNPSTATS programme (http://bioinfo.iconcologia.net/SNPstats) which also worked out the Hardy-Weinberg equilibrium statistics. The haplotypes and associated statistics were derived from SNPSTATS programme. The binary logistic regression analyses were performed (SPSS, version 13.0) with disease or fracture outcome as the dependent variables. The genotypes as categorical variables were used as specific genotypes; the other variables included in the regression analysis were age, height, weight, BMI, and key relevant biochemical parameters.

3. Results

The age, anthropometric, and BMD measurements for the men with osteoporosis and the control subjects are given in



1. SNP1: nt16 T/G (Ser37Ala)
2. SNP2 : nt287 G/T (Ala94Ser)
3. SNP3: nt224 A/T (Arg190Ser)

Figure 1: Schematic diagram of the three single nucleotide polymorphisms in BMP2 gene.

Table 1. The men with osteoporosis, who had age range of 30 to 86 yr, were on average 6 years younger than controls with the age range of 41 to 79 yr. For this reason when comparing bone density comparisons, to compensate for age-related decline in BMD, comparison with Z score was performed. The osteoporotic patients were also significantly shorter and lighter than the male control subjects, as well as having significantly lower BMD at the lumbar spine, total femoral and femoral neck ($P < 0.0001$).

3.1. Genotype and Allele Frequency. In both men with osteoporosis and control subjects the BMP2 gene SNP1 GG genotype was absent, and in the combined cohort only eight subjects were found to be heterozygotes, thus making this locus uninformative. SNP1 genotype, due to Ser37Ala variants that change a conserved amino acid, has been previously shown to have significant association with osteoporosis in both Danish and Icelandic cohorts [9]. However, in our cohorts the frequency of SNP1-related G allele was low in both the patients (0.004) and controls (0.015). SNP2, which gives rise to Ala to Ser change at 94th position, was rare, uninformative, and monomorphic, and of the three genotypes GG, GT, and TT, only GG was observed. SNP3 (Arg190Ser) was polymorphic, and distribution of genotypes and allele frequencies in both male controls and men with osteoporosis is shown in Table 2. In both cohorts the genotypes were in Hardy-Weinberg equilibrium. Genotype AA was slightly higher in controls (14.3% versus 10.2%, $P = 0.36$) while susceptibility genotype TT was higher in patients (38.1% versus 31.7%, $P = 0.28$). The odds ratio for BMP2 SNP3 genotypes and alleles *A and *T showed no statistically significant evidence of association with the risk of osteoporosis, although a trend towards carriers of the *T allele may be worth exploring.

Linkage disequilibrium (LD) was calculated for SNP1 and SNP3 using EM method. The D′ prime is 0.989 (P value = 0.0264), suggesting that there is a significant linkage disequilibrium between SNP1 and SNP3, but caution is warranted in view of the relatively small numbers and low allele frequencies especially at SNP1 locus which led to a low r^2 value (0.02). Haplotype analysis based on two SNPs (SNP1 and SNP3) showed that two common haplotypes (TA and TT) accounted for >99% of the alleles at these BMP2 loci (Table 3). Haplotype-based association analysis indicated higher odds ratio for T-T haplotype; however, it was nonsignificant (OR = 1.43, CI 0.96–2.15, $P = 0.08$).

Table 1: Age, weight, height and bone mineral density of control male subjects and men with osteoporosis.

	Controls ($n = 224$)	Patients ($n = 118$)
Age (yr)	65.0 (7.7)	59.1 (12.6)***
Height (cm)	173 (6)	171 (8)*
Weight (kg)	81.5 (10.8)	75.9 (14.6)***
Spinal BMD (g/cm^2)	1.113 (0.160)	0.797 (0.100)***
Spinal Z score	0.96 (1.49)	−2.06 (0.96)***
Spinal T-score	0.14 (1.46)	−2.69 (0.90)***
Total femoral BMD (g/cm^2)	1.02 (0.13)	0.783 (0.099)***
Total femoral Z score	0.71 (0.96)	−1.25 (0.81)***
Total femoral T-score	−0.32 (0.98)	−2.18 (0.75)***
Femoral neck BMD (g/cm^2)	0.85 (0.12)	0.692 (0.100)***
Femoral neck Z score	0.71 (1.09)	−1.05 (0.90)***
Femoral neck T score	−1.11 (1.09)	−2.57 (0.86)***

* $P < 0.01$ and *** $P < 0.0001$ using unpaired Student's t-test.

Table 2: The distribution of BMP2 SNP3 genotype and allele frequencies and odds ratio.

(a)

Genotypes	Patients (n, %)	Controls (n, %)
AA	12 (10.2)	32 (14.3)
AT	61 (51.7)	121 (54.0)
TT	45 (38.1)	71 (31.7)
Total	118	224
HWE P value	0.19	0.09
Alleles		
*A	0.36 (±0.029)	0.41 (±0.022)
*T	0.64	0.59

(b)

Allele/genotype	Odd ratios (95% confidence interval), P value
*A versus *T	1.25 (0.90–1.73) $P = 0.18$
AA versus AT	1.34 (0.65–2.79), $P = 0.43$
AA versus TT	1.69 (0.79–3.62), $P = 0.17$
AA versus AT + TT	1.47 (0.73–2.98), $P = 0.28$
Armitage's trend test	1.29, $P = 0.15$

3.2. BMP2 Genotype Association with Phenotype. The effect of BMP2 gene SNP3 genotypes on weight, height, and BMD at lumbar spine, femoral neck, and total femoral was analysed by ANOVA in both the male controls and men with osteoporosis, and genotype was found to lack any significant association (Table 4). The analysis of the pooled data, comprising controls and patients, also failed to demonstrate any significant association of SNP3 genotype with the BMD or anthropometric indices (data not shown).

3.3. Logistic Regression Analysis. The association of the SNP3 genotype was also determined by employing a binary logistic

Table 3: Basic haplotypes of SNP1 and SNP3 at BMP2 gene in men with osteoporosis and controls.

Haplotype	Cases no., freq.	Control no., freq.	Odds ratio	Chi	*P* value
T-A	0.344	0.431	0.69 (0.46–1.04)	3.16	0.07
T-T	0.651	0.565	1.43 (0.96–2.15)	3.09	0.08
G-A	0.00	0.004		0.809	0.36
G-T	0.005	0.000	19.01 (19.01–65938)*	0.984	0.321

D′ prime is 0.989 (*P* value = 0.0264) suggesting that there is a significant linkage disequilibrium between SNP1 and SNP3 but caution is warranted as numbers are small.
*This range is due to the small numbers involved.

Table 4: BMP2 SNP3 genotypes and age, weight, height, BMD and annualised bone loss in male controls and men with osteoporosis.

	Control AA ($n = 32$)	Control AT ($n = 121$)	Control TT ($n = 71$)	Patients AA ($n = 12$)	Patients AT ($n = 61$)	Patients TT ($n = 45$)
Age (yr)	66.1 (7.2)	64.0 (9.4)	65.4 (7.4)	65.7 (9.2)	59.0 (12.6)	57.1 (12.9)
Height (cm)	173 (7)	173 (7)	172 (5)	174 (9)	172 (8)	171 (7)
Weight (kg)	81.9 (9.6)	80.8 (12.4)	81.2 (12.5)	83.9 (25.9)	75.7 (11.8)	74.0 (14)
Spinal BMD (g/cm^2)	1.132 (0.195)	1.112 (0.156)	1.105 (0.152)	0.826 (0.058)	0.785 (0.095)	0.807 (0.113)
Spinal Z score	1.15 (1.85)	0.92 (1.46)	0.93 (1.38)	−1.71 (0.55)	−2.16 (0.88)	−2.03 (1.13)
Spinal T score	0.14 (1.46)	0.14 (1.46)	0.14 (1.46)	−2.43 (0.50)	−2.81 (0.86)	−2.60 (1.03)
Total femoral BMD (g/cm^2)	1.012 (0.133)	1.014 (0.119)	1.039 (0.135)	0.821 (0.121)	0.782 (0.091)	0.773 (0.114)
Total femoral Z score	0.65 (1.04)	0.64 (0.90)	0.86 (1.01)	−0.91 (0.77)	−1.24 (0.79)	−1.39 (0.87)
Total femoral T score	−0.39 (1.03)	−0.37 (0.93)	−0.21 (1.05)	−1.91 (0.95)	−2.19 (0.68)	−2.28 (0.83)
Femoral neck BMD (g/cm^2)	0.860 (0.132)	0.840 (0.132)	0.861 (0.125)	0.708 (0.095)	0.687 (0.087)	0.695 (0.114)
Femoral neck Z score	0.82 (1.24)	0.59 (1.03)	0.85 (1.10)	−0.74 (0.67)	−1.07 (0.87)	−1.10 (1.00)
Femoral neck T score	−0.39 (1.03)	−0.37 (0.93)	−0.20 (1.05)	−2.42 (0.87)	−2.62 (.076)	−2.54 (1.00)
Spinal bone loss (% per annum)	−0.37 (1.14) ($n = 20$)	−0.51 (1.47) ($n = 69$)	−0.45 (1.05) ($n = 56$)			
Femoral neck bone loss (% per annum)	−0.03 (1.77) ($n = 20$)	0.20 (1.59) ($n = 69$)	0.33 (1.42) ($n = 56$)			
Total femoral bone loss (% per annum)	0.00 (0.80) ($n = 20$)	0.16 (1.70) ($n = 69$)	0.15 (1.07) ($n = 56$)			

No significant effect of BMP2 genotypes on height, weight or BMD was seen using ANOVA ($P < 0.05$). Annualized loss was studied in a subgroups of subjects, the numbers indicated in appropriate cells. Results are given as mean, and SDs are given in parentheses.

regression model, which accounted for between 44.5% and 62.6% of the variance in disease status and the overall model successfully predicted 72% of osteoporotic cases correctly (Table 5). An analysis of fracture incidence with reference to genotypes and age, age at fracture, and biochemical parameters was performed. Age at fracture, weight, biochemical parameters, and genotypes did not show any interaction or independent effect in the fracture prediction. The haplotype analysis did not reveal any significant influence on BMD via logistic regression. The addition of covariates to the model resulted in very sparse data categories and led to wide confidence intervals.

4. Discussion

This investigation is the only one from the UK that has sought an association between BMP2 gene polymorphisms and BMD and susceptibility to osteoporosis. As far as we know this is the first such investigation carried out exclusively in men with idiopathic osteoporosis. However, the power of this study is low; therefore any conclusions drawn should be treated with caution. Post hoc power analysis, calculated using a Quanto programme and based on observed allele frequencies, sample size, and odds ratios, showed that the study had 67% power to detect an odds ratio of 1.50 (moderate level) and above. In order to achieve an odds ratio of 1.25 one would require a case-control sample size of 507 individuals in each group. In the original Icelandic study a significant effect of BMP2 gene SNP1 polymorphism, Ser37Ala, was demonstrated in a cohort of 201 subjects, comprising 153 controls and 58 subjects with fracture [4]. Just like the earlier observation we too found it to be a rare variant in our cohort with an allele frequency of 1.25% for Ala37. In previous reports, the allele frequency for Ala37 in the Icelandic cohort was 0.8% in controls and 3% to 4.9% in osteoporotic patients and 2.5% in the Rotterdam study. However, unlike the Icelandic study, we did not see a significant association or increase in the relative risk (RR) with the SNP1 genotype. In our cohort we did not see any GT or TT genotypes of the rare variant of the SNP2 genotype that arises from Ala94Ser. The gene frequency for

Table 5: Logistic analysis of BMP2 SNP2 genotypes.

	B	S.E.	Wald	*df*	Sig.	Exp(B)	95.0% C.I. for Exp(B)	
							Lower	Upper
AGE	−.069	.021	10.994	1	.001	.933	.896	.972
HT	−.054	.171	.101	1	.751	.947	.677	1.325
WT	−.944	16.025	.003	1	.953	.389	.000	2.0*E* + 13
BMI	.166	.539	.095	1	.758	1.181	.410	3.398
FNBMD	−3.359	2.205	2.319	1	.128	.035	.000	2.622
Spinal BMD	−20.656	2.929	49.731	1	.000	.000	.000	.000
SNP3 (AA-Ref)			1.213	2	.545			
SNP3 (AT)	−.518	.654	.626	1	.429	.596	.165	2.148
SNP3 (TT)	−.039	.697	.003	1	.955	.961	.245	3.770
Constant	26.453	27.867	.901	1	.342	2.0*E* + 11		

SNP3 polymorphism, due to Arg190Ser variants, constituting three genotypes, TT (Arg190Arg), AT (Arg190Ser), and AA (Ser190Ser), was akin to earlier reports in European populations. In both male controls and men with osteoporosis the genotypes were in Hardy-Weinberg equilibrium. In addition to studying the association of the BMP2 to risk of fracture and BMD, in a subset of subjects we also investigated the association of the genotype with annual bone loss at the lumbar spine, total femoral, and femoral neck. Both initial BMD as well as annual change in the BMD showed no association with the BMP2 genotype. This could be partly due to allele frequency differences in different geographical and gender cohorts. In our study we observed a higher frequency of susceptible T (Ser) allele (64% in patients and 59% in controls) which is comparable to HAPMAP frequencies (58%–73% in European populations), but other populations like Turkish, Korean, and African populations show a lower frequency [13–15].

The desirability of having a large cohort for SNP studies investigating polygenic disease association with putative candidate genes with phenotype or disease state is well established. This is due to the fact that in a polygenic disorder like osteoporosis many genes make a small, but significant contribution towards the attainment and maintenance of BMD. Therefore, many genes may be involved in the pathogenesis of osteoporosis and risk of osteoporotic fractures. In view of this small contribution of a large number of genes, the contribution of the genotypic influence is likely to be more clearly demonstrated in a sufficiently large cohort with the required level of statistical power. It is, however, becoming increasingly evident that the association of a large number of candidate gene SNPs with BMD and osteoporosis risk shows race-, geography- and gender-related differences [3, 16, 17]. We would also contend that, if a given genotype does make a substantial contribution to the attainment and maintenance of BMD and pathophysiology of disease then it should be possible to demonstrate such effects even in smaller homogenous cohorts. Indeed, in the original study on BMP2 the genotype association with osteoporosis was established in a small cohort [4]. We have been able to demonstrate associations of some novel genotypes in smaller cohorts and which were subsequently verified in much larger studies [5, 18, 19].

There have been only a handful of studies following the first report of bone morphogenetic protein 2 (BMP2) as a susceptibility gene for osteoporotic fractures and low BMD in Icelandic and Danish populations [9]. Even these relatively few studies of polymorphisms, within the BMP2 gene in relation to bone mineral density (BMD) and fracture, have produced inconsistent findings [4–9]. Neither a Rotterdam study of a large population-based cohort of Dutch whites nor a study in healthy American whites could find any contribution to BMD by variations in BMP2 genotypes [7, 8]. On the other hand, a SNP- and haplotype-based US family study showed highly suggestive associations with BMP2 [5]. In another analysis on a European cohort, variation in BMP2 genotype showed no association with BMD; it did, however, find a role in aspects of bone quality, which may be age and site dependent [9]. The 3′ region of the gene was significantly associated with the ultrasound parameters speed of sound and stiffness. Similarly in Turkish women study BMP2 polymorphisms did not substantially contribute to lumbar spine bone mineral density [14]. Overall, association studies have shown that Arg190Ser SNP is not associated with BMD in different populations and the initial study may have been a false positive association. Alternatively, it is entirely possible that the association may show gender-related variation, as the initial study had shown association in females and our study was carried out in an exclusively male population.

In conclusion, in a BMP2 candidate gene polymorphism study in the UK, comprising osteoporotic men and male control subjects, there were no genotypic or haplotypic effects on phenotype and fracture risk identified.

Abbreviations

BMP2: Bone morphogenetic protein 2
SNP: Single nucleotide polymorphism.

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Research Article

Evaluation of Osteoporosis in Hemophilic Arthropathy Patients: Correlation with Disease Severity and Serum Trace Minerals

Eiman Mahmoud Ghaniema,[1] Sahar Fathi Ahmed,[1] Irene Raouf Amin,[1] and Maryse Soliman Ayoub[2]

[1] *Rheumatology and Rehabilitation Department, Ain Shams University, Cairo 11566, Egypt*
[2] *Internal Medicine Department (Hematology Unit), Ain Shams University, Cairo 11566, Egypt*

Correspondence should be addressed to Sahar Fathi Ahmed, saharfathi_283@yahoo.com

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Objective. To find out the presence of osteoporosis in hemophilic arthropathy patients and its correlation with clinical severity and serum levels of magnesium, copper, and zinc. *Methods*. Joint score, functional assessment score, bone densitometry, and serum magnesium, copper and zinc were done in twenty male hemophilic arthropathy patients and twenty controls. *Results*. There was highly significant lower Z scores of lumbar spine and neck of femur in patients versus controls ($P < 0.011$). Z score of neck of femur correlated negatively with total joint score ($P = 0.013$) and functional assessment score ($P = 0.011$). Serum levels of copper and zinc correlated positively with Z score of neck of femur ($P = 0.004$, $P = 0.001$, resp.). *Conclusion*. Osteoporosis represents a frequent concomitant observation in hemophiliacs. Screening of young hemophiliacs for osteoporosis is recommended with measuring serum levels of magnesium, copper, and zinc for better management of the disease.

1. Introduction

Hemophilia is a coagulation disorder characterized by acute hemorrhages into the musculoskeletal system leading eventually to arthropathy and disability [1]. Deficiency of factor VIII (Hemophilia A) accounts for 85% of cases and 15% are due to factor IX deficiency (Hemophilia B). They are both inherited as x-linked recessive disorder [2]. Hemoarthrosis occurs in 75%–90% of patients with hemophilia. The most common target joints are knee, ankle, and elbow [3].

Acute bleeding increases the pressure in the synovial cavity and bone marrow which leads to severe pain and possible osteonecrosis or a pseudotumoral mass. Intra-articular bleeding produces a direct chemical effect on synovium, cartilage, and bone. Overtime, the blood becomes deposited in the form of hemosiderin in these tissues. Recurrent hyperemia of the joint in the growing child causes juxta articular osteoporosis and overgrowth of the epiphysis. About 50% of patients with hemophilia develop permanent joint deformities [4].

Patients with severe hemophilia may be at risk for developing reduced bone density in childhood and adolescence for a number of reasons as arthropathy and joint deformities result in prolonged immobilization and reduced physical activity which predisposes them for osteoporosis. This can lead to increasing tendency of bone fragility and fractures in patients after trivial trauma [5].

Osteoporosis is a multifactorial disease with particular considerations to calcium, magnesium (Mg), and other trace elements as copper (Cu) and zinc (Zn), as they are essential in bone metabolism as cofactors for specific enzymes for optimal bone matrix development and bone density sustenance [6].

Therefore, we aimed to assess the presence and severity of osteoporosis in patients with hemophilic arthropathy by determining dual energy X-ray absorptiometry (DEXA) and to correlate these findings with the extent of joint disease and serum levels of trace minerals as magnesium, copper, and zinc.

2. Patients and Methods

Twenty male patients with hemophilia A were enrolled randomly for this study. Their age ranged from 7 to 40 years compared to 20 controls matched for age and sex. Those patients and controls were presenting to the Internal Medicine and Physical Medicine, Rheumatology, and Rehabilitation outpatient clinics of Ain Shams University Hospitals. The diagnosis of hemophilia was made clinically and useful to classify them according to measured factor VIII activity in the plasma as severe <1 unit/dL, moderate 1–5 unit/dL, and mild >5 units/dL, according to Arnold and Hilgartner [7].

We excluded patients with cigarette smoking or alcohol abuse, patients with history of any chronic medical illness producing osteopenia/osteoporosis, thyroid or parathyroid disorders, history of chronic renal, hepatic, or gastrointestinal disease including parasitic infestations, patients with prolonged intake of steroids, antiepileptic medication, iron for anemia, Ca or Vit D supplementation, or any drug affecting bone metabolism.

All patients were subjected to the following.

(1) Joint Evaluation: Lower limb joints (ankles and knees) were assessed by using the clinical evaluation score of the world Federation of Hemophilia (WFH) which includes seven criteria and a total possible score of 12 [8]. Each joint was ascribed a score. Normal joints were scored as 0, the highest possible score for knees and ankles was 48. The sum of scores for both knees and ankles was used in the analysis [5].

(2) Juvenile Arthritis Functional Assessment Report (GAFAR): It is a single dimension scale developed by Howe et al. [9] based on 23 items used as a disability score to evaluate the functional status and daily living activities for each patient. The response to each activity was scored between 0 and 2 as follows: 0 = indicating that the activity could be done alone without any difficulty during the previous week; 1 = indicating that it could be done some of time and 2 = indicating that it was almost never done alone. The score based on the 23 items was calculated as the sum of all items, assuming a range between 0 and 46 with lower score indicating better function.

(3) Radiological Evaluation:

(a) The roentgenographic examination included an anteroposterior projection and lateral views of both knee joints. The X-ray were classified according to Pettersson et al. [10] for standardized examination. The maximum possible score of a given joint was 13. The total score for both knee joints was 26.

(b) Bone densitometry: in all subjects, bone mineral density (BMD) was measured at the femoral neck and lumbar spine (L_1–L_4) in the anterior and posterior projection using DEXA. Results were recorded for each patient as Z score (difference in SD from the mean of a healthy age- and gender-matched sample) [11].

(4) Laboratory Investigations:

(i) Hemoglobin for anemia.

(ii) Complete liver and kidney function tests.

(iii) Serological screening for HBs Ag and HCV.

(iv) Serum calcium (Ca), phosphorus, and alkaline phosphatase to rule out osteomalacia and other metabolic bone disorders.

(v) Stool analysis to rule out any parasitic infestations.

(vi) Serum magnesium level was assayed using ADVIA 1650 (Payer, Siemens Healthcare Diagnostics) using modified xylidyl blue reaction, described by Mann and Yoe [12]. Reagent Code 74064. Normal range for serum is 1.9–2.5 mg/dL.

(vii) Determination of copper and zinc in serum was done by using the flawless atomic absorption spectrometry (Perkin-Elmer Corp., Norwalk, Conn. Germany). 5 mL of blood was obtained after overnight fasting. Serum was diluted 1 : 1 with deionized water for copper and 1 : 4 with 1% glycerol for zinc determination [13, 14]. Results were expressed in μg/mL; normal levels for copper and zinc ranged from 0.7–1.0 μg/mL and 0.8–1.2 μg/mL, respectively.

2.1. Statistical Methods. SPSS statistical software package (V. 18, IBM Corp., USA, 2010) was used for data analysis. Data were expressed as mean ± SD for quantitative measures and both number and percentage for categorical data. Comparison between two independent groups of numerical parametric data was done using Student's t-test. Comparison between two independent groups of nonparametric data was done using Wilcoxon Rank Sum test. Ranked Spearman correlation test was done to study the possible association between each two variables among each group for nonparametric data. Probability of error at 0.05 was considered significant and highly significant at 0.001.

3. Results

This study included 20 patients with hemophilic arthropathy and twenty healthy male subjects served as the control group. The patients' age ranged from 7 to 40 years, with a mean of 21.7 ± 11.2 years.

The disease duration ranged from 2 to 33 years with mean of 14.7 ± 10.5. The annual number of bleedings during the last 5 years ranged from 2 to 6 attacks per year with a mean of 4.45 ± 1.43. As regards therapy, we use cryoprecipitate and/or purified plasma derived factor eight, around 500–5000 units/kg/year according to requirements.

The plasma factor level (VIII) ranged from 2–10 units/dL, with a mean of 5.05 ± 3.34 (Table 1). According to the factor level, 8 patients (40%) diagnosed as mild grade and 12 patients (60%) diagnosed as moderate grade of hemophilia. Four patients (20%) had positive hepatitis C virus.

The number of clinically affected joints ranged from 1–4 (both knees and ankles). The total joint score (TJS) ranged from 4–21 with mean of 13.5 ± 6.2. Functional assessment score (JAFAR) ranged from 0–9 with mean of 2.8 ± 3.4. The total X-ray score ranged from 6–19 with a mean of 12.5 ± 4.8 (Table 1).

Total joint score correlated positively with total X-ray score ($r = 0.58$, $P = 0.006$). In addition, both TJS and total X-ray score correlated positively with disease duration ($r = 0.46$, $P = 0.03$ and $r = 0.63$, $P = 0.002$, resp.), and correlated negatively with the factor level of hemophilia ($r = -0.64$, $P = 0.002$ and $r = -0.49$, $P = 0.02$, resp.).

Functional assessment score correlated positively with TJS and total X-ray score ($r = 0.81$, $P = 0.001$ and $r = 0.55$, $P = 0.01$, resp.) and correlated negatively with factor level ($r = -48$, $P = 0.03$).

Presence of osteoporosis assessed by DEXA (Table 2) revealed highly significantly lower Z scores of lumbar spine and neck of femur in hemophilic arthropathy patients versus controls ($P < 0.001$) (Table 2). While, there was no significant difference in Z score of lumbar spine and neck of femur between patients with or without hepatitis C virus ($P > 0.05$).

Z score of neck of femur correlated negatively with total joint score ($r = -0.547$, $P = 0.01$), functional assessment score ($r = -0.553$, $P = 0.01$), and total X-ray score ($r = -0.484$, $P = 0.03$).While, there was no significant correlation between Z score of lumbar spine and the clinical or radiological scores ($P > 0.05$).

In hemophilic arthropathy patients, a highly significant decrease was found in serum levels of Mg, Cu, and Zn compared to controls ($P < 0.001$), while there was no statistically significant difference as regards serum calcium levels ($P > 0.05$) (Table 3).

Serum levels of Zn correlated negatively with TJS, functional assessment score, and total X-ray score ($r = -0.51$, $P = 0.01$, $r = -0.66$, $P = 0.001$ and $r = -0.94$, $P = 0.001$, resp.).

Serum levels of Cu and Zn correlated positively with Z score of neck of femur ($r = 0.61$, $P = 0.004$ and $r = 0.83$, $P = 0.001$, resp.) (Figures 1 and 2). On the other hand, there was no significant correlation between serum levels of either calcium or magnesium and the severity of osteoporosis as measured by Z score ($P > 0.05$).

Table 1: Demographic, clinical, laboratory, and radiological data of hemophilic arthropathy patients.

	Patients ($n = 20$)	
	Range	Mean ± SD
Disease duration (years)	2–33	14.7 ± 10.5
Factor level (units/dL)	2–10	5.05 ± 3.3
Total joint score	4–21	13.5 ± 6.2
Functional assessment score	0–9	2.8 ± 3.4
Total X-ray score	6–19	12.5 ± 4.8

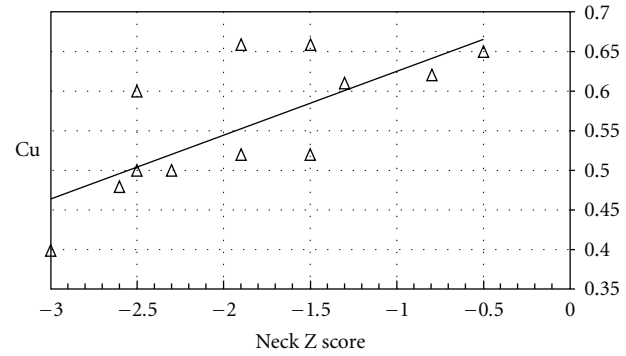

Figure 1: The positive correlation between serum cupper level and Z score of neck of femur among all patients.

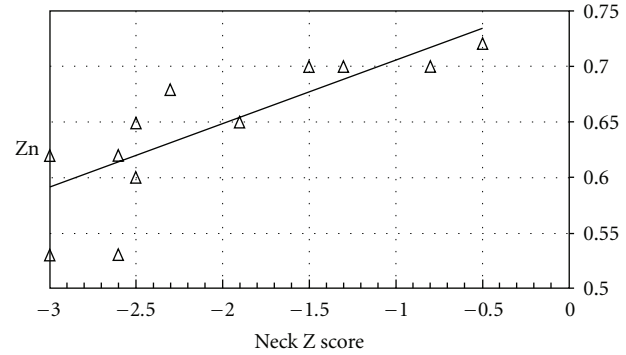

Figure 2: The positive correlation between serum zinc level and Z score of neck of femur among all patients.

4. Discussion

It has been suggested that the hemophiliacs may show a markedly lower bone mineral density (BMD) than the average population due to arthropathy that may induce lack of mobility as well as a pathological liver metabolism in those patients coinfected with a chronic hepatitis [15].

The possible role of functional interaction between hematopoietic and bone tissues in the development of age related osteoporosis is discussed by Gurevitch and Slavin [16]. Blood loss creating developmental pressure on hematopoietic system enhances production of hematopoietic growth factors and subsequently intensifies proliferation of hematopoietic progenitor cells, increasing the number of osteoclasts which intensifies resorption of bone tissue [17].

In addition, blood loss leads to the production of activity stimulating bone formation factors, extensive proliferation of osteogenic progenitor cells resulting in increased numbers of osteoblasts followed by new bone formation, and at same time increased production and maturation of osteoclasts which again enter the cycle of bone resorption together with exhaustion of osteogenic cell population for gradual development of osteoporosis [16]. Trace minerals may be important in maintaining bone quality through their role as metalloenzymes in the synthesis of collagen and other

Table 2: DEXA results.

	Patients ($n = 20$)		Controls ($n = 20$)		P
	Range	Mean ± SD	Range	Mean ± SD	
Z score lumbar	−2.7–(−0.9)	−2.1 ± 0.6	−0.2–1.3	0.7 ± 0.5	0.001
Z score femur	−3.0–(−0.5)	−1.8 ± 0.8	−0.4–1.4	0.7 ± 0.6	0.001

Table 3: Serum levels of Ca, Mg, Cu, and Zn.

	Patients ($n = 20$)		Controls ($n = 20$)		P
	Range	Mean ± SD	Range	Mean ± SD	
Calcium (mg/dL)	9.1–9.6	9.3 ± 0.18	9–9.5	9.2 ± 0.18	0.26
Magnesium (mg/dL)	1.5–1.8	1.6 ± 0.09	1.9–2.5	2.2 ± 0.21	0.001
Copper (μg/mL)	0.40–0.66	0.55 ± 0.08	0.7–1.0	0.84 ± 0.12	0.001
Zinc (μg/mL)	0.53–0.72	0.65 ± 0.05	0.8–1.2	1.0 ± 0.13	0.001

proteins that form the structure of bone [18]. They play important functional roles in bone metabolism and bone turnover. Zinc inhibits the differentiation of osteoclasts and promotes osteoblast activity affecting the formation of hard tissues. It could also increase bone growth factors and bone matrix protein, which are involved in the stimulation of bone formation and proliferation of osteoblastic cells [19].

Magnesium (Mg) appears to be important in bone cell activity. It is shown to be mitogenic for osteoblasts, and its depletion causes cellular growth inhibition in vitro [20]. Copper (Cu) is a cofactor for lysyl oxidase which is required in cross-linking of collagen and elastin. Cu deficiency causes inhibition of bone growth and osteoporosis as observed in Menkin's disease, an inherent inability to absorb Cu [21].

The present study aimed to find out the presence of osteoporosis in patients with hemophilic arthropathy and its correlation with clinical disease severity and serum levels of trace minerals as Zn, Cu and Mg.

In the present study, we used 3 scoring systems; clinical, functional and radiological scoring system to assess the extent of joint damage and related disability in 20 hemophilic patients. We scored only the lower limb major joints (knees and ankles), because these are the joints frequently affected by hemophilia, and damage of these joints will have a major impact on the patient's daily activities involving weight bearing. This was in agreement with, Gurcay et al. [1] who studied thirty one young patients with hemophilia aged between 3–18 years using the 3 scoring systems. They found that the most commonly affected joints in hemophilia are the hinge joints: knee, elbow, and ankles. Especially, the knees are most commonly affected in early childhood because of their weight bearing function.

60% of our patients diagnosed as moderate grade of hemophilia, our results showed that the total joint score correlated positively with total X-ray score. In addition, both total joint and total X-ray scores correlated positively with disease duration and correlated negatively with serum factor level of hemophilia. This was in accordance with the study done by Gurcay et al. [1] who diagnosed 21 from 31 patients (67.7%) as moderate grade and found that the clinical score correlated significantly with the radiological score. Their results were supported by those of Pettersson et al. [10] who concluded that Pettersson's score correlated very well with the clinical profile of patients with hemophilic arthropathy. In addition, they reported that total X-ray score increased with age. This may be primarily based on the duration of the disease and increased recurrent hemarthrotic attacks due to insufficient factor treatment.

In the current study, there was positive correlation between the JAFAR disability score, total joint score, and total X-ray score. Dalyan et al. [22] reported a similar observation, as their analysis showed significant correlation between the disability and the radiological scores. Also, other study [1] found strong association between the disability score, radiological and clinical findings. The most difficult activities reported by our patients were walking 50 feet without help, standing up on tiptoes, and picking up something from the floor from a standing position. This was in accordance with Gurcay et al. [1] who concluded that these activities were limited mainly due to involvement of the joints of lower extremities which may easily result in functional disability.

In our study, presence of osteoporosis assessed by DEXA revealed highly significant lower Z scores of lumbar spine and neck of femur in hemophilic arthropathy patients versus controls. This was in accordance with the study done by Wallny et al. [15] who found a relationship between hemophilia and osteoporosis and increased severity of hemophilia was associated with lower BMD in neck of femur. Also, Gallacher et al. [23] studied 19 patients with severe hemophilia A and showed significantly lower BMD values in comparison with controls.

In addition, Z score of neck of femur correlated negatively with total joint, functional assessment, and total X-ray scores. This was in accordance with Barnes et al. [4] who found a statistically significant association between areal BMD Z scores and objective lower limb joint evaluation results. Patients with more established changes resulting from hemophilic joint disease exhibited the lowest BMD. Also, other studies [5, 15] found that patients with severe

hemophilia are at risk of developing reduced BMD. Lastly, Khawaji et al. [24] stated that with increasing severity and number of affected joints, BMD significantly decreases.

There was no significant correlation between Z score of lumbar spine and either clinical or radiological scores. This was in accordance with Nair et al. [5] as the BMD of femoral neck showed over all better correlation with the examined variables than the BMD of the lumbar spine.

Similar observation was seen between our study and the previous studies done that no significant difference was found in Z scores of lumbar spine and neck of femur between patients with or without hepatitis C virus.

In our study, we found a highly significant decrease in serum levels of Mg, Cu, and Zn among hemophilic patients compared to controls, while there was no statistically significant difference as regards serum Ca levels. Moreover, serum levels of Cu and Zn correlated positively with Z score of neck of femur and serum levels of Zn correlated negatively with total joint, functional assessment, and total X-ray scores. On the other hand, there was no significant correlation between serum levels of either Ca or Mg and severity of osteoporosis as measured by Z score. Also, Mir et al. [19] found a significantly lower serum Zn concentration in men, with hip osteoporosis and concluded that zinc had a positive association with BMD in men and its deficiency was more common in osteoporotic

In addition, Mutlu et al. [6] found that serum Mg and Zinc were significantly lower in osteopenic women than in normal women. But no statistically significant differences were observed as regards copper levels. They also concluded that trace element supplementation especially with magnesium, zinc, and perhaps copper, may have beneficial effects on bone density. On the other hand, Gur et al. [18] stated that Mg, Cu, and Zn levels in serum of patients with postmenopausal osteoporosis were lower than those in controls demonstrating that deficiency of these trace minerals plays a major role in the development of osteoporosis when serum Ca and phosphorus were normal. Also, Odabasi et al. [21] reported significant differences between osteoporotic patients and controls as regards Mg concentration.

In conclusion, in hemophilic arthropathy patients, osteoporosis represents a frequent concomitant observation which may complicate the future treatment of these patients. Extensive joint disease is an important risk factor for the severity of osteoporosis. Trace minerals like Mg, Cu, and Zn are essential in bone metabolism as cofactors for specific enzymes that are essential for organic bone matrix synthesis. Screening of young hemophiliacs for reduced bone density is recommended with measuring the levels of Mg, Cu, and Zn for better assessment and management of the disease.

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Review Article

The Bone-Muscle Relationship in Men and Women

Thomas F. Lang

Department of Radiology and Biomedical Imaging, School of Medicine, University of California, San Francisco, San Francisco, CA 94143-0946, USA

Correspondence should be addressed to Thomas F. Lang, thomas.lang@ucsf.edu

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Muscle forces are a strong determinant of bone structure, particularly during the process of growth and development. The gender divergence in the bone-muscle relationship becomes strongly evident during adolescence. In females, growth is characterized by increased estrogen levels and increased mass and strength of bone relative to that of muscle, whereas in men, increases in testosterone fuel large increases in muscle, resulting in muscle forces that coincide with a large growth in bone dimensions and strength. In adulthood, significant age-related losses are observed for both bone and muscle tissues. Large decrease in estrogen levels in women appears to diminish the skeleton's responsiveness to exercise more than in men. In contrast, the aging of the muscle-bone axis in men is a function of age related declines in both hormones. In addition to the well-known age related changes in the mechanical loading of bone by muscle, newer studies appear to provide evidence of age- and gender-related variations in molecular signaling between bone and muscle that are independent of purely mechanical interactions. In summary, gender differences in the acquisition and age-related loss in bone and muscle tissues may be important for developing gender-specific strategies for using exercise to reduce bone loss with aging.

1. Introduction

Skeletal fractures occur when bones are subjected to mechanical loads which exceed their strength. Diminished skeletal strength is a primary risk factor for fracture, and gender differences in skeletal structure and strength play a powerful role in determining gender differences in fracture risk. Skeletal structure adapts to the long-term loads exerted on the skeleton exerted as a result of physical activity, and the most powerful loading forces are conferred by muscles, which must exert enough force to move bones while acting against extremely short lever arms. Thus, skeletal muscle is one of the most powerful determinants of bone strength and gender differences in the bone-muscle relationship are of key interest in understanding gender differences in bone growth, in age-related bone loss, and in risk of fracture. The close coupling between muscle and bone and the gender differences in the relationship are often viewed in the context of the the mechanostat theory, first elaborated by Frost [1, 2]. In this paradigm, the muscle-bone relationship, expressed as the "bone-muscle unit," is viewed as a mechanical relationship modulated by the systemic effects (e.g., hormones). Bones respond to the varying strains imposed by increases or decreases of mechanical loading, with sharp losses or modeling effects triggered when strains, respectively, fall below or exceed setpoints that are determined by the gender-specific interaction of systemic factors with bone tissue. These same endocrine factors also have direct gender-specific interactions with muscle tissues, altering muscle mass and strength and affecting the loads placed on bone. Finally, newer research points to direct two-way signaling between muscle and bone tissues, broadening the relationship beyond that of a purely mechanical perspective.

2. Gender Differences in the Bone-Muscle Unit in Childhood and Adolescence

Skeletal fragility in old age is a function of peak bone strength in young adulthood and age-related loss of bone strength.

In order to understand gender differences in fracture risk in elderly subjects, it is important to understand the gender differences in the conditions of accrual of peak bone strength during childhood and adolescence. The achievement of peak bone strength is a function both of accrual bone of mass and changes in bone geometry, and this differs strongly in males and females in relation to patterns of skeletal muscle growth.

The idea of the bone-muscle unit, derived from the mechanostat theory [1, 2], has been widely employed to account for gender-specific trends in acquisition of peak bone strength in relation to growth of muscle area [3, 4]. From this point of view, bone structure evolves to match increased tissue strains occurring as a function of growth. During the period of rapid growth in adolescence, bone structure constantly adapts to maintain stability in the presence of mechanical loads and a rapidly changing hormonal environment. In this phase, changes in mechanical loads occur as bones grow longitudinally, resulting in higher lever arms and increasing bending moments. The increasing size and strength of muscles result in larger deformation forces on bone. The increased mechanical stimulation due to the combination of longitudinal growth and muscle contraction results in bone growth primarily due to periosteal bone formation.

Gender differences in the relation of muscle and bone growth are generally not evident in early childhood, and studies show little to any differences in the relation of muscle to bone area. However, gender-variant patterns emerge during adolescence, reflecting the different musculoskeletal effects of testosterone and estrogen in males and females [5]. In males, the changes of bone and muscle during puberty are dominated by the increasing levels of testosterone and IGF-1, which result in increased muscle mass and strength. The combination of higher deformation forces and the higher bending moments due to longitudinal growth leads to a bone growth pattern dominated by periosteal apposition. Thus, in men, the growth in muscle and bone is more parallel in nature and the peak values of cortical area and muscle cross-sectional area tend to coincide within half a year in men. In girls, with lower levels of testosterone, and higher levels of estrogen, bone mass, but not total cross-sectional area, tends to increase more rapidly in relation to muscle area. The increase in bone mass appears to take the form of increased endosteal apposition, rather than periosteal apposition. A study examining gender differences in bone structure in young men and women at the hip, distal tibia, and distal radius found that men have higher total and cortical bone cross-sectional area, but volumetric density values similar to those observed in women [6]. When the data are adjusted for differences in body height, gender differences in cortical thickness and area are highly attenuated, but differences in total bone cross-sectional area remain large. The higher total bone area is consistent with higher muscle cross-sectional area found in young men compared to young women. In young adulthood, there are apparent gender differences in the correlation of muscle area to bone area. In men, more of the variation in bone dimensions is explained by muscle area in men [7]. Women have higher values of bone in relation to muscle, but a lower percentage of the variation in cortical area in women is explained by muscle mass [8].

3. Aging, Physical Activity, and Skeletal Integrity

After attainment of peak bone and muscle strength, both men and women begin to lose both bone and muscle tissue with age. In women, age-related bone loss begins in the early to mid-thirties. This process is greatly accentuated by the rapid decrease of estrogen levels occurring as a result of the menopause. Men have a lower rate of bone loss that continues throughout the lifespan that is also influenced by age-related decreases in estradiol levels. Both men and women undergo age-related muscle loss associated with decline in testosterone levels, with men undergoing a larger lifetime loss of muscle mass and strength.

In the aging process, the bone-muscle relationship is affected by gender differences in the rate of loss of bone and muscle and in the mechanosensitivity of bone. In males, aging is characterized by large declines in testosterone, and men experience cross-sectional and longitudinal losses of muscle strength and mass that are twice what is observed in women [9, 10]. Women, on the other hand, experience an over 50% larger lifetime loss of bone mass and strength, driven by loss of age- and menopause-related loss of estrogen [6, 11]. Although bone and muscle show sharply different age-related changes in men and women, the critical factor for the bone muscle relationship is the change in bone mechanosensitivity that occurs in women as a function of estrogen loss. At the cellular level, mechanical loading involves a series of molecular events that depend on the estrogen receptor alpha (ER-α). ER-α number declines with menopause, reducing the ability of mechanical loading to induce an osteogenic response [12]. This picture is consistent with gender differences in the relationship of muscle mass with bone density, with men tending to show higher correlations between muscle mass and areal bone density [13]. It is also consistent with observations that the effect of strenuous exercise on bone mineral density is attenuated in older compared to younger women and that among older subjects, evidence seems to point to more robust exercise effects on bone in men. Overall, the relationship of muscle mass to bone structure and strength is more preserved in men than in women. While exercise can be of high relevance in reducing the rate of age-related bone loss in both genders, the effect is especially important in men.

4. Muscle and Bone Tissues as Individual Targets of Systemic Hormone Action

IGF-1 is a hormone that targets both muscle and bone tissue and is considered to be of particularly high importance in the development of osteoporosis and sarcopenia in males. IGF-1 stimulates the proliferation of muscle progenitor cells and their integration with existing fibers during the muscle repair process [14]. It also affects pathways controlling the calcium-induced contractility of muscle fibers. IGF-1 is also anabolic

for bone. Mice with overexpression of IGF-1 show higher cortical tissue properties. In men, increasing IGF-1 levels are associated with increasing femoral neck density [15]. The expression of IGF-1 in muscle tissue may be associated with the positive skeletal effects of exercise in both young and elderly men, and the age-related decrease of IGF-1 levels may lead to decreased mechanosensitivity as reflected in the lower effects of exercise on bone in elderly men.

Androgens play a significant role in the development and maintenance of muscle and of skeletal integrity in both men and women. Androgens stimulate the skeletal modeling process by inhibition of RANKL action on osteoclasts both through their own receptors and through aromatization to estrogen. In the growth process, androgens are responsible for large increases in formation of trabecular bone and are in particular associated with bone size, in both men and women [16]. Androgen loss has a particularly important effect in men; eugonadal men undergo severe bone loss, which can be partially recovered through androgen replacement therapy. Androgens also have a particularly important role in skeletal muscle in men. Increased testosterone levels are associated with increased muscle mass in men, and low levels of androgen lead to loss of muscle mass and reduced growth of muscle mass in boys. Androgens are also important for skeletal and skeletal muscle development in women. Women with low testosterone levels show higher degrees of menopause-related bone loss, a condition that can be counteracted through androgen supplementation [17].

While estrogen is central to skeletal growth and maintenance of skeletal integrity in women, it is also a significant factor for men. Estrogen inhibits the action of proresorption cytokines. Decrease in estrogen levels, in both genders, results in increased bone resorption, but low levels of estrogen also affect the skeleton by decreasing mechanosensitivity. Thus, as with androgens, estrogens affect the muscle bone system by decreasing the effect of muscle contractions on bone, leading potentially to decreased efficacy of resistance exercise in men as well as women with increasing age.

5. Molecular Signaling between Muscle and Bone

Emerging research indicates that muscles release factors that are detected by bones and that may affect bone structure and strength independently of mechanical loads. In a study of mice lacking a muscle-specific phosphatase (MIP/MTMR14;MIPKO), Brotto et al. reported increases in intracellular phosphate accompanied by impaired calcium homeostasis, decreases in the function of skeletal, cardiac, and smooth muscle, as well as deterioration of trabecular structure with no effect on cortical bone [18]. The skeletal effects of ablation of MIP were gender-specific. Female knockout mice of 12–14 months showed severe trabecular bone loss, but this knockout did not appear to have a similar effect on male mice. Further investigation in this area is underway, and a potential gender difference in muscle-bone signaling, which is independent of the mechanical loads on bone exerted by muscle, may have importance for gender-specific strategies for prevention of muscle and bone loss.

6. Summary

In conclusion, the interaction between bone and muscle, in the process of growth and development and in the process of aging, differs between men and women. In females, the growth process is characterized by increased mass and strength of bone relative to that of muscle, whereas in men, increases in testosterone fuel large increases in muscle, resulting in muscle forces that coincide with a large growth in bone dimensions and strength. In both genders, aging causes pronounced losses in both tissues, but the large decrease in estrogen levels in women appears to diminish the skeleton's responsiveness to exercise more than in men. In contrast, the aging of the muscle-bone axis in men is a function of age-related declines in both hormones. The gender differences in the acquisition and age-related loss in bone and muscle tissues may be important for developing gender-specific strategies for using exercise to reduce bone loss with aging.

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Research Article

Testosterone and the Male Skeleton: A Dual Mode of Action

Mieke Sinnesael,[1] Steven Boonen,[2,3,4] Frank Claessens,[5] Evelien Gielen,[2,3,4] and Dirk Vanderschueren[1,4,6]

[1] *Experimental Medicine and Endocrinology, Department of Experimental Medicine, K. U. Leuven, 300 Leuven, Belgium*
[2] *Division of Geriatric Medicine, Leuven University Hospital, Leuven, 300 Leuven, Belgium*
[3] *Gerontology and Geriatrics Section, Department of Experimental Medicine, K. U. Leuven, 300 Leuven, Belgium*
[4] *Leuven University Centre for Metabolic Bone Diseases, 300 Leuven, Belgium*
[5] *Molecular Endocrinology Laboratory, Department of Molecular Cell Biology, K. U. Leuven, 300 Leuven, Belgium*
[6] *Laboratory for Experimental Medicine and Endocrinology, Leuven University Hospital , Herestraat 49, 300 Leuven, Belgium*

Correspondence should be addressed to Dirk Vanderschueren, dirk.vanderschueren@uz.kuleuven.be

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Testosterone is an important hormone for both bone gain and maintenance in men. Hypogonadal men have accelerated bone turnover and increased fracture risk. In these men, administration of testosterone inhibits bone resorption and maintains bone mass. Testosterone, however, is converted into estradiol via aromatization in many tissues including male bone. The importance of estrogen receptor alpha activation as well of aromatization of androgens into estrogens was highlighted by a number of cases of men suffering from an inactivating mutation in the estrogen receptor alpha or in the aromatase enzyme. All these men typically had low bone mass, high bone turnover and open epiphyses. In line with these findings, cohort studies have confirmed that estradiol contributes to the maintenance of bone mass after reaching peak bone mass, with an association between estradiol and fractures in elderly men. Recent studies in knock-out mice have increased our understanding of the role of androgens and estrogens in different bone compartments. Estrogen receptor activation, but not androgen receptor activation, is involved in the regulation of male longitudinal appendicular skeletal growth in mice. Both the androgen and the estrogen receptor can independently mediate the cancellous bone-sparing effects of sex steroids in male mice. Selective KO studies of the androgen receptor in osteoblasts in male mice suggest that the osteoblast in the target cell for androgen receptor mediated maintenance of trabecular bone volume and coordination of bone matrix synthesis and mineralization. Taken together, both human and animal studies suggest that testosterone has a dual mode of action on different bone surfaces with involvement of both the androgen and estrogen receptor.

1. Introduction

The major circulating androgen in men is testosterone. Testosterone, like adrenal androgens, is a C19 steroid synthesized from cholesterol. Both gonadal and adrenal testosterone can be converted into estrogens (C18 steroids) by the P 450 aromatase, encoded by CYP 19, which is present in many peripheral tissues, including bone. Bone cells express androgen receptor (AR) as well as estrogen receptor-α (ERα) and $-\beta$ (ERβ) [1]. Therefore, androgen action on male bone may be explained by AR activation or, alternatively, activation of ERα and $-\beta$ (Figure 1).

2. Evidence from Human Studies

The importance of estrogen receptor alpha activation as well as of aromatization of androgens into estrogens was highlighted by a number of cases of men suffering from an inactivating mutation in the estrogen receptor alpha or in the aromatase enzyme [2–4].

Table 1 shows the age of diagnosis, bone phenotype, and effect of estrogen treatment (if appropriate) in these men. All these men had low bone mass as measured by DEXA, high bone turnover, and open epiphyses and the distal radius despite normal to elevated testosterone concentrations. Estrogen treatment resulted in closure of the epiphyses, increased bone density, and reduced bone

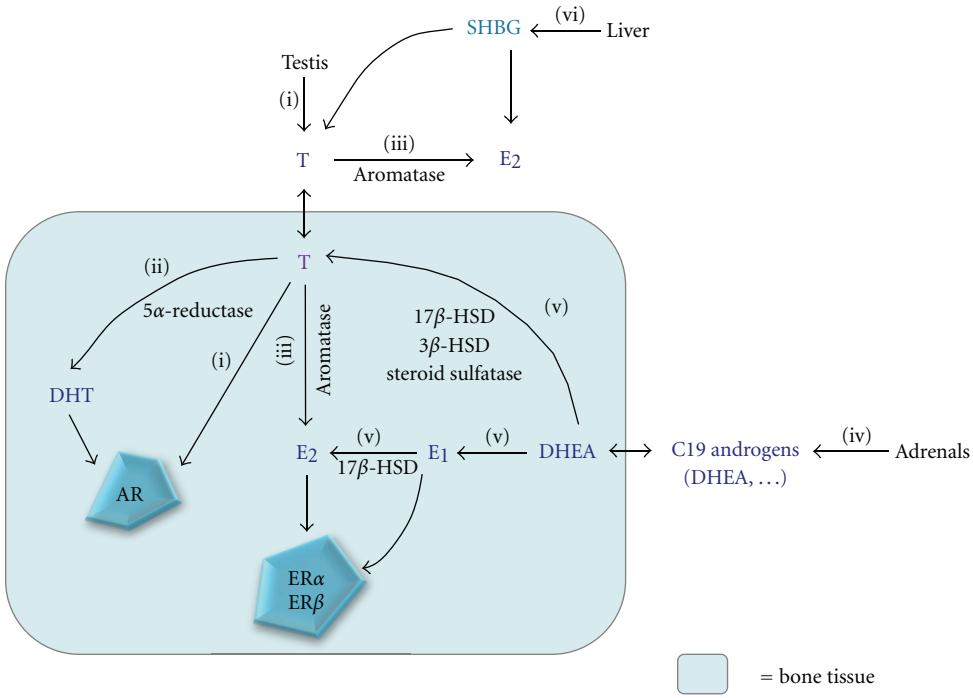

Figure 1: Metabolism of sex steroids. (i) Testosterone (T), that is secreted by the testes, can directly act on its receptor, the androgen receptor (AR), present in bone cells. (ii) T can also be converted locally to dihydrotestosterone (DHT) by 5α-reductase. (iii) In addition, T can undergo aromatization to 17β-estradiol (E_2) by aromatase. E_2 acts on one or both estrogen receptors (ERα or ERβ). (iv) The adrenals secrete C19 androgens including dehydroepiandrosterone (DHEA) that can also be converted to (v) estrone (E_1) and E_2 by aromatase, 17β-hydroxysteroid dehydrogenase (17β-HSD), and 3β-HSD or to T by 17β-HSD and/or 3β-HSD. (vi) In men and women, T and E_2 are predominantly bound to sex hormone binding globulin (SHBG), synthesized by the liver.

turnover. One patient diagnosed at much younger age was followed by peripheral computerized tomography during estrogen treatment over a period of three years. During estrogen treatment, the cortical area expanded as a result of periosteal expansion. There was no effect on trabecular bone density suggesting that the main action of estrogen on male growing bone is on the cortical and not the cancellous compartment [5]. However, it has been suggested that trabecular bone may require higher levels for its regulation. For cortical bone, estrogen deficiency is the major cause of age-related bone loss [6]. It is clear, therefore, that estrogen is important for epiphyseal closure as well as periosteal bone formation and hence cortical bone mineral acquisition during male growth. Observations in a single patient suffering from an inactivating mutation in the androgen receptor suggest that estrogen may also increase mineral apposition at the endocortical surface [7]. During adult life, estrogens mediate endosteal bone apposition and volumetric bone density, without marked influence on periosteal bone apposition. The finding of a bone size intermediate between male and female supports a role for testosterone as an essential mediator for periosteal bone expansion, but not as the sole stimulus for bone expansion during growth, supporting the concept that estrogen is also involved in bone maintenance after growth in men.

Table 2 shows cohort studies in men in which both estradiol and testosterone were measured, with either tandem mass spectrometry (LC-MS-MS) or with immunoassay, and in relation to bone loss and fractures [8–12]. Most but not all (Dubbo Osteoporosis Epidemiology study) of these studies showed an association between estradiol and fractures in elderly men [14].

While serum estradiol and testosterone are inversely related to fracture risk in older men, serum-sex-hormone binding globulin (SHBG) shows a positive relationship. Low serum estradiol, low serum testosterone, and high SHBG predict clinical vertebral fractures, nonvertebral osteoporosis fractures, and hip fractures. For estradiol, a threshold effect has been documented, below which estradiol is related to fracture risk [13]. Low estradiol induces bone resorption independently of testosterone, presumably reflecting a net increase in endocortical bone resorption [15, 16].

The effect of estrogen on bone resorption was illustrated by administration of an aromatase inhibitor to men receiving a GnRH agonist in combination with testosterone, resulting in increased bone resorption markers (deoxypyridinoline, N-telopeptide of type I collagen). Bone resorption markers increased significantly in the absence of both testosterone and estrogen and were unchanged in men receiving both hormones. Estrogen prevented the increase in the bone resorption markers, whereas testosterone had no significant effect. By contrast, serum osteocalcin, a bone formation marker, decreased in the absence of both hormones, and both estrogen and testosterone maintained osteocalcin levels. Taken together, according to this one study in men, estrogen may be important in regulating bone resorption,

Table 1: Clinical parameters of men with aromatase deficiency or estrogen resistance.

	Aromatase deficiency	Estrogen resistance
Age of diagnosis	Newborn, 24–38 yrs	28.5 yrs
Bone phenotype	(i) Persistent linear growth	(i) Continuing linear growth into adulthood
	(ii) Unfused epiphyseal cartilages	(ii) Unfused epiphyses
	(iii) Delayed bone age	(iii) Delayed bone age
	(iv) Osteopenia/osteoporosis	(iv) Osteoporosis
	(v) Eunuchoid proportion of the skeleton	(v) Progressive *genu valgum*
	(vi) Progressively worsening of bilateral *genu vagum*	(vi) Eunuchoid proportions of the skeleton
Hormonal analysis	(i) Serum estradiol below the range of detection	(i) High concentration of serum estradiol, estrone, FSH, LH
	(ii) Gonadotropins and circulating testosterone ranging from normal to elevated	(ii) Normal serum testosterone
	(iii) Impaired glucose metabolism	
	(iv) Insulin resistance	
Effect of estrogen treatment	(i) Complete epiphyseal closure	No changes
	(ii) Spinal BMD increase	
	(iii) Skeletal maturation	

Table 2: Overview of cohort studies in men.

	Average age of men	Number of men	Study duration	LC-MS-MS	Immunoassay	Main result
Rancho Bernardo study [8]	66	352	12 yr		x	(i) E: strong correlation with fractures (ii) T: no correlation with fractures
Dubbo study [9]	>60	609	16 yr	x		(i) T: no correlation with fractures (ii) E: strong correlation with fractures
Tromso [10]	50–84	1364	8.4 yr		x	T, E, SHBG: no correlation with fractures
Rotterdam study [11]	67.7 ± 6.8	178	6.5 yr		x	T, E, SHBG: no correlation with fractures
Framingham study [12]	71	793	18 yr		x	(i) E: strong correlation with fractures (ii) T + E: strong correlation with fractures
MrOS study [13]	75	2639	3.3 yr	x		E, SHBG: strong correlation with fractures

E: estrogen; T: testosterone; SHBG: sex hormone binding globulin.

whereas estrogen and testosterone may both be important in maintaining bone formation [17]. Another study in younger men, however, concluded that both androgens and estrogens play independent and fundamental roles in regulating bone resorption. In this study, young men (20–44 yrs) were divided in 3 groups. The first group received only a GnRH analog, the second group a GnRH analog plus testosterone, and the third group a GnRH analog plus an aromatase inhibitor. Bone resorption markers increased in the group who received a GnRH analog alone and the group who received a GnRH analog plus an aromatase inhibitor. Bone formation markers increased more in the first group than in the second group. Overall, these findings suggest that both estrogens and androgens play independent and fundamental roles in regulating bone resorption in men. This study also suggests that androgens may play an important role in the regulation of bone formation in men [18]. Genetic polymorphism in the Cyp 19 or in ESR genes, encoding for aromatase and estrogen receptor, respectively, may further mediate the risk for bone loss induced by low estradiol in men [19].

In humans, in contrast to rodents, circulating testosterone and estradiol are bound to sex-hormone-binding globulin, with testosterone more tightly bound than estradiol. The role of free (not bound to any protein) or bioavailable (not bound to SHBG) versus total testosterone remains controversial [20]. SHBG, which increases with age, has been associated with bone loss in elderly men [21]. However, other studies in younger men have shown a positive rather than a negative association between peak bone mass

Table 3: Relative effects of testosterone, dihydrotestosterone, estradiol, and selective estrogen receptor modulator on body weight, appendicular skeletal growth, cancellous bone mass, and on cortical bone area.

	Body weight gain	Appendicular skeletal growth	Cancellous bone mass	Cortical bone area
Orch + T	=,↑	=, ↑	↑	↑
Orch + DHT	=,↓	=,↑	↑	=, ↑
Orch + E_2	=, ↓	=	↑	=, ↑
Orch + SERM	↓	NA	↑	↑

Orch: orchidectomy; T: testosterone; DHT: dihydrotestosterone; E_2: estradiol; SERM: selective estrogen receptor modulator.

and SHBG [22–24]. However, the exact role and contribution of SHBG in bone gain and loss in men remains to be clarified.

Compared to estradiol levels (see Table 1), testosterone concentrations are less consistently associated with bone loss/fractures in men, except for very low levels in hypogonadal men (especially men following chemical and surgical castration) who show a significant increase in bone turnover, bone loss, and fracture risk [25]. In these men, testosterone inhibits bone resorption and maintains bone mass [26] whereas its effect in elderly men with borderline low testosterone or low normal testosterone concentrations is more controversial [27].

With selective estrogen receptor modulators (SERM's), very few data are currently available in men. In one study in men receiving a GnRH agonist for prostate cancer, raloxifene increased bone mineral density of the hip and, to a lesser degree, the spine. In this study, Raloxifene reduced serum concentrations of aminoterminal propeptide of type I collagen, a marker of bone formation and also tended to reduce urinary excretion of deoxypyridinoline, a marker of bone resorption [28]. Short-term administration (12 weeks) of an aromatase inhibitor in elderly men was found to increase testosterone and reduce estrogen levels but with hardly any effect on bone metabolism [29]. This lack of an effect may be due to the concomitant increase in testosterone production, the relative modest effect on estradiol production, or a combination of both factors. However when the aromatase inhibitor was administered for a longer term (12 months) there was a decrease in bone mineral density [30]. Therefore, aromatase inhibition does not improve skeletal health in aging men with low or normal testosterone levels. With selective androgen receptor modulators (SARMs), capable of selectively stimulating the androgen receptor in bone and muscle but not in prostate, no clinical bone data are available in men [31].

3. Evidence from Animal Studies

To further investigate the relative importance of androgen receptor-mediated testosterone actions compared to estradiol effects, an increasing number of animal experiments have been published, in particular in the orchidectomized rodent, a well-characterized model for hypogonadal osteoporosis. Following orchidectomy, bone resorption increases at cancellous and endocortical surfaces and results in reduced cancellous and cortical bone volume. Periosteal bone formation during growth is decreased in orchidectomized rodents as well and further lowers bone strength [1]. A number of animal experiments have investigated the bone phenotypic changes induced by androgens, nonaromatisable androgens and estrogens in orchidectomized rodents (mice, rat and growing/non-growing). Table 3 shows the relative effects of androgens [32–34], non-aromatisable androgens [32–36], estrogens [33, 36, 37] on bone turnover, bone density, and periosteal bone formation in male orchidectomized rats.

From these studies, it is clear that non-aromatisable androgens can also stimulate periosteal bone formation and inhibit cancellous bone, although less than testosterone, and that estradiol exerts potent effects on different bone surfaces [36]. However, what is not always clear is to what extent the effects of these hormones are pharmacological or physiological and, if physiological, to what extent the effects can be extrapolated to the human condition, in a context of higher estradiol concentrations than in mice [38].

Other animal experiments have investigated the bone phenotype of transgenic male animals with KO of AR (ArKO), ER alpha (ERKO), beta (BERKO), or both (DERKO), and this in combination with orchidectomy with or without replacement with androgens and estrogens. The latter is needed because the ArKO and ERKO models may have an impact on respective concentrations of androgens/estrogens [26, 39] (Table 4). Overall, available evidence from these studies suggests that, ER activation, not AR activation, is involved in the regulation of male longitudinal appendicular skeletal growth in mice. ERα and AR but not ERβ enhance cortical radial bone growth. The AR, not Erβ, is required for the maintenance of cancellous bone mass. AR and ERα, but not ERβ, can independently mediate the cancellous bone-sparing effects of sex steroids in male mice [26].

These studies have greatly increased our understanding of the role of estrogen receptor and androgen receptors in male skeletal growth and maintenance in male rodents. Both AR and ERα are involved in male skeletal growth and maintenance, supporting a dual mode of action for testosterone, either directly on the AR or indirectly on the ERα through aromatization.

In summary, both AR and ERα activation appear to stimulate periosteal bone formation and cortical bone growth. ERα is also involved in longitudinal bone formation but its action on periosteal surface as well as growth plate may be mediated indirectly by the GH-IGF-I axis [40]. On trabecular bone surfaces, AR activation may be most critical, at least in mice, as elegantly illustrated with a double KO

Table 4: Summary of the skeletal phenotypes in mice with different sex steroid-related gene inactivations.

				Cancellous bone		
	Longitudinal skeletal growth	Cortical bone area	Intact mice	Effect of E in Orch	Effect of T in Orch	Effect of DHT in Orch
BERKO	0	0	0	Yes	ND	Yes
ERKO	−	−	+	No	Yes	Yes
DERKO	−	−	+	No	ND	Yes
ArKO	−	−	−	Yes	ND	ND
Tfm	?	?	−	Yes	Partial	ND

+: Increased; −: decreased; 0: no effect; conflicting results; ND: not determined; Orch: orchidectomy; E: treatment with physiological levels of estrogen; T: treatment with physiological of testosterone; DHT: treatment with physiological levels of 5α-dihydrotestosterone.

Table 5: Overview bone parameters in osteoblast-specific AR knockout mice.

	Tb. N (/mm)	Tb. Th (μm)	BV/TV (%)	Osteoid surface (%BS)	OCL surface (%BS)	MAR (μm/day)	BFR (μm_2 /μm/day)
Col 2.3-cre AR KO	↓	↑	↓	=	=	=	=
Osteocalcin-cre KO	↓	↓	↓	↑	=	=	=

Tb. N: trabecular number; Tb. Th: trabecular thickness; BV/TV: trabecular bone volume; OCL: osteoclast; MAR: mineral apposition rate; BFR: bone formation rate.

AR-ER in comparison with either AR or ERα disruption alone. Combined AR and ERα inactivation further reduced cortical bone and muscle mass and AR activation was found to be solely responsible for the development and maintenance of male trabecular bone mass. However, both AR and ERα activation appeared to be essential to optimize the acquisition of cortical bone and muscle mass [41]. Erβ, on the other hand, seems not to be relevant for bone growth and maintenance in male mice [42]. To further document the target cell of AR and ER in mice, the AR was recently selectively knockedout in bone cells by cre-lox technology. In two studies (Table 5), the osteoblast was targeted which resulted in a trabecular and no cortical phenotype, suggesting that the osteoblast is the target cell for androgen-receptor-mediated maintenance of trabecular bone volume and coordination of bone matrix synthesis and mineralization [43, 44].

This assumption was further supported by a coculture experiment were the *in vitro* osteoclastogenesis was assessed using osteoclast precursor cells from bone marrow and calvaria osteoblasts from male WT and ARKO mice. When the AR was absent in osteoclast precursor cells, osteoclastogenesis was unaffected [45]. Osteoclastogenesis, in response to 1α,25$(OH)_2D_3$ after activation by RANKL and M-CSF (macrophage-colony stimulating factor), also seemed unaffected in AR-deficient osteoclasts. However, AR inactivation in osteoblasts potentiated osteoblastic functions that promote osteoclastogenesis in the presence of inducers. RANKL turned out to be upregulated in these AR-deficient osteoblasts, suggesting that the suppressive function of AR on RANKL gene expression mediates the protective effects of androgens on bone remodelling through inhibition of bone resorption. It would seem therefore that intact AR function is required for the suppressive effects of androgens on the osteoclastogenesis supporting activity of osteoblasts, but not osteoclasts [45].

In conclusion, both human and animal experiments suggest that testosterone has a dual mode of action on different bone surfaces. Activation of both ERα and AR seems to be involved.

Conflict of Interests

The authors have no conflict of interests.

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Research Article

Elevated Incidence of Fractures in Solid-Organ Transplant Recipients on Glucocorticoid-Sparing Immunosuppressive Regimens

B. J. Edwards,[1] A. Desai,[2] J. Tsai,[1] H. Du,[2] G. R. Edwards,[1] A. D. Bunta,[1] A. Hahr,[1] M. Abecassis,[3] and S. Sprague[2]

[1] *Bone Health and Osteoporosis Center, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA*
[2] *NorthShore University HealthSystem, Evanston, IL 60201, USA*
[3] *Kovler Transplant Center, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA*

Correspondence should be addressed to B. J. Edwards, bje168@northwestern.edu

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This study was conducted to assess the occurrence of fractures in solid-organ transplant recipients. *Methods*. Medical record review and surveys were performed. Patients received less than 6 months of glucocorticoids. *Results*. Of 351 transplant patients, 175 patients provided fracture information, with 48 (27.4%) having fractured since transplant (2–6 years). Transplants included 19 kidney/liver (50% male), 47 kidney/pancreas (53% male), 92 liver (65% male), and 17 pancreas transplants (41% male). Age at transplant was 50.8 ± 10.3 years. Fractures were equally seen across both genders and transplant types. Calcium supplementation ($n = 94$) and bisphosphonate therapy ($n = 52$) were observed, and an association with a lower risk of fractures was noted for bisphosphonate users (OR = 0.45 95% C.I. 0.24, 0.85). Fracture location included 8 (16.7%) foot, 12 (25.0%) vertebral, 3 (6.3%) hand, 2 (4.2%) humerus, 5 (10.4%) wrist, 10 (20.8%) fractures at other sites, and 7 (14.6%) multiple fractures. The estimated relative risk of fracture was nearly seventeen-times higher in male liver transplant recipients ages 45–64 years compared with the general male population, and comparable to fracture rates on conventional immunosuppressant regimens. *Conclusion*. We identify a high frequency of fractures in transplant recipients despite limited glucocorticoid use.

1. Introduction

Within the past 3 decades, organ transplantation has become an established therapy for end-stage diseases of the kidney, liver, and lung. Survival after solid-organ transplantation has improved markedly mainly because of the addition of calcineurin inhibitors, cyclosporine A (CsA), and tacrolimus, to posttransplantation immunosuppressive regimens. With improved survival has come a greater appreciation of complications such as osteoporosis and fractures that negatively influence patients' quality of life. The pathogenesis of transplantation osteoporosis is complex and incompletely understood. It is probably related to a combination of noxious effects to the skeleton that occur both before and after organ transplantation. Cardiac, kidney, lung, and liver failure each have unique pathophysiologies that influence bone and mineral metabolism before transplantation. Additional factors such as aging, nutritional deficiencies, immobility, diabetes mellitus, tobacco, and alcohol may affect the skeletons of these transplant recipients before and after transplantation. In the posttransplant period, patients are then subjected to a drug regimen that usually includes high doses of glucocorticoids, the most common cause of secondary osteoporosis. Glucocorticoids are prescribed in combination with other immunosuppressive agents, such as calcineurin inhibitors (cyclosporine A or tacrolimus), rapamycin, mycophenylate mofetil, and azathioprine. Of these agents, both cyclosporine A and tacrolimus are thought to have specific adverse effects upon skeletal integrity. It is thought that the independent and interconnected skeletal

effects of glucocorticoids and calcineurin inhibitors lead to a form of bone disease characterized by rapid bone loss and high rates of fractures [1–6].

While most transplant centers have used triple therapy consisting of a calcineurin-inhibitor (CNI), an antimetabolite, and steroids as induction and maintenance regimens, steroid sparing regimens have been developed due to the concern in the transplant community about the importance of steroid-related morbidity [7, 8]. The purpose of this study was, therefore, to evaluate whether glucocorticoid-sparing immunesuppressive regimens are associated with a reduced risk of fractures. Our prior work has shown that conventional immunosuppressant regimens were associated with a 13-fold higher risk of fracture than age- and gender-matched rates form a nationally representative sample (National Health Interview Survey).

2. Materials and Methods

2.1. Patients. The Kovler Transplant Center at Northwestern University is located at Northwestern Memorial Hospital. An extensive clinical database is maintained at Northwestern Memorial Hospital. The status of all patients in the database is maintained as part of the regular posttransplantation care at the respective hospitals. The Institutional Review Board approved this study and all participants provided informed consent.

Inclusion. 18 years of age and older recipients of solid-organ transplants between January 1, 2001 and December 31, 2007, survivals for at least 2 years after transplant. Use of glucocorticoids limited to the initial 6 months of immunosuppressive regimen.

Exclusion. prolonged glucocorticoid therapy, inability to provide informed consent, fractures of digits or toes, and skull fractures. The cohort for this study includes 351 patients who received pancreas, kidney-pancreas, and liver transplants and survived. Adequate fracture information was obtained in 175 subjects. There were 92 liver, 47 kidney-pancreas, 19 kidney-liver, and 17 pancreas transplants preformed and evaluable during the study interval. Kidney-only transplants and cardiac transplants were excluded due to prolonged glucocorticoid use.

2.2. Fracture Ascertainment and Verification. Information in symptomatic incident fractures was obtained retrospectively in the organ transplant cohort. All patients were contacted by telephone (88%) or at the clinic visit (12%) and queried about fracture occurrence since the transplant. All fractures identified were verified by review of the medical record for formal radiographic reports or other relevant documentation. Asymptomatic fractures not located in the thoracic spine/rib cage (visualized on chest X-ray) may have been missed because routine thoracic and lumbar spine films were not obtained for spinal morphometry. Fractures of the face and digits were excluded from analysis.

2.3. Data Analysis. Descriptive statistics such as mean ± SD (standard deviation) were used to summarize patient characteristics for continuous variables whereas frequency and percentage were used for categorical variables. Incidence rate per person-year of fracture was calculated, using the observed fracture frequency in this study cohort as the numerator, and a product of the individuals at risk and the time units as the denominator. The time units were defined as the years since the transplant till whatever happened first during the followup, fracture date, or the interview date. All interviews were conducted between July 1, 2007 and December 31, 2009. Overall person-year fracture incidence rate was computed, as well the age- and gender-specific person-year fracture incidence rate. Age was also stratified as <18, 18–24, 25–44, 45–64, and 65–69, according to 1994 NHIS grouping. Organ-, age-, and gender-specific numbers and rates, and person-years of observation were calculated. Weighted age- and gender-specific fracture rates from 1994 NHIS (National Health Interview Survey) were applied to the number of person-years of observation for each organ-specific age and gender category of transplant patients to calculate an expected number of fractures. The ratio (expressed as an estimated relative risk) of observed and expected number of fractures was used to compare fracture of transplant patients to that of the national sample of the 1994 NHIS.

A Chi-square or Fisher's exact test was used to assess the association between categorical variables (i.e., gender) and the occurrence of fracture. A two-sample t-test was used to assess the difference between fractured and nonfractured subjects, with respect to continuous variables (i.e., age at transplant). Time to event analysis was applied to estimate the average time to fracture after transplant among organ transplant recipients. Kaplan-Meier plot was used to depict the time to fracture difference between male and female, between age at transplant ≥45 and <45 patients, and among organ transplant types. Log-rank test was used to determine the P values. A two-sided significance level 0.05 was used. Data were stored in excel format, and the statistical analysis was carried out using SAS 9.1.

3. Results

The specific patient characteristics stratified by type of organ transplant are shown in Table 1. Of 298 transplant patients (Jan 2001–Dec 2007), 175 patients provided fracture information, with 48 (59%) having fractured since transplant (2–6 years). Nonrespondents were similar in demographic characteristics as well as comorbidities. Calcium supplementation ($n = 94$) and bisphosphonate therapy ($n = 52$) were observed and an association with a lower risk of fractures was noted for bisphosphonate users (OR 0.45 95% C.I. 0.24, 0.85). Transplants included 19 kidney/liver (50% male), 47 kidney/pancreas (53% male), 92 liver (65% male), and 17 pancreas transplants (41% male). Age at transplant was similar ($P = 0.146$) in fracture (47.8 ± 11.3 years) and nonfracture groups (50.4 ± 10.0 yrs). Fractures were equally seen ($P = 0.224$) in both genders (23 of 71 female (32.4%) and 25 of 104 male (24.0 %)). Fractures were equally distributed ($P = 0.582$) across all transplant types (15.8%, 29.8%, 27.2%, and 35.3%, resp.). Fracture location included

Table 1: Characteristics of organ transplant recipients and patients with fractures.

Patient features	Kidney/liver	Kidney/pancreas	Liver	Pancreas
Number of patients	19	47	92	17
Gender (% female)	52.6	46.8	35	58.8
Age at transplant in years (mean ± SD) (*n*)	54.9 ± 7.5	43.9 ± 8.2	53.2 ± 10.3	41.1 ± 7.3
Number with fractures	4	15	22	7
Men	1/9	7/25	16/60	1/7
	11%	**28**%	**27**%	**14**%
All women	3/10	8/22	6/32	6/10
	30%	32%	19%	60%
Postmenopausal women**	2/10	5/22	5/22	0/1
	20%	23%	23%	0%

**Menopause assumed if age at fracture ≥50 years.

Table 2: Age- and gender-specific fracture incidence rates in *all* transplant recipients.

	Women				Men			
Age in years	Person-years at risk	Observed number of fractures	Expected number of fracture	Estimated relative risk	Person-years at risk	Observed number of fractures	Expected number of fractures	Estimated relative risk
<18	0	0	—	—	0	0	—	—
18–24	2.8	1	—	—	0	0	—	—
25–44	106.1	6	0.33	18.2	127.0	9	0.83	10.9
45–64	155.1	16	0.47	34.4	280.3	12	0.81	14.8
65–69	14.1	0	0.06	0	31.1	3	0.09	0

Table 3: Age and gender specific fracture incidence rates in the US population. National Center for Health Statistics, National Health Interview Survey (NHIS) 1994.

Age in Years	Women	Men
<18	5.9	10.7
18–24	1.8	10.3
25–44	3.1	6.5
45–64	3.0	2.9
65–69	4.6	2.8

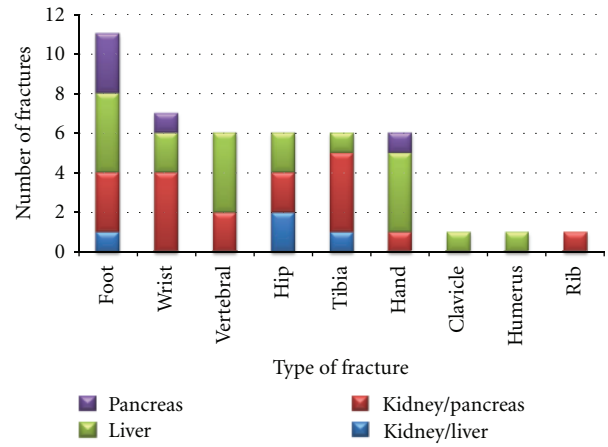

Figure 1: Type of fracture by type of solid-organ transplant.

8 (17.5%) foot (metatarsal), 8 (16.7%) vertebral, 12 (25.0%) vertebral, (6.25%) hand 3, 2 (4.2%) humerus, 5 (10.4%) wrist fractures, 10 (20.8%) occurred at other sites, and 7 (14.6%) occurred at multiple sites (Figure 1). Of the 25 men with fractures, the incidence of fractures was 0.065 per person-year among the 733.8 person-years of observation.

Patient characteristics were stratified by type of organ transplant (Table 1). Four (21%) of the 19 kidney-liver transplant recipients had symptomatic initial fractures during the 76.9 person-years of observation for a crude fracture rate of 0.039 per year of observation. The mean age at the time of the initial symptomatic fracture was 56.5 ± 9.2 years (median: 58; range: 47–65), and the mean time from transplant to initial fracture was 54.1 ± 18.8 months (median: 54; range: 41–67). Both axial ($n = 2$) and limb ($n = 1$) fractures occurred in this small group.

Fifteen of 47 kidney-pancreas (32%) recipients had their initial symptomatic fracture during the 207.1 person-years of observation for a crude fracture rate of 0.068 per year of observation. The mean age at the time of initial fracture was 48.3 ± 9.3 years, (median: 50; range: 32–63), and the mean number of months from transplant to initial fracture was 51.0 ± 23.2 months (median: 59; range: 5–80). The most common site of fracture in this group was the foot, and occurred equally in males and females 28.0% versus 31.8%, respectively. Five axial (rib and spine) fractures occurred compared with 7 limb fractures in this group as compared to 15 limb fractures. Two of the 3 patients who reported multiple fractures were women.

Table 4: Age- and gender-specific fracture incidence rates in *kidney/liver* transplant recipients.

	Women				Men			
Age in years	Person-years at risk	Observed number of fractures	Expected number of fractures	Estimated relative risk	Person-years at risk	Observed number of fractures	Expected number of fractures	Estimated relative risk
<18	0	0	—	—	0	0	—	—
18–24	0	0	—	—	0	0	—	—
25–44	7.0	0	0.02	0	5.5	0	0.04	0
45–64	30.1	2	0.09	22.1	28.8	2	0.08	12.0
65–69	5.3	0	0.02	0	0	0	—	—

Table 5: Age- and gender-specific fracture incidence rates in *kidney/pancreas* transplant recipients.

	Women				Men			
Age in years	Person-years at risk	Observed number of fractures	Expected number of fractures	Estimated relative risk	Person-years at risk	Observed number of fractures	Expected number of fractures	Estimated relative risk
<18	0	0	—	—	0	0	—	—
18–24	0	0	—	—	0	0	—	—
25–44	44.5	2	0.14	7.2	62.3	4	0.40	9.9
45–64	43.8	6	0.13	45.7	52.4	4	0.15	26.2
65–69	0	0	—	—	0	0	—	—

Twenty-two (24%) of 92 liver transplant recipients had fractures during the 383.3 person-years of observation, for a crude fracture rate of 0.065 per year of observation. The mean age at the time of initial symptomatic fracture was 54.4 ± 12.2 years (median: 57; range: 25–71), and the mean number of months from transplant 36.0 ± 28.9 months (median: 30; range: 3–86). Nine axial (vertebral and hip) fractures occurred compared with 18 limb fractures. Four patients receiving liver transplants sustained multiple fractures.

Seven (41%) of the 17 pancreas transplant recipients had fractures during the 66.5 person-years of observation for a crude fracture rate of 0.090 per year of observation. The mean age at the time of initial symptomatic fracture was 40.6 ± 6.5 years (median: 42; range: 31–50), and the mean number of months from transplant to initial fracture was 41.7 ± 22.1 months (median: 42; range; 26–57). All 3 fractures in this group were limb fractures (1 wrist and 2 foot (metatarsal)), and none sustained multiple fractures.

Age- and gender-specific fracture incidence rates representative of the US civilian, noninstitutionalized population from the 1994 NHIS are shown in Tables 2, 3, 4, 5, 6, and 7. The comparison of age- and gender-specific incidence rates for initial symptomatic fractures in solid-organ transplant recipients and the US population were shown in Tables 3, 4, 5, 6, and 7.

The male liver transplant recipients aged 45–64 years at the Kovler Transplant Center cohort had 100 person-years of observation. The estimated relative risk of fracture was nearly seventeen-times higher in male liver transplant recipients ages 45–64 years compared with the general male population of those age groups.

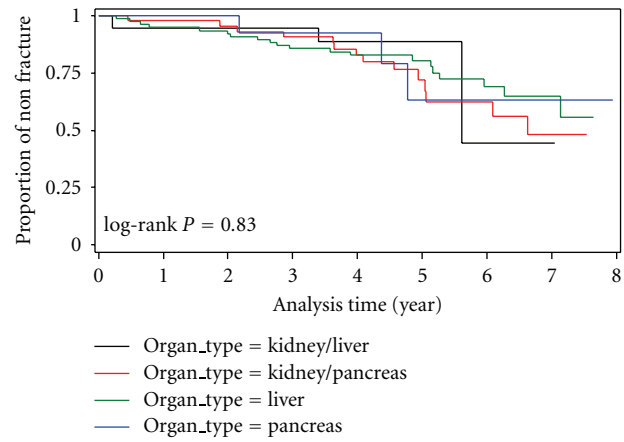

Figure 2: Proportion of fracture among all patients over time.

For those 175 transplant recipients who provided valid fracture data (yes or no), the median followup was 4.46 years (range: 0–7.93 years), 75% nonfracture time was 5.04 years (95% confidence interval: 4.08–5.37 years). Figure 2 demonstrated that among those 175 transplant recipients about 5% sustained fracture within the first 12 months while by 5 years about 25% of transplant recipients have sustained a fracture. The time to fracture was not different between females and males, neither between patients with age <45 and ≥45 years of age at time of transplant, nor between organ transplant types.

Table 6: Age- and gender-specific fracture incidence rates in *liver* transplant recipients.

	Women				Men			
Age in years	Person-years at risk	Observed number of fractures	Expected number of fractures	Estimated relative risk	Person-years at risk	Observed number of fractures	Expected number of fractures	Estimated relative risk
<18	0	0	—	—	0	0	—	—
18–24	2.8	1	0.005	201.5	0	0	—	—
25–44	33.2	1	0.10	9.7	33.3	4	0.22	18.5
45–64	75.3	7	0.23	31.0	189.2	6	0.55	16.4
65–69	8.8	0	0.04	0	31.1	3	0.09	34.5

Table 7: Age- and gender-specific fracture incidence rates in *pancreas* transplant recipients.

	Women				Men			
Age in years	Person-years at risk	Observed number of fractures	Expected number of fractures	Estimated relative risk	Person-years at risk	Observed number of fractures	Expected number of fracture	Estimated relative risk
<18	0	0	—	—	0	0	—	—
18–24	0	0	—	—	0	0	—	—
25–44	25.0	4	0.08	51.7	25.8	2	0.17	6
45–64	5.9	1	0.02	56.5	9.9	0	0.03	0
65–69	0	0	—	—	0	0	—	—

4. Discussion

This study is the first to quantify the magnitude of excess fractures occurring in solid-organ transplant recipients (liver) on glucocorticoid-sparing immuno-suppressive regimens. An elevated fracture risk (17-fold increased risk of fractures) was identified, in fact, the fracture incidence was similar to our prior studies of transplant recipients on conventional antirejection regimens [9]. As survival after organ transplantation has improved, skeletal complications are becoming increasingly troubling, with some studies showing fracture incidence of 20–40% post transplant [10–12]. Immuno-suppressive regimens with limited glucocorticoid use have been developed in an attempt to minimize adverse events. Prior studies have assessed kidney transplants, showing that glucocorticoid-free protocols have resulted in less bone loss, although fractures have not been reported as primary outcomes [13–15]. Glucocorticoids and calcineurin inhibitors have specific adverse effects on the skeleton [16–18], and have been associated with increased risk for fracture. Glucocorticoids contribute to posttransplant bone loss, especially the rapid loss that occurs in the first 6–12 months, by inhibiting bone formation. Thus, organ transplantation has been revolutionized in the era since the routine use of immune suppressive therapy with glucocorticoids, calcineurin inhibitors (cyclosporine A or tacrolimus), and either mycophenolate mofetil or azathioprine.

Post transplantation osteoporosis is a complex and multifactorial disease with preexisting bone loss associated with end-stage organ failure, immobility, aging, high-dose glucocorticoid use, and immune suppressant therapy having been implicated. End-stage kidney or liver disease, and congestive heart failure are associated with low bone mineral density, fractures, and abnormalities of mineral metabolism [19–23]. Metabolic disturbances such as abnormalities in calcium, phosphorus, and parathyroid hormone persist for many months or years following kidney transplantation [24]. Diabetes mellitus, a common cause for pancreas and kidney transplants, results in alterations in bone metabolism and fractures [19, 25–27]. Hypogonadism common in older males has been associated with bone loss and fractures [28–30]. Immobility due to end-stage organ disease has profound effect on bone metabolism [31].

Additionally, we identified that calcium supplementation and vitamin D was common in transplant recipients, but failed to reduce fracture risk. Bisphosphonate therapy however, was identified as being associated with a reduction in fracture risk. These preliminary findings suggest important treatment options in transplant candidates with suitable kidney function such as CKD stage 2-3 (GFR > 35 cc/min/ 1.73 m^2).

Our data on four self-reported symptomatic fractures in 19 liver-kidney transplant recipients represents both genders. Symptomatic axial fractures were less frequently reported than limb fractures. There is a paucity of studies assessing the incidence of fractures in these transplant recipients. All four fractures occurred in patients with pretransplant chronic hepatitis, none of these patients had diabetes mellitus. We reported on 15 fractures occurring in 47 kidney-pancreas recipients, all of whom had diabetes mellitus and end-stage kidney disease. In the kidney pancreas transplant recipients

followed for a mean time of 6.3 years we noted more limb than axial fractures. Our findings confirm the high incidence of fractures of 20–45% in prior studies [19, 32–34]. Diabetes mellitus results in low bone formation bone disease, with consequent fractures [27, 35, 36].

We described 22 symptomatic fractures in 92 liver transplant recipients, this cohort was followed for a mean of 6 years, the crude fracture rate was 0.063 per year. These data are comparable to the frequency of postliver-transplant fractures reported in other studies ranging from 20–40% [10–12]. Up to 21% of patient who receive a liver transplant sustain a fracture within the first two years [37]. Thus this data demonstrate that rate of fracture on glucocorticoid-sparing regimen remains unchanged. Fractures were most common in patients with hepatocellular carcinoma (3/3, 100%), than in patients with chronic hepatitis (13/49, 26%) or alcoholic-induced cirrhosis (2/9, 22%).

We also reported on seven fractures in 17 pancreas transplants with diabetes and followed for a mean of 4 years. The most common site affected was the foot. Patients who received kidney pancreas and pancreas transplants were diabetic and 10 years younger than other solid-organ transplant recipients. In all cases, fractures were limb fractures.

Calcineurin inhibitors have allowed for improved survival and reductions in glucocorticoid therapy in transplantation. However, the calcineurin inhibitors, cyclosporine (CsA), and tacrolimus (FK506) have also been implicated in posttransplant bone disease. These drugs stimulate loss of bone mass independent of glucocorticoid therapy, with high-turnover bone metabolism noted in rat models [38–40]. Specifically, CsA administration has resulted in marked increases in bone resorption and formation as well as greater losses of trabecular bone [38–40]. Furthermore, its direct effects on calcineurin genes expressed in osteoclasts may affect bone turnover [41, 42]. FK506 has also been shown in rat models to cause loss of trabecular bone volume in rats [38–40]. Comparison of the two drugs in rat models has demonstrated more severe bone loss with the use of CsA than FK506. In liver transplant patients [43], there is a more favorable long-term effect on bone mass evolution with the use of FK506 up to 2 years posttransplantation [44]. Both have been noted to cause significant bone resorption in kidney transplant recipients [17]. However, FK506 has been noted to protect bone mineral density better than CsA when both have been administered with combined steroid therapy over 1 year [45]. Less is understood of the effects of other immunosuppressive agents on bone loss. Few studies have evaluated the effects of mycophenolate mofetil (MMF) and sirolimus on bone metabolism [46]. In rat models, short-term use of MMF did not result in decreased bone volume [46, 47]. In humans, comparison of CsA to sirolimus resulted in less bone turnover and less bone resorption with sirolimus [47] in the short term. Longer-term data is warranted.

There are several limitations with this analysis as comparisons between the fracture rates in the transplant cohort and the NHIS data should be interpreted with caution. First, the NHIS data includes self-reported fractures that were not verified. We used a more stringent case finding procedure in the transplant cohort as we verified the patient's self-report of fracture. Thus, our observed number of fractures is more conservative than the NHIS data. Second, the number of person-years of observation in some of our strata are small, especially for kidney pancreas and kidney-liver recipients. Thus, the expected number of fractures are small, resulting in relative risk estimates that are unstable and liable to inflation from a very small number of events. Thus, we did not use test of significance for these results. Fractures rates for patients included only the initial posttransplant fracture while some patients experienced more than one fracture. Therefore, the number of fracture events in the transplant cohort represent the lowest estimate of the problem. In the strata with at least 100 person-years, we determined that fractures in transplant patients were increased nearly seventeen-fold compared with expected numbers from national data. The frequency of fractures in this transplant cohort clearly represent the lower boundary of the problem because routine surveillance for asymptomatic vertebral fractures was not performed. Nevertheless, using our conservative estimate from the occurrence of symptomatic fractures, these data demonstrate the magnitude of this excess risk.

There is limited information in medical records about potential risk factors for increased fracture rate observed in this study. Previous studies of those risk factors reveal inconsistent findings. For kidney transplant patients, risk factors associated with fracture included low BMD, prior parathyroidectomy, higher glucocorticoid use, and longer interval between transplant and fracture [48]. A population-based study showed that age and diabetic nephropathy were independent predictors of fracture risk while higher activity status was protective [49]. Future well characterized studies will allow better definition of specific risk factors for transplant-related fractures in this heterogeneous cohort.

Patients with kidney/pancreas and pancreas transplantation appear to be at higher risk of fracture. Diabetics are predisposed to low bone turnover bone disease, neuropathy, and osteopenia. Factors uniquely associated with osteopenia in diabetics include chronic hypocalcemia, insulin deficiency, hypomagnesemia, relative hypoparathyroidism, negative protein balance, immobility, hypogonadism, and metabolic acidosis [19, 27, 35, 36].

In liver transplant recipients, initial BMD, interval change in BMD, menopause, primary biliary cirrhosis, long-term glucocorticoid use, calcineurin inhibitors, underlying pretransplant disease severity, multiple fractures, and pre-transplant fracture have been identified as risk factors for fractures [10, 22, 37, 50].

5. Conclusion

This study is the first to quantify the magnitude of excess fractures occurring in solid-organ transplant recipients on glucocorticoid-sparing immuno-suppressive regimens. Liver transplant recipients have a 17-fold increased risk of fractures as compared to age- and gender-matched controls. Additional research must be conducted to clarify pathogenesis of bone loss and fractures and the development of suitable preventive strategies.

Abbreviations

CsA: Cyclosporine
FK506: Tacrolimus
MMF: Mycophenolate mofetil
CNI: Calcineurin inhibitor
NHIS: National Health Interview Survey.

Conflict of Interests

A. Desai, J. Tsai, H. Du, G. R. Edwards, A. D. Bunta, A. Hahr, M. Abecassis, and S. Sprague declare no conflict of interests.

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Research Article

Calcium and Vitamin D Supplementation in Men

Evelien Gielen,[1,2,3] Steven Boonen,[1,2,3] Dirk Vanderschueren,[3,4] Mieke Sinnesael,[4] Annemieke Verstuyf,[4] Frank Claessens,[5] Koen Milisen,[1,6] and Sabine Verschueren[7]

[1] *Division of Geriatric Medicine, Leuven University Hospital, Herestraat 49, 3000 Leuven, Belgium*
[2] *Gerontology and Geriatrics Section, Department of Experimental Medicine, K.U.Leuven, 3000 Leuven, Belgium*
[3] *Leuven University Centre for Metabolic Bone Diseases, 3000 Leuven, Belgium*
[4] *Experimental Medicine and Endocrinology, Department of Experimental Medicine, K.U.Leuven, 3000 Leuven, Belgium*
[5] *Molecular Endocrinology Laboratory, Department of Molecular Cell Biology, K.U.Leuven, 3000 Leuven, Belgium*
[6] *Centre for Health Services and Nursing Research, K.U.Leuven, 3000 Leuven, Belgium*
[7] *Research Centre for Musculoskeletal Rehabilitation, Department of Rehabilitation Sciences, K.U.Leuven, 3000 Leuven, Belgium*

Correspondence should be addressed to Steven Boonen, steven.boonen@uzleuven.be

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Calcium and vitamin D supplements reverse secondary hyperparathyroidism and are widely prescribed to prevent osteoporotic fractures, with proven antifracture efficacy when targeted to individuals with documented insufficiencies. Men who should particularly be considered for calcium and vitamin D supplements include elderly or institutionalized individuals, patients with documented osteoporosis on antiresorptive or anabolic medication, and individuals receiving glucocorticoids. Benefits are most apparent when a daily dose of 1000–1200 mg calcium is complemented with 800 IU vitamin D. Compliance is the key to optimizing clinical efficacy. While (conventionally dosed) vitamin D has not been associated with safety concerns, recent meta-analytic data have provided evidence to suggest that calcium supplements (without coadministered vitamin D) may potentially be associated with cardiovascular risks.

1. Introduction

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility [1]. Together with the age-related increase in the risk of falling, this compromised bone strength results in an increased fracture risk [2]. Key determinants of this age-related bone fragility are calcium intake and levels of vitamin D, which promotes intestinal calcium absorption [3]. In older individuals, low dietary calcium intake and vitamin D deficiency are common because of less sunlight exposure, reduced capacity of the skin for vitamin D synthesis, inadequate vitamin D dietary intake, or less efficient intestinal absorption of vitamin D, resulting in a negative calcium balance. This stimulates the secretion of parathyroid hormone (PTH) and induces age-associated secondary hyperparathyroidism, which enhances bone turnover and accelerates bone loss [4]. Additionally, vitamin D deficiency leads to muscle weakness and increases the risk of falling [5], low physical performance [6], and dynamic and postural instability [7].

Taken together, low serum calcium and vitamin D deficiency increase fracture risk by enhancing bone metabolism as well as by increasing the risk of falling. Substitution with calcium and vitamin D reduces both bone loss and the risk of falling and is therefore recommended as first-line strategy in the prevention and treatment of osteoporosis and osteoporotic fractures. Guidelines typically apply this recommendation to both men and women, although most individual trials and meta-analyses have only or mainly included women, with limited data in men [8]. This paper reviews the existing evidence for the effect of calcium and vitamin D supplementation on bone mineral density (BMD), muscle strength, and the risk of falls and fractures in men.

2. Effect of Calcium and Vitamin D on Secondary Hyperparathyroidism, Bone Mineral Density, and Bone Turnover

Both calcium and vitamin D supplements *reverse the age-associated secondary hyperparathyroidism* in older individuals. This reduction in serum PTH is greater with combined calcium and vitamin D supplementation than with vitamin D alone [3] and depends on baseline calcium balance, with the greatest effect of supplementation observed in individuals with the lowest calcium intake and/or vitamin D levels [9]. Gender, however, apparently is an independent determinant of serum vitamin D [10], and to keep PTH within the normal range, women seem to require higher levels of vitamin D than men [11].

A decrease in levels of PTH is associated with an *increase in BMD*, as has been demonstrated in a recent meta-analysis in more than 40000 men and women aged 50 years and older [12]. In this meta-analysis of 24 trials reporting BMD as an outcome, 19 trials included only women, while four trials included men and women [13–16], and one trial included only men [17]. Of the five trials that included men, two trials determined the difference in bone loss between calcium in monotherapy (±750 mg per day) and placebo [13, 15], while the others compared calcium (500 mg to 1000 mg per day) plus vitamin D (500 IU to 1000 IU per day) with placebo [14, 16, 17]. Overall, treatment with calcium plus vitamin D or with calcium alone was associated with a significantly reduced rate of bone loss at the hip and spine. No subgroup analyses were performed to evaluate the influence of sex on treatment effect on BMD, but in line with the overall conclusion of this meta-analysis, reduced bone loss was seen in most of the studies that included men [13–16]. However, the one available men-only trial could not demonstrate a reduction of bone loss in spite of supplementation with 1000 mg calcium and 1000 IU vitamin D per day during three years [17]. This negative result may reflect the high baseline dietary calcium intake in the men included in the study (1160 mg per day) compared to lower doses of calcium in the other trials, such as a daily dose of 700 mg calcium [14] or less (550 mg [15]), supporting the concept that substitution therapy only makes sense when calcium balance is negative.

The decrease in PTH with calcium and vitamin D supplementation is also associated with *reduced bone turnover* [15, 16]. Meier et al. observed that calcium and vitamin D supplementation during winter prevented seasonal changes in PTH and bone loss in both men and women, although the effect on bone turnover was stronger in women. In this context, it should be noted that seasonal changes of bone turnover markers are only significant in women and almost absent in men [16].

3. Effect of Calcium and Vitamin D on Muscle Strength and the Risk of Falls

Supplementation with calcium and vitamin D not only reduces bone loss but also reduces the risk of falling by improving muscle strength, muscle function, and balance [7, 18, 19]. Vitamin D receptors (VDRs) are present in skeletal muscle cells and may account for these effects [20, 21]. In a recent meta-analysis, vitamin D therapy (200–1000 IU per day) lowered the risk of falling with 14% (RR 0.86, 95% CI 0.79–0.93) compared with calcium or placebo [22]. In this meta-analysis of ten trials, the number needed to treat with vitamin D to prevent one fall was 15, and—although a dose of 800 IU vitamin D or greater was most effective—a significantly lower fall risk was also seen with 400 IU [22].

In an earlier meta-analysis of eight double blind randomized controlled trials, on the other hand, a 19% fall reduction (RR 0.81, 95% CI 0.71–0.92) was only observed with a dose of at least 700 IU vitamin D per day and a 23% fall reduction (RR 0.77, 95% CI 0.65–0.90) with serum vitamin D concentrations of at least 60 nmol/L (24 ng/mL), while less than 700 IU vitamin D per day did not reduce fall risk (RR 1.10, 95% CI 0.89–1.35) [23]. Subgroup analyses were unable to document a significant reduction in falls in men but the subset was small ($N = 211$) and the analyses underpowered. In an earlier meta-analysis of the effect of vitamin D on falls, sex-specific subgroup analyses suggested that the fall-preventing effect of vitamin D is independent of sex: vitamin D reduced the odds ratio of falling in men by 21% (OR 0.79, 95% CI 0.57–1.1; $P = 0.17$) and in women by 19% (OR 0.81, 95% CI 0.65–1.00; $P = 0.05$). Again, the result of the sex-specific subgroup analysis in men was not significant due to the small number of men in the included trials, but numerically similar to the reduction seen in women [24].

Future research should determine the optimal dose of vitamin D to prevent falls, but available evidence suggests that men benefit to a similar extent as women.

4. Antifracture Efficacy of Calcium and Vitamin D

A negative calcium balance contributes to fracture risk by enhancing bone degradation through secondary hyperparathyroidism and by increasing the risk of falling through a negative effect on muscle strength, muscle function, and balance. Substitution with calcium and vitamin D is therefore considered the first-line strategy in the prevention of osteoporotic fractures. To establish the antifracture efficacy of substitution, several individual trials and meta-analyses have been performed with calcium or vitamin D alone or combined calcium plus vitamin D.

4.1. Effect of Calcium or Vitamin D Alone on Fracture Risk. Meta-analyses comparing the effect of calcium alone with placebo showed that calcium in monotherapy does not significantly reduce fracture risk [25, 26]. Of these meta-analyses, one included only women [25], while the other included both genders and conducted sex-specific analyses on the reduction of hip fracture risk [26]. Although data in men were limited, this meta-analysis did not reveal a differential effect of calcium in men or women: in both sexes calcium in monotherapy was unable to reduce (hip) fracture risk.

The same holds true for the effect of vitamin D alone versus placebo: a meta-analysis of four randomized controlled trials ($N = 9083$) showed that vitamin D alone was insufficient for fracture prevention, even when trials with vitamin D used in higher dose (700–800 IU per day) were evaluated separately [27]. A more recent meta-analysis came to the same conclusion: irrespective of sex, 400 to 800 IU vitamin D alone is no more effective in preventing fractures than placebo [28].

These results should not come as a surprise because the negative calcium balance in older and institutionalized adults is often the result of insufficiencies in both calcium and vitamin D. For example, community-dwelling French women aged 75–90 years had a mean daily calcium intake of just 569 mg and 39% had a serum vitamin D less than 30 nmol/L (12 ng/mL) [29]. In another trial, 66% of institutionalized women had a daily calcium intake less than 800 mg and a serum vitamin D less than 30 nmol/L [30]. As a result, in these elderly and institutionalized individuals, supplementation with calcium alone or vitamin D alone would fail to restore calcium balance and prevent fractures [8].

4.2. Effect of Combined Calcium and Vitamin D Supplementation on Fracture Risk. A meta-analysis of six randomized controlled trials ($N = 45509$) comparing the effect of combined calcium and vitamin D supplementation with placebo [27] showed that this combination therapy, contrary to calcium or vitamin D alone, significantly reduced fracture risk. The risk reduction was 12% for all nonvertebral fractures, 18% for hip fractures, and 21% for hip fractures when the one trial in this meta-analysis that did not use 700 to 800 IU but 400 IU vitamin D [31] was excluded. Similarly, a recent Cochrane review found that calcium plus vitamin D prevented hip fractures in frail elderly [32] and the DIPART group concluded that the combination of calcium and vitamin D significantly reduced the risk of any fractures and hip fractures and probably reduced the risk of clinical vertebral fractures [28]. In their meta-analysis, the DIPART group adjusted analyses for several factors including sex and found that the risk reduction of combined calcium and vitamin D was independent of sex [28].

Compared with vitamin D alone, combined calcium and vitamin D supplementation reduced the risk for hip fractures by 25% in an indirect comparison of meta-analyses [27]. This might be explained by the greater effect of combined calcium and vitamin D on secondary hyperparathyroidism and bone loss [3]. In this context, it should be noted that the only trial that directly compared the effect of combined calcium and vitamin D supplementation with vitamin D [33] could not document a beneficial effect of the combination therapy, but this study—like many individual trials in this field—suffered from a lack of statistical power, a lack of targeting of the supplements to individuals with documented insufficiencies and a lack of compliance [27], which we will discuss in more detail later.

Finally, one meta-analysis including 17 trials compared the effect of calcium plus vitamin D with calcium alone on fractures [12]. Six of these trials included men and women, 11 included only women, and there were no men-only trials. Calcium or calcium plus vitamin D was associated with a 12% risk reduction in fractures of all types (RR 0.88, 95% CI 0.83–0.95). This treatment effect was similar across women and men, as suggested indirectly (since there were no men-only trials) by the comparison of the women-only to the mixed-sex trials. In this meta-analysis, subgroup analyses showed that reduction in fracture risk was greater in individuals with low dietary calcium intake (<700 mg per day) and in those with low serum vitamin D concentration (<25 nmol/L or <10 ng/mL). Treatment effect was most effective with at least 1200 mg calcium and at least 800 IU of vitamin D. In this particular analysis, the combination of calcium and vitamin D (RR 0.87, 95% CI 0.77–0.97) and calcium in monotherapy (RR 0.90, 95% CI 0.80–1.00) were equally effective ($P = 0.63$) in the prevention of osteoporotic fractures, a finding that is difficult to reconcile with evidence from other meta-analyses that calcium alone does not significantly reduce fracture risk [25, 26].

Overall, there is increasing evidence from several meta-analyses for a beneficial effect of combined calcium and vitamin D supplementation on fracture risk with no firm reasons to assume that men would respond differently from women. However, results of individual trials assessing fracture reduction with combined calcium and vitamin D supplementation have been inconsistent. Some of these individuals trials, such as the Women's Health Initiative (WHI) trial [31] and the RECORD trial [33], failed to demonstrate a significant reduction in fracture risk, whereas other trials found a beneficial effect of calcium and vitamin D supplementation on fracture risk [14, 30, 34]. These inconsistencies can be attributed to several factors, including differences in targeting of the supplementation and differences in compliance [8].

4.3. Determinants of Antifracture Efficacy of Calcium and Vitamin D Supplementation. To be effective, supplementation with calcium and vitamin D has to be targeted to men with documented or particularly at risk of calcium and/or vitamin D insufficiencies, while general supplementation in the community is not necessary. Except for extremely minor subsets, baseline vitamin D status was not assessed in participants of the WHI trial and the RECORD trial [31, 33]. In fact, most of these study participants were mobile, healthy, and community-dwelling, who are less likely to have vitamin D insufficiency and therefore less likely to benefit from substitution. This may have contributed significantly to the negative results of these studies [8]. Men who will benefit most from substitution therapy are older (>75 years of age) or institutionalized persons in whom calcium and vitamin D insufficiency is highly prevalent, as well as men with documented osteoporosis or receiving glucocorticoids. The addition of calcium and vitamin D to antiresorptive or anabolic therapy in patients with established osteoporosis is essential, given that calcium and vitamin D insufficiency is common in patients with osteoporosis and osteoporosis medication is most effective in calcium and vitamin D

replete individuals. Glucocorticoids suppress intestinal and renal calcium absorption and increase urinary calcium excretion, resulting in a negative calcium balance [35]. Therefore, patients on glucocorticoids are particularly prone to fractures and supplementation therapy should be initiated as soon as glucocorticoids are prescribed [3, 8]. Most meta-analyses recommend a combination of 1000 to 1200 mg calcium with 700 to 800 IU vitamin D per day [12, 27] or at least a dose in excess of 400 IU (482–770 IU) vitamin D [36]. The aim is to increase serum levels of vitamin D to the 50–75 nmol/L (20–30 ng/mL) range [37, 38].

In addition to adequate targeting of supplementation, compliance with calcium and vitamin D is critical as well, to optimize clinical efficacy. Within 6 weeks after calcium and vitamin D have been discontinued, bone remodeling resumes to pretreatment levels [39]. In line with these findings, any positive effects of calcium and vitamin D on bone density will not persist after discontinuation of the supplements [40]. Therefore, to prevent osteoporotic fractures, compliance and persistence with calcium and vitamin D are essential. However, even in relatively healthy trial participants in studies like the WHI and the RECORD trial, a significant proportion of individuals did not comply with supplementation: in both trials, estimated compliance was only 40–60% [31, 33]. The negative outcome of these trials can, at least partly, be explained by this lack of compliance and emphasizes the importance of adherence to treatment [8].

5. Safety Concerns about Supplementation

While (conventionally dosed) vitamin D has not been associated with safety concerns, a recent meta-analysis has provided evidence to suggest that calcium supplements (without coadministered vitamin D) may potentially be associated with cardiovascular risks. This safety concern may potentially be even more of an issue in men than in women. However, in the meta-analysis, sex-specific subgroup analysis showed this elevated cardiovascular risk to be independent of sex [41]. Although these findings constitute a safety signal that has to be taken seriously, the data have to be interpreted with some caution. When dietary intake was taken into account, there was no significant correlation between calcium intake and risk of infarction. In addition, there was no effect of calcium on strokes or death, none of the trials had adjudicated cardiovascular outcomes in a standardized manner, and the statistical outcome was only borderline significant. Finally, there are numerous large studies of calcium plus vitamin D that have shown no increased risk of cardiovascular events [31, 42] and studies of calcium alone that did not provide evidence of an increased cardiovascular risk with daily supplemental intake of 1200 mg calcium in women [43] or 1000 mg calcium in men [44]. Nevertheless, reassessment of the role of calcium supplements to prevent osteoporotic fractures is warranted. Because, in general, men are more at risk of heart disease than women, future studies with supplements should include cardiovascular endpoints and carefully assess safety in men.

6. Conclusion

Age-associated bone loss due to secondary hyperparathyroidism and an increased risk of falling are key determinants of osteoporosis and osteoporotic fractures. Substitution with calcium and vitamin D reduces bone loss by reversing secondary hyperparathyroidism and prevents falls by improving muscle strength, muscle function, and balance. As a result, calcium and vitamin D supplementation is generally recommended in the prevention of osteoporotic fractures. In men, data on the effect of calcium and vitamin D supplements on BMD and the risk of falls and fractures are limited. However, from the mechanism of bone loss and from the available evidence, there is no reason to assume that men would respond differently to calcium and vitamin D supplementation compared to women. Combined supplementation with 1000 to 1200 mg calcium and 800 IU vitamin D per day should be particularly considered in older or institutionalized men, men receiving glucocorticoids, and in male osteoporosis patients on antiresorptive or anabolic medication.

Conflict of Interests

The authors have no conflict of interests.

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Clinical Study

Predictors of Fracture Risk and Bone Mineral Density in Men with Prostate Cancer on Androgen Deprivation Therapy

Katherine Neubecker,[1] Beverley Adams-Huet,[2] Irfan M. Farukhi,[3] Rosinda C. Delapena,[3] and Ugis Gruntmanis[4]

[1] *Department of Medicine, University of Texas, Southwestern Medical Center, Dallas, TX 75390, USA*
[2] *Departments of Clinical Sciences and Medicine, University of Texas, Southwestern Medical Center, Dallas, TX 75390, USA*
[3] *Department of Nuclear Medicine, Dallas Veterans Affairs Medical Center, Dallas, TX 75216, USA*
[4] *Department of Medicine, Dallas Veterans Affairs Medical Center and University of Texas, Southwestern Medical Center, Dallas, TX 75216, USA*

Correspondence should be addressed to Ugis Gruntmanis, ugis.gruntmanis@utsouthwestern.edu

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Decrease of bone mineral density (BMD) and fracture risk is increased in men with prostate cancer receiving androgen deprivation therapy (ADT). We looked at possible predictors of decreased BMD and increased fracture risk in men with prostate cancer; most of whom were on ADT. In a retrospective study, we analyzed serum, BMD, and clinical risk factors used in the Fracture Risk Assessment (FRAX) tool and others in 78 men with prostate cancer with reported height loss. The subjects were divided in two groups: 22 men with and 56 without vertebral fractures. 17 of the 22 men with vertebral fractures on spine X-rays did not know they had a vertebral fracture. Of those 17 men, 9 had not previously qualified for treatment based on preradiograph FRAX score calculated with BMD, and 6 based on FRAX calculated without BMD. Performing spine films increased the predictive ability of FRAX for vertebral fracture. Vertebral fracture was better predicted by FRAX for other osteoporotic fractures than FRAX for hip fractures. The inclusion of BMD in FRAX calculations did not affect the predictive ability of FRAX. The PSA level showed a positive correlation with lumbar spine BMD and accounted for about 9% of spine BMD.

1. Introduction

220,000 men are diagnosed with prostate cancer each year, and more than 40 percent receive androgen deprivation therapy (ADT) as initial treatment [1, 2]. ADT has been shown to decrease bone mineral density (BMD) as measured by dual-energy X-ray absorptiometry (DXA) as well as increase risk of fracture. After initiating ADT, BMD decline starts shortly after [3, 4]. A meta-analysis of almost 117,000 men demonstrated that patients on ADT had lower total BMD, 30% increased risk of osteoporosis, and 70% increased risk of fractures [5]. In a study of over 47,000 men with prostate cancer by the National Cancer Institute (NCI) and Medicare, those treated with ADT or bilateral orchiectomy within six months of diagnosis had a 5-year fracture risk of 19.4% versus 12.6% in controls matched for patient and tumor characteristics [6]. A systematic review of five studies on prostate cancer patients in the US and abroad, which included the NCI-Medicare study, found a 23% increase in fracture risk for men treated with ADT [7].

Fracture Risk Assessment (FRAX) is an algorithm developed by the World Health Organization to determine fracture risk of men and women [8]. The risk factors in the algorithm include age, current height, prior fracture, parental history of fracture, BMD of the femoral neck, and secondary osteoporosis risk factors such as hypogonadism. Yet, inclusion of secondary causes such as hypogonadism does not change FRAX-calculated risk of fracture when femoral neck BMD is included. The National Osteoporosis Foundation's recommended treatment thresholds based on FRAX score are 3% for hip ("Hip FRAX") and 20% for major osteoporotic fractures ("Osteoporotic FRAX") [9].

Prior studies of men with prostate cancer on ADT by Saylor et al. and Adler et al. [10, 11] demonstrated that the inclusion of BMD values in the FRAX calculation led to lower FRAX scores, and thus fewer people qualified for treatment. These studies also found that the majority of men who met treatment thresholds did so because of the hip FRAX rather than osteoporotic FRAX. Men younger than 70 [10] and of African-American ethnicity [11] were less likely to meet treatment thresholds.

Prior studies have addressed factors that affect treatment qualifications in men on ADT based on FRAX scores. The utility FRAX for predicting incidence of fracture in men with prostate cancer has yet to be determined. Many of vertebral fractures are clinically unrecognized due to lack of complaints, yet, they are strong predictors of future fractures at all sites. Two and half inches or greater height loss in men is associated with increased risk of vertebral compression fractures [12]. The utility of height loss in predicting vertebral fracture in men with prostate cancer has not been established.

2. Materials and Methods

2.1. Subjects. This was a retrospective study of 78 male patients with prostate cancer (median age 77: 55 Caucasian, 19 African-American, 3 Hispanic, and 1 Spanish) at the Dallas Veterans Affair Medical center (DVAMC) approved by the Institutional Review Board of DVAMC, Dallas, Tex, USA.

Subjects had been referred to the Osteoporosis Clinic by the Department of Urology for management of bone health, either while receiving ADT ($N = 63$) or before initiating ADT ($N = 15$). All men at DVAMC receiving ADT or about to initiate ADT are referred by Urology Department to Osteoporosis clinic regardless of whether they have history of osteoporosis. At initial visit at Osteoporosis clinic, spine X-rays are obtained on all patients whose measured height in clinic is at least one inch less than their self-reported young adult height. Inclusion criteria were height loss and having spine X-rays, which were performed near or at the time of the initial Osteoporosis Clinic visit. Height loss was determined by the height measured at the initial Osteoporosis Clinic visit compared to self-reported young adult height. Patients with pathologic fractures thought to be secondary to bone metastases were excluded. The primary outcome of the study was whether height loss predicted fracture and BMD in men with prostate cancer on ADT. Secondary endpoints: in how many men would FRAX score change with finding compression fracture on spine X-ray, and if the duration of ADT and level of prostate-specific antigen (PSA) could predict BMD and fractures at any site.

2.2. Statistical Analysis. Comparisons of subjects with and without vertebral fractures were made with the Wilcoxon Rank Sum test. Univariate associations between continuous variables were assessed with Spearman correlation coefficients. Logistic regression analysis was used to assess predictors of fractures and to estimate unadjusted and adjusted odds ratios for fracture risk. Multiple linear regression analysis was conducted to further evaluate the relationship between spine BMD as the dependent variable and PSA while controlling for age, weight, and use of vitamin D supplements. Data with positively skewed distributions were log-transformed prior to parametric analysis. Statistical analysis was performed with SAS version 9.2 (SAS Institute, Cary, NC, USA).

To assess the utility of FRAX scores in predicting of vertebral fractures, receiver operating characteristic (ROC) curves which plot 1-specificity versus the sensitivity of the diagnostic value of the model were constructed, and the area under these curves (AUC) and 95% confidence intervals were calculated. An ROC AUC of 0.5 indicates chance performance, and an ROC AUC of 1.0 is the ideal, maximum AUC [13].

2.3. Radiography/Laboratory Measurements. BMD of the femoral neck, total hip, and lumbar spine were measured by DXA (Hologic QDR 4500A, Waltham, Mass, USA) in the DVAMC Nuclear Medicine Department. The least significant change in g/cm^2 for regions of interest in grams per square centimeter was as follows lumbar spine 0.031, total hip 0.037, and femoral neck 0.029. Serum total testosterone was measured by electrochemiluminescence immunoassay, alkaline phosphatase by the p-nitrophenyl phosphate method, 25-hydroxy vitamin D by liquid chromatography/tandem mass spectrometry, PTH by chemiluminescent immunoassay, and calcium by indirect potentiometry. Intra-assay coefficients of variation for all of the assays were less than three percent, and interassay coefficients of variation were less than 8 percent.

3. Results and Discussion

At baseline, men with prostate cancer, with or without vertebral fracture, were not statistically different, except for total testosterone levels and FRAX scores (Table 1).

Testosterone levels between groups were statistically different ($P = 0.01$), yet, levels in both groups were profoundly low. The FRAX scores for hip fractures and other osteoporotic fractures were also statistically different (Table 2), as expected, when men with vertebral fractures and no vertebral fractures were compared.

None of the primary outcomes were positive. The mean difference of height loss between men with and without vertebral fractures group was only −0.19 cm (95% CI: −0.83 to 0.45). Degree of height loss did not predict either bone density or fractures at any site; risk of vertebral fracture based on height loss produced an odds ratio of 1.1 (95% CI: 0.76 to 1.65). This can likely be explained by our inclusion criteria. In our clinic we order spine X-rays in men who have lost more than one inch of height, and, therefore, we could not compare men with and without height loss.

Yet, 17 of the 22 subjects with vertebral fractures had no known history of vertebral fracture until they were discovered incidentally on spine films ordered due to a loss of height. Had these fractures not been detected by spine X-rays, nine (about 41%) of the 22 men with fractures would have not qualified for treatment based on FRAX calculated with BMD or six (about 27%) based on FRAX calculated without BMD.

Table 1: Baseline characteristics stratified by presence of vertebral fracture.

	No vertebral fracture				Vertebral fracture				
	$n = 56$				$n = 22$				
Variable	Median	Mean	SD	Range	Median	Mean	SD	Range	P value*
Duration of ADT (months)	16.5	39.6	44.7	0.0–10.5	10.5	30.3	43.5	0.0–156.0	0.21
Age (years)	77.0	76.5	8.2	58.0–77.5	77.5	78.0	6.7	63.0–88.0	0.53
Weight (kg)	83.5	84.8	17.1	54.5–86.7	86.7	87.1	18.0	54.8–119.8	0.46
Height (cm)	172.7	171.7	6.1	152.4–171.4	171.4	172.5	7.1	157.5–182.9	0.83
Height loss (cm)	5.1	5.8	3.0	0.8–5.8	5.8	6.4	3.6	1.3–14.0	0.58
BMI (kg/m^2)	28.4	28.5	4.9	18.2–28.6	28.6	28.7	5.5	18.3–40.4	0.76
25-(OH) Vitamin D (ng/mL)	31.1	30.3	14.6	7.0–26.1	26.1	27.1	10.9	8.0–57.8	0.42
Serum calcium (mg/dL)	9.7	9.5	1.3	0.7–9.6	9.6	9.5	0.5	7.9–10.6	0.52
Parathyroid hormone (pg/mL)	44.0	49.5	28.1	16.7–37.3	37.3	45.9	28.8	15.8–135.0	0.49
Alkaline phosphatase (U/L)	80.0	92.4	80.6	49.0–73.0	73.0	74.2	17.0	47.0–112.0	0.16
Prostate-specific antigen (PSA) (ng/mL)	0.3	6.2	15.1	0.1–0.3	0.3	2.8	6.7	0.1–30.7	0.49
Testosterone (ng/mL)	0.1	0.6	1.3	0.1–0.2	0.2	1.4	1.7	0.1–5.9	0.01

* P values are from the Wilcoxon rank sum test.

Table 2: BMD and FRAX stratified by presence of vertebral fracture.

	No vertebral fracture				Vertebral fracture				
	$n = 56$				$n = 22$				
Variable	Median	Mean	SD	Range	Median	Mean	SD	Range	P-value*
BMD, femoral neck	0.7	0.7	0.1	0.5–1.0	0.7	0.7	0.1	0.5–0.9	0.34
T score, femoral neck	−1.8	−1.9	0.7	−3.3–−0.7	−2.2	−2.0	0.8	−3.4–−0.6	0.33
BMD, total hip	0.9	0.9	0.1	0.6–1.2	0.8	0.8	0.2	0.5–1.2	0.22
T score, total hip	−1.2	−1.3	0.9	−3.0–0.3	−1.4	−1.5	1.1	−3.7–0.0	0.51
BMD, lumbar spine	1.0	1.0	0.2	0.8–1.6	0.9	0.9	0.2	0.5–1.3	0.11
T score, lumbar spine	−1.1	−0.9	1.6	−4.0–4.6	−1.8	−1.6	1.8	−5.2–2.1	0.10
FRAX (Osteo) without BMD (%)	9.3	10.8	6.2	1.8–33.0	15.0	16.0	6.8	6.8–31.0	0.001
FRAX (Hip) without BMD (%)	4.0	5.5	4.8	0.1–24.0	6.8	8.1	6.0	1.7–25.0	0.03
FRAX (Osteo) with BMD (%)	7.5	9.5	6.9	1.9–33.0	11.0	14.0	7.8	5.4–41.0	0.002
FRAX (Hip) with BMD (%)	3.0	4.5	5.1	0.1–28.0	4.5	6.6	7.1	1.1–36.0	0.03

* P values are from the Wilcoxon rank sum test.
BMD measurements are in g/cm^2.

Table 3: Summary of multiple regression analysis for variables predicting spine bone mineral density.

	Variable	PSA*	Age	Weight	Vitamin D supplement	Race	R^2
Model 1	*B (SE)*	0.02 (0.01)	—	—	—	—	0.06
	P	0.03	—	—	—	—	
Model 2	*B (SE)*	0.02 (0.01)	0.005 (0.003)	—	—	—	0.08
	P	0.05	0.08	—	—	—	
Model 3	*B (SE)*	0.02 (0.01)	0.005 (0.003)	0.0007 (0.0005)	—	—	0.09
	P	0.05	0.04	0.17	—	—	
Model 4	*B (SE)*	0.02 (0.01)	0.004 (0.002)	—	−0.13 (0.04)	—	0.22
	P	0.045	0.12	—	0.001	—	
Model 5	*B (SE)*	0.02 (0.01)	0.004 (0.002)	—	−0.12 (0.04)	0.04 (0.04)	0.23
	P	0.05	0.13	—	0.001	0.33	
Model 6	*B (SE)*	0.02 (0.01)	0.004 (0.003)	0.0004 (0.0005)	−0.12 (0.04)	—	0.23
	P	0.04	0.04	0.40	0.003	—	

B = regression coefficient, SE = standard error.
*$\log_e$ transformed PSA.
Race is modeled as African-American versus non-African-American.

Table 4: FRAX score in the prediction of vertebral fracture in men with prostate cancer.

		All Subjects ($N = 78$; vertebral Fx = 22/78 = 24.4%)		Non-African-American ($N = 59$; vertebral Fx = 18/59 = 31%)		African-American ($N = 19$; vertebral Fx = 4/19 = 21%)	
		ROC AUC	95% CI	ROC AUC	95% CI	ROC AUC	95% CI
FRAX (Hip) without BMD	before X-ray	0.55	0.39–0.70	0.59	0.42–0.77	0.42	0.15–0.70
	after X-ray	0.66	0.53–0.78	0.63	0.47–0.78	0.72	0.42–1.00
FRAX (Osteo) without BMD	before X-ray	0.59	0.43–0.75	0.66	0.48–0.84	0.50	0.22–0.78
	after X-ray	0.74	0.62–0.85	0.73	0.59–0.87	0.94	0.82–1.00
FRAX (Hip) with BMD	before X-ray	0.53	0.37–0.69	0.60	0.42–0.79	0.64	0.18–1.00
	after X-ray	0.66	0.52–0.80	0.63	0.47–0.79	0.74	0.39–1.00
FRAX (Osteo) with BMD	before X-ray	0.54	0.38–0.69	0.62	0.43–0.82	0.69	0.30–1.00
	after X-ray	0.72	0.60–0.84	0.70	0.55–0.84	0.95	0.84–1.00

ROC: receiver operating characteristic; AUC: area under the curve; CI: confidence interval.
BMD: Bone mineral density; Osteo: osteoporotic.

Discovery of incidental vertebral fractures on spine films increased the predictive ability for vertebral fracture of all types of FRAX scores: hip FRAX with or without BMD and osteoporotic FRAX with or without BMD (Table 4). All post-X-ray hip FRAX and osteoporotic FRAX were statistically significant (AUC significantly different from null of 0.50). Osteoporotic FRAX demonstrated greater predictive ability for vertebral fracture than hip FRAX, 0.74 versus 0.66 AUC, respectively, without BMD ($P = 0.01$) and 0.72 versus 0.66 AUC, respectively, with BMD ($P = 0.04$). The ROC models for predictive ability of FRAX were not significantly changed by including a factor accounting for whether the patient was already on ADT. Thus, data for all 78 patients (63 already on ADT, 15 not on ADT) is displayed.

The inclusion of BMD in FRAX calculations did not affect the predictive ability of FRAX. FRAX scores calculated with and without BMD were significantly related (Table 2). Spearman correlation coefficients were rho = 0.80 ($P < 0.001$) for osteoporotic FRAX (Figure 1(a)) and rho = 0.71 for hip FRAX ($P < 0.001$) (Figure 1(b)).

There were too few fractures in the African-American group to give adequate precision so conclusions cannot be reliably drawn from the African-American-specific data. ROC AUC appears high in AAs because of high specificity due to low incidence of vertebral fracture (Table 4). Race was not correlated with spine BMD in multivariate linear regression models controlling for age, PSA level, and use of vitamin D supplementation. The PSA level showed a positive correlation with lumbar spine BMD (Figure 2) in both the univariate linear regression model ($P = 0.03$) and the multivariate linear regression models controlling for age, weight, and use of vitamin D supplements ($P = 0.04$), as displayed in Table 3, and accounted for about 9% of spine BMD. Weight correlated with BMD in the spine (rho = 0.25, $P = 0.03$), femoral neck (rho = 0.43, $P < 0.0001$), and total hip (rho = 0.43, $P = 0.0001$) (data not shown). Age had a positive correlation with BMD at lumbar spine ($P < 0.02$) but not at femoral neck or total hip.

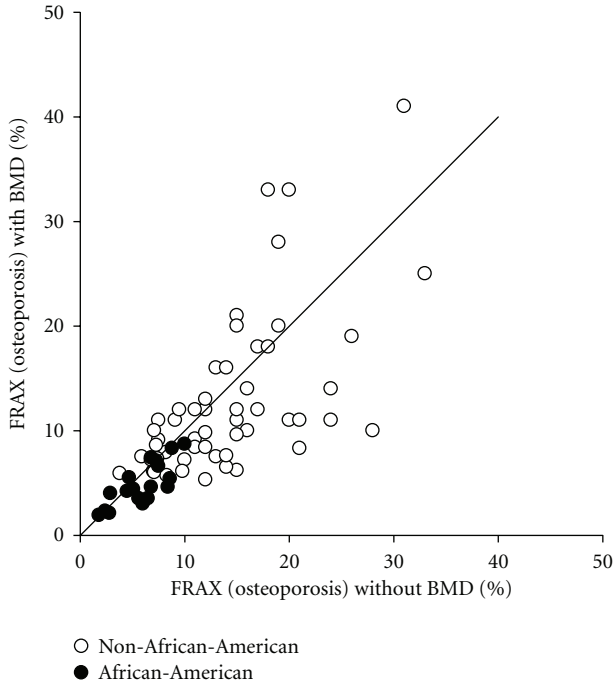
From other secondary outcomes, neither duration of ADT, level of 25-hydroxyvitamin D, parathyroid hormone, total alkaline phosphatase, nor total testosterone levels predicted BMD or fractures at any site in our study population. 25-hydroxyvitamin D level was found to be below 20 ng/mL in 31% and 20–30 ng/mL in 20% of men. In the other 49% of men, 25-hydroxyvitamin D was above 30 ng/mL.

4. Conclusions

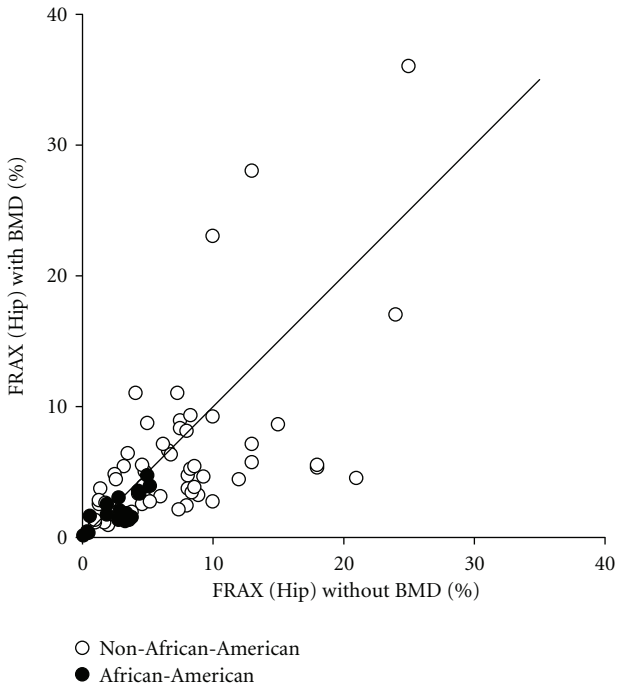
The utility of height loss and FRAX scores as predictors of fractures in men with prostate cancer had not been previously studied. In our study population of older men with prostate cancer, most of whom were on ADT, height loss was similar in men with and without compression fractures and other osteoporosis-related fractures. In conclusion, our study cannot suggest a threshold of height loss for which spine X-rays or perhaps vertebral fracture assessment with DXA should be used.

As with prior studies by Adler et al. [11] and Saylor et al. [10], fewer men met treatment criteria when BMD was included in FRAX score calculation. Also, fewer men met treatment threshold for major osteoporotic fracture risk than for hip fracture risk. Compared to Adler et al., our study population had similar age (both with median age 77 years) and weight (85.5 kg versus 88.1 kg in Adler et al.) and excluded men with fractures secondary to bone metastases (versus 30% with bone metastases in Saylor et al.). Our study population was 24% African-American population, compared to 58% in Adler et al. and 5% in Saylor et al.

The utility of FRAX in predicting fracture incidence in men with prostate cancer had not been studied previously. An advantage of the current study is that incidence of vertebral fracture was known due to spine radiographs,



(a)



(b)

Figure 1

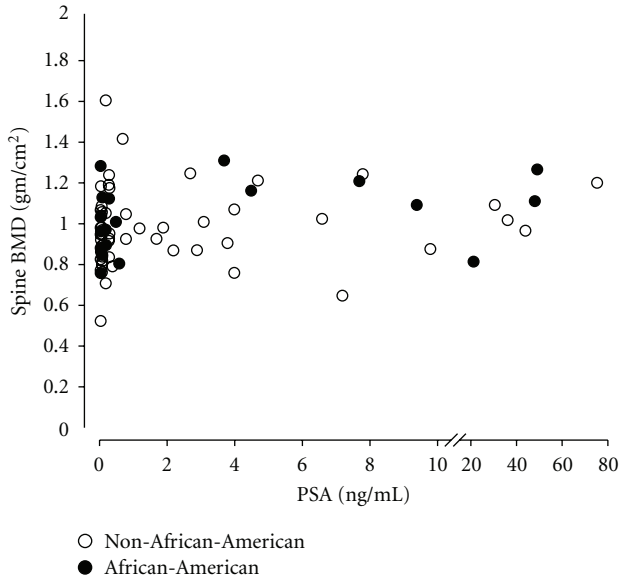


Figure 2

so the predictive ability of FRAX scores with or without BMD, for hip versus osteoporotic, and across ethnic groups could be analyzed. Our study demonstrated that discovery of incidental vertebral fractures on routine spine films increased the predictive ability for vertebral fracture of all types of FRAX scores. Another novel finding was that vertebral fracture in men with prostate cancer was better predicted by osteoporotic FRAX than hip FRAX. The FRAX score (without knowledge of spine fractures) calculated with BMD underestimated therapeutic threshold in nine men with previously unknown spine fractures in our study. As a result we feel that most men with prostate cancer on ADT, and with any height loss, should have spine X-rays taken to estimate their future fracture risk accurately.

It should be mentioned that hypogonadism is one of the "secondary causes" in the FRAX calculator but does not change the FRAX score if and when BMD of the femoral neck is used in calculation. We believe that patients with prostate cancer on ADT may be different from "typical" patients with hypogonadism. It is well known that patients with prostate cancer on ADT have mostly undetectable levels of testosterone and as the result have low estradiol levels. Both testosterone and estradiol are imperative in preserving bone density, quality, and in predicting the risk of future fractures. Moreover, the effects of ADT on BMD and fracture risk persist for years after ADT is completed [6].

The positive correlation of age and spine BMD may be an artifact of falsely elevated BMD in lumbar spine due to vertebral body degeneration since the group without vertebral fractures had lower spine T scores (higher spine BMD) than the group with vertebral fractures. The significance of the positive correlation of PSA level and lumbar spine BMD is unclear. PSA accounted for 9% of the variability in lumbar BMD. The mechanism by which PSA affects bone metabolism is not clearly defined, but it seems to regulate the biologic activity of parathyroid-hormone-related protein (PTHrP). PSA specifically cleaves PTHrP, which subsequently eliminates the ability of PTHrP to stimulate cAMP production [14]. Moreover, PSA appears to promote osteoblast differentiation via transcription factor Cbfa1 [15]. PSA prevents bone resorption by inducing osteoprotegerin activity and inhibiting the receptor activator of nuclear factor-kB ligand (RANKL) expression on osteoblasts

[16]. Osteoblasts produce cytokines including IL-6 that appear to lead to androgen-independent induction of PSA genes [17]. Increases in PSA may lead to greater production of endothelin-1, a protein that stimulates osteoblasts and inhibits osteoclasts in the presence of androgen-insensitive prostate cancer cells [18]. All of the above factors may explain why spine BMD may be higher in men with elevated PSA.

Of note, Vitamin D levels were fairly replete in both groups of men, those with and without fractures. This is likely because the Urology Clinic at the Dallas VAMC often empirically starts Calcium plus Vitamin D 500 mg/250 U replacement twice a day before we see men in the Osteoporosis clinic.

Limitations of our study include the small sample size, retrospective data collection, and possible selection bias from inclusion criteria. Based on prior studies discussed above, one would expect BMD and fracture risk to be associated with testosterone levels and the duration of ADT.

However, we did not find it in our study. The cross-sectional nature of the study is not optimal for evaluating predictors of fracture since fractures are already present at the time the variables are assessed. Our inclusion criteria likely generated some selection bias due to the inclusion criteria of height loss. A future study could include subjects without height loss to compare groups with or without height loss. The range of height loss was relatively small with over 50% of subjects with height loss of 2 inches or less.

Including BMD of distal 1/3 radius has been shown to increase sensitivity of DXA scans in detecting osteoporosis [3] but is not included in routine DXA scans at DVAMC.

Going forward it would be important to study prospectively if the FRAX calculator is a precise predictor of fractures in men with prostate cancer on ADT. A future study with a prospective approach could better determine predictors of fracture by performing spine films at two points in time to assess for new fractures. Further study in a larger sample is needed to address the predictive ability of FRAX across ethnic groups.

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Research Article

The Evidence for Efficacy of Osteoporosis Treatment in Men with Primary Osteoporosis: A Systematic Review and Meta-Analysis of Antiresorptive and Anabolic Treatment in Men

Peter Schwarz,[1,2] Niklas Rye Jorgensen,[1,3] Leif Mosekilde,[4] and Peter Vestergaard[4]

[1] *Research Center of Aging and Osteoporosis, Department of Medicine, Glostrup Hospital, 2600 Glostrup, Denmark*
[2] *Faculty of Health Science, Copenhagen University, Copenhagen, Denmark*
[3] *Department of Clinical Biochemistry, Glostrup Hospital, 2600 Glostrup, Denmark*
[4] *Department of Endocrinology and Internal Medicine, MEA, THG, Aarhus University Hospital, Denmark*

Correspondence should be addressed to Peter Schwarz, petsch02@glo.regionh.dk

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Purpose. Fragility fractures in men constitute a major worldwide public health problem with a life-time risk of 13%. It cannot be directly inferred that antiosteoporotic drugs effective in women have the same effect in men. Our aim was to appraise the existing evidence for efficacy of osteoporosis treatment in men. *Methods*. This study was a systematic review of the published literature on the clinical efficacy of medical osteoporosis therapy in the reduction of fracture risk in men (age > 50 years). Studies included were randomised, placebo-controlled trials of men. *Results*. Five BMD studies of antiresorptive treatment were included. All studies showed an increase in BMD, but there was only a nonsignificant trend in the reduction of clinical fractures. Three BMD studies of anabolic treatment with teriparatide were also included. These showed a significant mean increase in spine BMD and for vertebral fractures a non-significant trend towards a reduction was seen. *Conclusion*. The evidence of medical osteoporosis treatment in men is scant and inconclusive due to the lack of prospective RCT studies with fracture prevention as primary end point. So far, all evidence is based on BMD increases in small RCT studies showing BMD increases comparable to those reported in postmenopausal women.

1. Introduction

Fragility fractures in men constitute a major worldwide public health problem [1] although the incidence and gender ratio varies between countries [2]. The life-time risk of any fracture in the hip spine or distal forearm in men aged >50 years has been estimated to be 13% compared with 40% in females [3] The fractures occur 5–10 years later in men than in women [4], but the increasing longevity in men is likely to increase the public health burden of the fractures [2]. Follow-up studies, including the osteoporotic fractures in men (MrOS) cohort, have established that 1 SD deviation in areal bone mineral density (aBMD) equally predict fracture risk for spine and hip in men and in women [2, 5]. Therefore, the lower incidence of fractures in males compared with females in all probability reflects that at any, age fewer males than women have compromised biomechanical competence because of smaller bones, lower volumetric BMD (vBMD), thinner cortices, thinner trabeculae, microfractures with disruption of trabecular structure, or higher bone turnover [2]. Moreover, the etiology differs between males and females. Hypogonadism is a risk factor for osteoporosis in both sexes, but the prevalence and progression of sexhormone deficiency differs. Testosterone deficiency is a risk factor for male osteoporosis, whereas estradiol deficiency is a triggering factor in both sexes. Furthermore, the influence of environmental factors like alcohol, smoking, and risk of falling may differ between sexes. Because of the described gender differences in risk factors, pathophysiology, and bone structure, it cannot be directly inferred that anabolic or antiresorptive drugs that prevent BMD loss and osteoporotic fractures in females [6–26] have the same effect in males. However, only few small randomized controlled trials (RCTs) on the treatment efficacy of antiosteoporotic drugs have been

performed in men. It is, therefore, important to appraise the existing evidence of the impact of osteoporosis treatment in elderly and old men.

2. Objectives

This is a systematic review and meta-analysis of the published literature on RCT studies of clinical efficacy of antiresorptive and anabolic therapy in the reduction of fracture risk in elderly and old men. The following end points were used: RCT studies on vertebral fracture reduction, nonvertebral fracture reduction, and hip fracture reduction for men with primary osteoporosis.

3. Materials and Methods

3.1. Eligibility Criteria for Study Inclusion. Studies should be randomised placebo-controlled trials of at least 12 months duration (anti-resorptive treatment) or of at least 6 months duration (anabolic therapy). The antiresorptive medications included as exposure variables in the search were strontium ranelate, bisphosphonates, denosumab, and miacalcic. Strontium ranelate was here categorized as antiresorptive although there is growing evidence that it also may exert anabolic properties. The anabolic treatments included the truncated PTH(1–34) analog teriparatide and the full length PTH(1–84) preotact. Of the bisphosphonates, we included all commercially available medications for oral or intravenous treatment. That is, etidronate, ibandronate, risedronate, alendronate and zoledronate.

Only RCT studies where the primary end-points were vertebral, nonvertebral or hip fracture risk reductions, and/or BMD changes were included.

3.2. Search Methods. An electronic search of PubMed (1951 and onwards), Embase (1974 and onwards), Science Citation Index (1945 and onwards), and the Cochrane Central Register of Controlled Trials was performed. The search date was December 19, 2010.

Abstracts of all possibly relevant articles were reviewed for potential eligibility (assessed by P.Schwarz and P. Vestergaard). Discrepancies were solved through discussion. Those deemed eligible and those that did not had adequate information to confirm their inclusion underwent a full text review. The retrieval was based on published papers only. We examined reference lists of retrieved studies for further relevant publications. If several publications were reported based on the same trial data we chose the report with the longest followup. Pooled analyses and subgroup analyses were not included due to their weak statistical value. No contacts were made with lead authors or pharmaceutical companies.

The keywords producing the majority of results, that is "osteoporosis," "treatment," and "men" were chosen. This search gave 10.314 trials (Table 1). Subsequently, a search was made separately for each of the respective drugs. This method did not produce any articles with fracture reduction as end point in men, so the same search was repeated with BMD as a substitute endpoint for fracture risk reduction. Concerning antiresorptive treatment, this method produced 13 potential papers of which 7 reported open-labelled and/or not randomised studies, leaving 6 papers to be included.

Table 1: Identifying key words.

Osteoporosis AND Treatment AND Men	10.314
AND alendronate	495
AND risedronate	215
AND ibandronate	63
AND didronate	300
AND zoledronic acid	127
AND strontium ranelate	50
AND denosumab	28
AND miacalcic	81
AND teriparatide	175
AND PTH(1–84)	17
AND preotact	1

As to anabolic treatment, 5 potential papers were identified. However, one study only reported data with a mixture of men and women without the possibility of extracting data solely on men, leaving 4 papers for evaluation.

All data were summarised in a formula including number of patients, age, gender, BMI, BMD, duration, and main outcomes measured (Table 2).

3.3. Statistical Analyses. The meta-analysis was performed as a random effects model using the inverse of the standard deviation of the individual BMD and fracture risk parameters from each study as weights for the estimates as proposed by Böhning [33]. Tests for heterogeneity and publication bias were performed. $P < 0.05$ was considered statistically significant.

4. Results

4.1. Antiresorptive Drugs. Five antiresorptive drugs, alendronate (2 studies), risedronate (1 study), ibandronate (1 study), zoledronate (2 studies), and nasal miacalcic (1 study), have been investigated in male populations with osteoporosis (Table 2) [27–32, 34]. The study zoledronate study of Orwoll et al. [32] was excluded, as it was not placebo-controlled, and the zoledronate study of Lyles et al. [34] was a mixture of men and women, and data on men could not be extracted. The remaining five studies had BMD as their primary end-point (Table 3).

4.1.1. Changes in BMD. Orwoll et al. [27] reported a significant increase in bone mineral density of 7.1 ± 0.3% at the lumbar spine, 2.5 ± 0.4% at the femoral neck, and 2.0 ± 0.2% for the total body ($P < 0.001$ for all comparisons with baseline). The increase in BMD in the alendronate group was greater than that in the placebo group at all

Table 2: Baseline characteristics of included studies.

Study	Intervention (plus calcium and/or vitamin D)		Number of patients	Age (±SD)	BMI	BMD (lumbar; total hip; femoral neck) in g/cm^2	BMD (lumbar; total hip; femoral neck) T score	Duration (months)	Outcomes measured	Lost to follow-up (intervention versus control)
Alendronate										
Orwoll et al. [27]	Placebo or 10 mg/d of alendronate (500 mg/d and 400–450 IU/d)	Placebo Alendronate	95 146	63 (12) 63 (13)	25 (3) 25 (3)		2.2; 2.1; 2.3 2.0; 2.1; 2.2	24	BMD Vertebral fractures. nonvertebral fractures (secondary endpoints)	17% versus 14%
Gonnelli et al. [28]	Placebo (calcium) or 10 mg/d of alendronate (1000 mg/d)	Placebo Alendronate	38 39	56.6 (10.4) 57.2 (9.9)	24.3 (2.9) 24.9 (2.4)	0.737 (0.103); 0.770 (0.099); 0.632 (0.100) 0.725 (0.110); 0.762 (0.101); 0.622 (0.090)		36	BMD. QUS	6 versus 7
Risedronate										
Boonen et al. [29]	Placebo or 35 mg of risedronate (1000 mg/d and 450–500 IU/d)	Placebo Risedronate	93 191	62 (11) 60 (11)	25 (4) 25 (4)	0.824 (0.96); 0.763 (0.106); NA 0.809 (0.99); 0.768 (0.111); NA	−3.1 (0.9); −2.0 (0.7); NA −3.3 (0.9); −2.0 (0.7); NA	24	Lumbar spine BMD BMD at other sites. new vertebral fractures. clinical fractures (secondary endpoints)	16 versus 18
Ibandronate										
Orwoll et al. [30]	Placebo or 150 mg Ibandronate/month	Placebo Ibandronat	47 85	65.0 (10.6) 63.9 (11.2)	24.8 (3.4) 25.9 (4.1)		−2.1 (0.68) −1.8 (0.70) −2.3 (0.55) −2.1 (0.61) −1.7 (0.68) −2.2 (0.50)	12	BMD	1 3
Miacalcic										
Trovas et al. [31]	Placebo or 200 IU/d of miacalcic (1000 mg/d)	Placebo Miacalcic	13 15	51.6 (10.5) 53.3 (13.7)	25.7 (3.1) 26.1 (2.4)	0.847 (0.190); NA; 0.753 (0.162) 0.866 (0.124); NA; 0.737 (0.116)		12	BMD Vertebral fractures	0

Table 2: Continued.

Study	Intervention (plus calcium and/or vitamin D)		Number of patients	Age (±SD)	BMI	BMD (lumbar; total hip; femoral neck) in g/cm^2	BMD (lumbar; total hip; femoral neck) T score	Duration (months)	Outcomes measured	Lost to follow-up (intervention versus control)
Teriparatide										
Trovas et al. [31]	Placebo or 20 or 40 μg Teriparatide/d versus Placebo	Placebo	147	59 (13)	25 (4)	0.85 (0.14)	−2.4 (1.2) −1.9 (0.8) −2.7 (0.8)	11	BMD	24
		Teriparatide 20 μg/d	151	59 (13)	25 (4)	0.89 (0.15)	−2.0 (1.3) −1.8 (0.8) −2.6 (0.8)			11
		Teriparatide 40 μg/d	139	58 (13)	25 (4)	0.87 (0.14)	−2.2 (1.2) −1.9 (0.9) −2.7 (0.8)			20
Orwoll et al. [32]	Placebo versus Teriparatide 32 μg	Placebo	10	54.5 (2.6)	25.9 (1.5)	0.746 (0.03) 0.781 (0.02) 0.650 (0.03) 0.731 (0.03)	−3.3 (0.3) −1.7 (0.2) −2.0 (0.2) −3.5 (0.2)	18	BMD	0
		Teriparatide	13	49.5 (2.9)	24.3 (1.0)	0.774 (0.03) 0.644 (0.02)	−1.7 (0.2) −1.9 (0.2)			0

Fx, number of patients with one or more fractures.

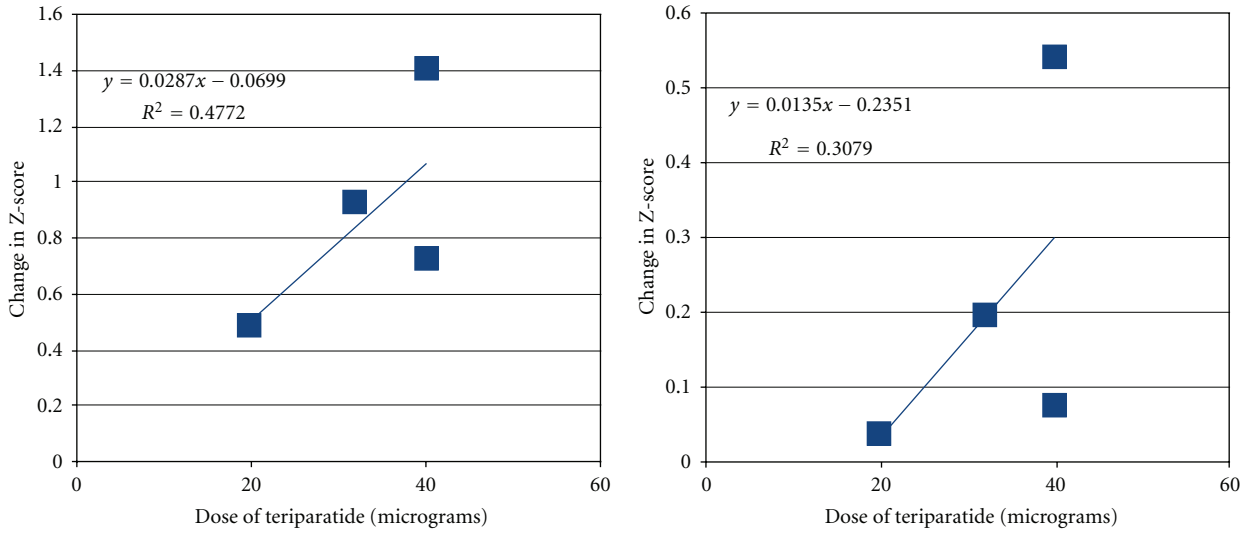

Figure 1: Increase in spine (a) and femur (b) BMD in the Teriparatide studies by daily dose.

measurement sites (Table 3, $P < 0.001$). In a 3-year RCT, Gonnelli et al. [28] reported an increase in lumbar spine BMD of 4.2% at year 1, 6.3% at year 2, and 8.8% at year 3. BMD at the femoral neck and total hip increased 2.1% and 1.6%, respectively, at year 1, 3.2% and 2.9% at year 2, and 4.2% and 3.9% at year 3. In a 2-year RCT Boonen et al. [29] reported that treatment with risedronate resulted in a significant 4.5% (95% CI: 3.5–5.6%; $P < 0.001$) increase in lumbar spine BMD compared with placebo. In a 1-year RCT study, Orwoll et al. [30] reported an increase in lumbar spine BMD of 3.5% ($P < 0.001$). BMD at the total hip increased by 1.8% ($P < 0.001$) and femoral neck 1.2% ($P < 0.012$) [30]. Trovas et al. [31] performed a 12-month RCT with nasal miacalcic. The men who were treated with calcitonin had a mean increase in BMD of 7.1 ± 1.7% at the lumbar spine. The increase in lumbar BMD in the calcitonin group was significantly greater than that in the placebo group ($P < 0.05$).

4.1.2. Changes in Risk of Fractures. Three studies reported fractures as secondary endpoints. All studies had included few patients with a low mean age, and they all had a relatively short duration of 12–36 months (Table 2).

The studies of Orwoll et al. (alendronate) [27] and Boonen et al. (risedronate) [29] both reported incidences of vertebral fractures (Table 3). Orwoll et al. found a significant reduction ($P = 0.02$) in vertebral fractures determined by quantitative methods and no effect on non-vertebral fractures. Boonen et al. found 2 new vertebral fractures after 2 years each in the risedronate group. There was a nonsignificant trend towards a reduction in all fractures (placebo 6 patients (6.5%); risedronate 9 patients (4.7%)).

4.2. Anabolic Drugs. Five studies were available on anabolic treatment with teriparatide in men [9, 35–38]. However, the study of Finkelstein et al. was not placebo controlled and therefore excluded [36], and the study of Kaufman et al. was based on the same men as reported in the study of Orwoll et al. [9] and therefore excluded as well. In addition, a newly published study report on both Japanese men and women was available [37]. However, data on men cannot be extracted from this publication and the included numbers of men were low (5 in the placebo group and 9 in the treatment group), this study was excluded as well [37]. No studies in men were available for preotact or any other anabolic medication. In all three included papers, the primary end point was BMD (Table 2).

4.2.1. Changes in BMD. Compared with placebo Orwoll et al. [9] found a significant increase in lumbar spine ($P < 0.001$) and femoral neck ($P = 0.029$) BMD in the group receiving 20 μg/day of teriparatide (Table 3). In the 40 μg/day group, the increase in BMD compared with placebo was significant at the lumbar spine ($P < 0.001$), the total hip ($P < 0.001$), and the femoral neck ($P < 0.001$). The increase was higher in the 40 μg/day than in the 20 μg/day group at the lumbar spine ($P < 0.001$), the total hip ($P = 0.009$) and the femoral neck ($P = 0.023$). In the PTH-treated group, Kurland et al. [35] found a gain in lumbar spine BMD at 18 months of 13.5 ± 3.0% ($P < 0.001$ compared with placebo), whereas the increase in the femoral neck was 2.9 ± 1.5% ($P < 0.05$) (Table 3).

The mean increase in BMD in all studies ($n = 3$) and subgroups ($n = 4$ in 3 studies) combined was 0.58 ± 0.02, $P < 0.01$ for spine BMD Z-score and 0.05 ± 0.01, $P < 0.01$ for femoral neck Z-score (Figure 1).

4.2.2. Changes in Risk of Fractures. Orwoll et al. [9] reported non-vertebral fractures as side effects in 6 patients (3 among 147 placebo treated, 2 among 151 treated with 20 micrograms of teriparatide, and 1 among 139 treated with 40 micrograms of teriparatide). Kurland et al. [35] reported

Table 3: Anti-fracture effects.

Study	Vertebral fractures				Nonvertebral fractures				HIP fractures				BMD (lumbar; total hip; femoral neck)
	Treatment	*N* (%)	RR (95% CI)	NNT	Treatment	*N* (%)/*n*	RR (95% CI)	NNT	Treatment	*N* (%)	RR (95% CI)	NNT	Mean difference % (95% CI) or Percent change (SD)
Alendronate													
Orwoll et al. [27]	Placebo	NA (7.1)			Placebo	5 (5.3)							5.3 (4.3–6.3); 2.6 (1.5–3.7); 2.6 (1.5–3.7)*
	Alendronate	NA (0.8)			Alendronate	6 (4.1)							
Gonnelli et al. [28]	Placebo	NA											10; 4.2; 5.4*
	Alendronate	NA											
Risedronate													
Boonen et al. [29]	Placebo	0											4.5 (3.5–5.6)*; results for total hip and femoral neck in figures
	Risedronate	2											
Ibandronate													
Orwoll et al. [30]	Placebo	2			Placebo	0							NA
	Ibandronate	1			Ibandronate	2							
Miacakcic													
Trovas et al. [31]	Placebo	2											2.47; −0.68; NA
	Miacalcic	1											7.13; 0.41; NA
Teriparatide													
Trovas et al. [31]	Placebo	0			Placebo	3							0.52 (3.90) 0.54 (2.70) 0.31 (4.10)
	Teriparatide 20 μg	0			Teriparatide 20 μg	2							5.87 (4.50)*** 1.17 (2.94) 1.53 (3.95)**
	Teriparatide 40 μg	0			Teriparatide 40 μg	1							9.03 (6.46)*** 2.33 (4.41)*** 2.93 (6.34)***
Orwoll et al. [32]	Teriparatide	1 (17)				0							13.5 (3.0)*** NA
	Placebo	2 (17)				0							2.9 (1.5)**

*Difference between groups (95% CI); ($P < 0.001$ in favour of active treatment).
**Difference between groups (SD); $P < 0.05$ in favour of active treatment.
***Difference between groups (SD); $P < 0.001$ in favour of active treatment.
NA: not available.

Table 4: Adverse events.

Study		General (N (%))				Specific (N (%))		
		Any AE	Any serious AE	Death	Withdrawals due to AE			
Alendronate							Placebo	Alendronate
Orwollet al. [27]	Placebo		22 (23)		10 (11)	Any upper GI AE	21 (22)	37 (25)
						Dyspepsia	1 (1)	9 (6)
	Alendronate		27 (18)		4 (3)	Abdominal pain	4 (4)	12 (8)
						Esophagitis	1 (1)	1 (1)
						Reflux	5 (5)	7 (5)
Gonnelli et al. [28]	Placebo Alendronate			NA			NA	
Risedronate							Placebo	Risedronate
Boonen et al. [29]	Placebo ($n = 93$)	68 (73)	15 (16)	3 (3)	9 (9.7)	Any upper GI AE	17 (18)	16 (8)
						Constipation	5 (5)	16 (8)
						Back pain	2 (2)	13 (7)
	Risedronate ($n = 191$)	134 (70)	29 (15)	2 (1)	7 (3.7)	Arthralgia	8 (9)	11 (6)
						Influenza	5 (5)	11 (6)
						Nasopharyngitis	5 (5)	11 (6)
						Headache	0 (0)	10 (5)
Ibandronate							Placebo	Ibandronate
Orwoll et al. [30]	Placebo	4 (9)	(7)	2		Any	4 (9)	16 (19)
	Ibandronate	16 (19)	(9)	1		Abdominal pain	(0)	(3)
Miacakcic								
Owroll et al. [30]	Placebo Miacalcic	Very well tolerated and no significant side effects		0	0		NA	
Teriparatide							Placebo	Teriparatide	Teriparatide
Trovas et al. [31]	Placebo	39 patients withdrew due to side-effects	3	0	7				
	Teriparatide 20 μg		3	2	14	Nausea	3.4	5.3	18.7
	Teriparatide 40 μg		0	0	18	Headache	NA	N A	10. 8
Orwoll et al. [32]	Teriparatide		0	0	0	Hypercalcemia	0	2	
	Placebo		0	0	0	Redness at inj. site	0	5	

data on the incidence of vertebral fractures (1 new fracture) among 6 PTH treated and 2 patients among 12 placebo-treated had new vertebral fractures (one and three new fractures, resp.). In average, the studies of Orwoll et al. and Kurland et al. yielded a reduction in risk of vertebral fractures of RR = 0.60, 95% CI: 0.29–1.22, P for heterogeneity 0.71.

4.2.3. Adverse Events. Focusing on adverse events in the anti-resorptive treatment group, the study of Orwoll et al. [27] showed that the incidence of overall GI adverse events was higher in the placebo group compared with the risedronate group (18% versus 8%). Also, withdrawal from the study because of adverse events was more frequent in patients taking placebo (9.7% versus 3.7%) [29]. For alendronate [27, 28], the results resemble the results in women. In the miacalcic study [31], no specific data are reported (Table 4). Among the anabolic studies, Orwoll et al. [9] reported 2 deaths in the teriparatide 20 μg group. None of these was considered related to study drug or procedures. Three cancers occurred in the placebo group, three in the teriparatide 20 μg group and none in Teriparatide 40 μg group. There were no cases of osteosarcomas. In the two studies, it was concluded that the medication was well tolerated [9, 35].

5. Discussion

There is evidence that both antiresorptive and anabolic treatment compared with placebo increase BMD in osteoporotic males. However, fracture data in men are scant at all sites (vertebral, non-vertebral, and hip fractures), and there are no RCTs that evaluate antiresorptive or anabolic osteoporosis treatment in men with fractures as primary end point. Furthermore, studies with fractures as secondary end points are inconclusive. As a consequence, there is at present no well-established documented treatment for idiopathic osteoporosis in men. However, the fact that one in five men aged $\geq$50 years will suffer an osteoporotic fracture during their lifetime underscore the necessity to appraise the antifracture efficacy of various treatment modalities in men.

The strength of this study is the systematic inclusion of all studies available in men receiving anti-resorptive treatment as well as anabolic osteoporosis treatments.

The limitations are the very low number of studies included in the meta-regression makes the evidence based on the method limited. Not only are the number of studies limited and the follow-up time short, the power of the studies to reveal significant effects on fracture risk is also low because of the limited number of patients included. Due to this we are not able to definitely conclude if one medication is in favor of others among men with primary osteoporosis.

In conclusion, the evidence of medical osteoporosis treatment in men is scant at all sites and inconclusive due to the lack of prospective large RCT studies with fracture prevention as primary endpoint. All evidence so far is based on BMD findings in small RCT studies showing increases comparable to those observed in studies in postmenopausal women.

Conflict of Interests

The authors have no conflict of interests.

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Review Article

Aromatase Activity and Bone Loss in Men

Daniela Merlotti, Luigi Gennari, Konstantinos Stolakis, and Ranuccio Nuti

Department of Internal Medicine, Endocrine-Metabolic Sciences and Biochemistry, Viale Bracci 1, 53100 Siena, Italy

Correspondence should be addressed to Daniela Merlotti, merlotti4@unisi.it

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Aromatase is a specific component of the cytochrome P450 enzyme system responsible for the transformation of androgen precursors into estrogens. This enzyme is encoded by the *CYP19A1* gene located at chromosome 15q21.2, that is, expressed in ovary and testis, but also in many extraglandular sites such as the placenta, brain, adipose tissue, and bone. The activity of aromatase regulates the concentrations of estrogens with endocrine, paracrine, and autocrine effects on target issues including bone. Importantly, extraglandular aromatization of circulating androgen precursors is the major source of estrogen in men. Clinical and experimental evidences clearly indicate that aromatase activity and estrogen production are necessary for longitudinal bone growth, the attainment of peak bone mass, pubertal growth spurt, epiphyseal closure, and normal bone remodeling in young individuals. Moreover, with aging, individual differences in aromatase activity may significantly affect bone loss and fracture risk in men.

1. Background

Sex steroid hormones are important for the acquisition and maintenance of bone mass in both sexes [1, 2]. Alterations in their levels can be relevant in the pathogenesis of osteoporosis and fractures because their deficiency may lead to suboptimal acquisition of peak bone mass in young individuals or bone loss in adulthood. While estrogens effects in bone in females have been well established (as estrogen deficiency after menopause leads to an imbalance between bone resorption by osteoclasts and bone formation by osteoblasts), the role for estrogen in male skeletal health has only recently become appreciated [3–6]. In fact, even though alterations in circulating androgen have been associated with low bone mass and impaired bone strength [7], their primacy has been increasingly questioned as direct and indirect evidence has emerged suggesting that estrogens also play a major role in male skeletal health [3–7]. These new observations underscore the normal biosynthetic pathway by which estrogens are made in men, via the activity of aromatase (a cytochrome P450 product of the *CYP19A1* gene) on circulating androgenic precursors (Figure 1).

Of interest, several clinical and experimental studies on estrogen and/or aromatase deficiency also reinforced the hypothesis of a threshold estradiol level for skeletal sufficiency in the male [1–4]. In fact, either in cross-sectional or longitudinal analysis in aging men bone mineral density (BMD) and rates of bone loss at different skeletal sites were unrelated to serum estradiol concentrations when the latter were above the median value, while they were clearly associated with estradiol at concentrations below the median [8–10]. This hypothesis gained further support from a study in which raloxifene (a selective estrogen receptor modulator) was given to men of varying estradiol levels [11]. Subjects with serum total estradiol levels below 96 pmol/liter responded to raloxifene with a decrease in bone resorption markers while above this estrogen value, raloxifene caused an increase in bone resorption markers [11]. Overall, these observations clearly indicate that men need a sufficient concentration of estrogen, defined as a threshold value, for normal skeletal remodeling. In all these studies, the required concentration appeared to be remarkably similar, ranging from 90 to 110 pmol/liter in case of total estradiol or from 40 to 55 pmol/liter in case of bioavailable estradiol [5]. This apparent threshold value is higher than typical estradiol concentrations for postmenopausal women who are not receiving exogenous estrogens. On the other hand, premenopausal women and young men are typically above

this apparent threshold level, while up to 50% of middle-aged men fall below this estradiol threshold and, thus, are at higher risk of bone loss as shown in several cross-sectional and longitudinal studies [1–4]. Importantly, estradiol levels above a given threshold are also important to prevent fractures, as recently demonstrated in a prospective study of men from the MrOS cohort [12].

This review summarizes the evidence that aromatase activity plays an important role in the skeleton in men, either in young individuals or in the elderly, by its actions to convert androgens to estrogens in bone and other peripheral tissues.

2. Aromatase and Estrogen Production in Men

Aromatase is a specific component of the cytochrome P450 enzyme system that converts the delta 4-3-one A ring of C19 androgen precursors into the corresponding phenolic A ring characteristic of C18 estrogenic compounds

While in fertile women the ovary represents the major source of circulating estrogen, which functions as a circulating hormone to act on distal target tissues, in men the testes account at most for 15% of circulating estrogens, while the remaining 85% is due to peripheral aromatization of circulating androgen precursors in different tissues [13, 14]. These include the adipose tissue, the brain, the skin, the endothelium, and the bone. It has been demonstrated that testicular androgen precursors contribute more to the total amount of circulating estradiol than adrenal androgens [15]. In fact, dexamethasone-induced suppression of adrenal steroid synthesis moderately decreases estradiol concentrations [16], whereas orchidectomy (ORX) leads to a more dramatic suppression of plasma estradiol [17, 18]. Clearly, these extragonadal sites of estrogen biosynthesis lack the ability to synthesize C19 precursors from cholesterol, hence, their estrogen-producing activity totally depends on the availability of these circulating C19 androgenic steroids [19]. Moreover, the estrogen synthesized within these extragonadal compartments may be also locally active in a paracrine or intracrine fashion [13, 20].

In human bone, aromatase is expressed in osteoblast or osteoblast-like cells from fetal and normal tissue [21–23], in articular cartilage chondrocytes, in adipocytes adjacent to bone trabeculae, and in osteocytes, but not in osteoclasts [23]. Importantly, a recent study demonstrated that aromatase gene can be expressed in bone tissue in consistent amounts, at levels similar to those found in adipose tissue [24]. In particular, osteoblasts are the major source of aromatase within the bone microenvironment [24].

Aromatase is encoded by the *CYP19A1* gene located at chromosome 15q21.2 [25] (Figure 2). Despite the presence of a common *CYP19A1* gene, in a tissue-specific fashion, a number of untranslated initial exons are found in aromatase transcripts due to differential splicing by at least 10 different tissue specific promoters [26–28]. Only the 30-kb 3′ region of the gene (containing exons 2 to 10) encodes aromatase while a larger 93 Kb 5′ flanking region serves as the regulatory unit of the gene [27, 28]. Thus all the multiple exons 1 are not translated, so that the different splicing patterns lead to transcripts that are all translated as the same protein. Importantly, this complex structure of the promoter region of the gene defines the tissue-specific regulation of aromatase activity and estrogen biosynthesis. Thus, the ovary, testes, adipose tissue, brain, and bone each utilize their own promoters and associated enhancers and suppressors leading to different amounts of mRNA transcripts, mRNA stability and/or protein translation [26, 29, 30].

In the skeleton, the majority of aromatase transcripts contain exon 1.4 and exon 1.6 [31–33]. Some minor transcripts by promoter I.3, PII and If have been also described [31]. Interestingly, experiments in osteoblast cell lines demonstrated that cortisol may induce aromatase gene expression transiently and that 1,25 dihydroxyvitamin D can maintain its expression, dependent on vitamin D receptor density [34–36]. Moreover, a more recent study demonstrated that Runx2 (a key regulator of osteoblast differentiation) directly increases aromatase gene expression in human osteoblast cells by increasing promoter I.4 and I.6 activity [37]. In keeping with this in vitro evidence, a marked decrease in skeletal aromatase expression has been described in Runx2-deficient mice [37].

3. Aromatase Deficiency and the Male Skeleton

During the past 2 decades, several clinical and experimental observations underscored the importance of local and peripheral aromatization of androgens into estrogen for skeletal homeostasis in males.

3.1. Aromatase Deficiency Syndrome. The discovery of human cases of aromatase deficiency preceded the construction of aromatase knockout animals and, in fact, provided the first insight into the role of estrogens and aromatase in male skeletal physiology.

Human aromatase deficiency is a very rare autosomal recessive syndrome characterized by congenital estrogen deprivation caused by loss-of-function mutations in the *CYP19A1* gene [38]. In both genders, the overall severity of the phenotype may be variable according to residual enzyme activity [38, 39]. While affected females generally have ambiguous genitalia at birth and fail to develop secondary sexual characteristics, the affected male individuals have normal male sexual differentiation and pubertal maturation, and their clinical phenotype mostly develops after puberty [5, 40]. So far, there are at least 9 known cases of inactivating mutations in the *CYP19A1* gene and aromatase deficiency in men [5, 41–49]. All these patients generally showed markedly low or undetectable estrogen levels while androgens were normal or even elevated. Interestingly, skeletal maturation and bone metabolism were severely impaired in all these subjects, with a similar phenotype to a previously described male case of loss of function mutation at the estrogen receptor alpha (*ESR1*) gene [50]. Common skeletal characteristics include tall stature and continued longitudinal growth due to unfused epiphyses, delayed bone age, lack of pubertal growth spurt, eunuchoid skeletal proportions, genu valgum, elevated bone resorption markers, and low bone mass. Moreover, other extraskeletal characteristics, such as lipid

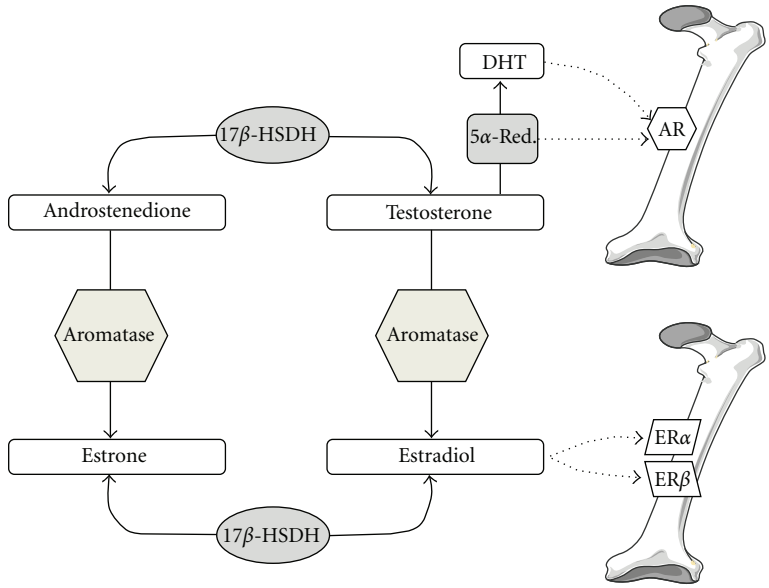

Figure 1: Proposed models of sex steroid hormones action on bone.

abnormalities, increased body weight, hyperinsulinemia, and various degrees of glucose impairment (including diabetes and acanthosis nigricans in 1 and 2 cases, resp.) have been also reported in these men.

As in part expected, in 2 of these cases, treatment with intramuscular testosterone did not give skeletal benefit to these patients, since aromatase deficiency does not lead to low testosterone levels [5, 47]. Conversely, estrogen treatment was associated with marked improvements in the skeletal phenotype. In fact, epiphyses closed quickly, longitudinal growth ceased, and BMD increased consistently at all assessed skeletal sites [41, 42, 47, 51, 52]. As counterpart, estrogen replacement in the male case of loss of function mutation at the *ESR1* gene did not improve bone outcomes [5, 50]. Of interest, consistent with the threshold estradiol hypothesis for skeletal health, a clear dose-dependent effect of estrogen replacement therapy on bone mass was evidenced, since a very low dose of estradiol (below 25 mcg twice weekly) was not sufficient for maintaining a normal BMD in one of these aromatase-deficient men [52].

In a more recent study, the skeletal phenotype of a 16-year-old boy with aromatase deficiency was investigated by both DXA and peripheral quantitative computed tomography (pQCT) of the radius [46]. The use of the later technique allowed to assess additional characteristics of bone strength, such as cross-sectional area (CSA), cortical thickness, trabecular volumetric BMD, and cortical volumetric BMD. Consistent with the previous observations, estrogen replacement in this boy was associated with the normalization of sex hormone concentrations, reduced bone turnover rate, and increases in lumbar spine (+23%) and femoral neck (+14%) areal BMD. However, the gain in volumetric BMD (either estimated by the calculation of the bone mineral apparent density from DXA or assessed directly by pQCT) was limited at the lumbar spine and even absent at the femoral neck and the radius. Conversely longitudinal bone growth, cross-sectional area, and cortical thickness (as measured by pQCT) increased significantly by 8.5%, 46%, and 12%, respectively. Thus, it was clear that the observed increase in areal BMD was mainly driven by an increase in bone size, rather than bone density, particularly at peripheral sites. Interestingly, these changes are similar to those associated with normal pubertal growth and support the notion that in growing bones, except for the spine true density does not increase [53, 54]. On the contrary, periosteal diameter continues to expand and cortical thickness increases during normal male puberty because of reduced endocortical expansion and accelerated periosteal apposition [55]. These effects lead to increased bone size, and have classically been attributed to androgens, accounting for the greater areal density that is typical of the male skeleton. In fact, when females enter puberty, periosteal apposition is inhibited, an action classically believed to be an estrogen effect. However, according to the effects of estrogen administration on cross-sectional area and cortical thickness in this young boy with aromatase deficiency [46], some actions on bone size, previously attributed to androgens, must at least in part be an estrogen effect. Thus, it is likely that a biphasic, dose-dependent effect of estrogen at the periosteum could exist. At low levels (as observed in males and in early pubertal females) estrogen may stimulate periosteal apposition and increase bone size, whereas at higher concentrations (as observed in late pubertal and adult females) estrogen may inhibit cross-sectional bone growth.

Recently, the concomitant presence of mild hypogonadism in a man with aromatase deficiency has offered a useful model to study the effects of testosterone and estradiol replacement, separately or in combination [56]. As expected, in this man, estradiol treatment alone increased BMD with a greater gain than the one obtained with testosterone alone. However, the combination of testosterone (6 mg/day) and estrogen (25 mcg twice weekly) replacement led to a further

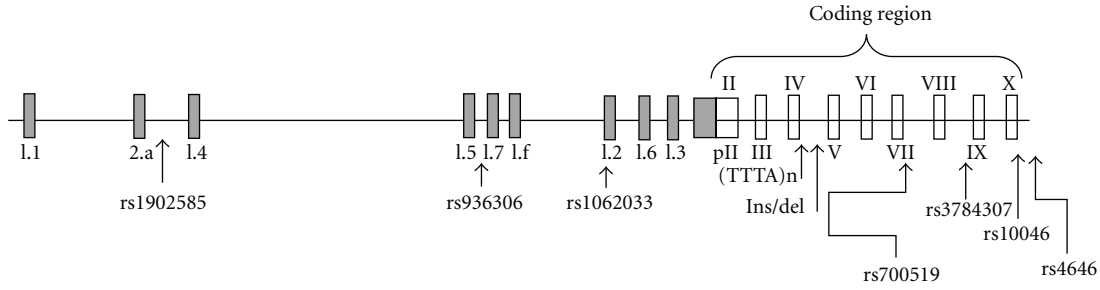

Figure 2: Aromatase gene (*CYP19A1*) with its promoters and untranslated first exons. Major polymorphic variants of the *CYP19A1* gene are indicated.

increase in cortical thickness at the radius and the tibia as measured by pQCT, further supporting the concomitant importance of both sex steroids for periosteal apposition. In this case, an increase in volumetric BMD at the tibia and the radius as well as an increase of areal BMD of the lumbar spine and the femoral neck was also described after 2 years of combined therapy.

The skeletal consequences of aromatase deficiency have been illustrated further by studies of the aromatase knockout mouse (ArKO) models [57–59]. Overall, these experimental observations generally provided the confirmation of the human gene disorders suggesting that estrogen may be more protective in the growing skeleton than androgens in man, but left open the issues of what, if any, are the roles of estrogen in regulating bone remodeling and bone loss in adult males.

3.2. Inhibition of Aromatase Activity: Clinical Studies in Adult Men. A first indirect indication about the importance of aromatase and the relative roles of estrogen versus testosterone in the adult male skeleton came from cross-sectional and longitudinal observations in middle-aged and elderly men. In fact, in most of these studies BMD and bone loss were more directly related to declining estrogen levels than declining androgen levels, particularly, when circulating bioavailable fractions of these steroids were considered [8–10, 60–66]. Moreover, in one of these studies, the ratio between estradiol and testosterone presumed to be an indirect index of aromatase activity, increased significantly with age, and was higher in normal than in osteoporotic subjects or in men with fragility fractures [10]. Consistent with these findings, other clinical evidences underlined the importance of estrogen on the adult male skeleton. Thus, in a preliminary observation, Taxel and Raisz described significant reductions in bone resorption markers in 9 elderly men treated short term with either 0.5 mg or 2.0 mg daily of micronized 17β-estradiol [67]. In a different study, Anderson et al. treated 21 eugonadal osteoporotic men with intramuscular testosterone and found a significant increase in lumbar spine BMD, which was correlated with changes in serum estradiol, but not circulating testosterone [68].

In order to definitively dissect out estrogen versus testosterone effects on the adult male skeleton, more dynamic short-term interventional observations have been performed, where aromatase activity was suppressed through the use of aromatase inhibitors. In a first study, Falahati-Nini et al. [69] examined the differential effects of estrogen versus testosterone replacement in a group of 59 elderly men (mean age 68 yr) following the induction of hypogonadism (by the use of a GnRH agonist, Lupron) and aromatase inhibition (with letrozole 2.5 mg daily). Of interest, the increase in bone resorption markers (deoxypyridinoline and N-telopeptide of type I collagen) associated with the use of the GnRH agonist was almost completely prevented by estradiol but not by testosterone therapy alone, indicating that the increase in bone resorption was due primarily to estrogen loss, not to testosterone loss. In the case of bone formation markers, there was evidence for stimulatory effects of both estrogen and testosterone on serum osteocalcin but not the amino-terminal propeptide of type I procollagen that only increased with estrogen replacement. Since osteocalcin is produced primarily by mature osteoblastic cells and osteocytes [70], these findings are consistent with an important role for both estrogen and testosterone in maintaining the functional integrity of these cells. Type I collagen, by contrast, is produced by cells of the entire osteoblastic lineage [71], and these data would suggest that it is primarily estrogen that regulates osteoblast differentiation. These results were in part replaced in a similar study performed in younger individuals, [72] following the induction of the hypogonadal state by the GnRH agonist, goserelin acetate. In this model, evidence was provided for independent effects of testosterone and estrogen on bone resorption. A subsequent study by Taxel et al. [73] with a longer observation period (9 weeks) also gave similar results, further indicating that treatment of elderly men with an aromatase inhibitor (in this specific-case anastrozole 2 mg/day) produces significant increases in bone resorption and decreases in bone formation. These effects on bone turnover may be less evident with lower doses (i.e., anastrozole 1 mg/day or less), or in case of borderline hypogonadism (testosterone levels less than 350 ng/dl), at least over a short-term period of treatment [74]. To this regard, a more recent study assessed the 12 months effects of a low dose of anastrozole (1.0 mg/day) on BMD and bone turnover markers in 69 men (aged 60 yr or older) with low or low-normal testosterone levels [75, 76]. Interestingly, with this longer observation period, despite an increase in testosterone levels at all time points, a statistically significant decrease in posterior-anterior spine BMD versus placebo was described, likely due to the parallel mild decrease in serum

estradiol. Qualitatively similar changes, although nonsignificant, were observed at the other bone sites. Conversely, bone turnover markers were not significantly affected by aromatase inhibition, in this study.

4. Variation in Aromatase Activity and Bone Metabolism in Men

All the above clinical and experimental models (in which aromatase activity is absent or inhibited) have clearly shown that aromatase and estrogen production are important factors in male skeletal health. These models, however, were mainly based on conditions of complete estrogen deficiency, while they did not address the potential skeletal implications of interindividual variation in aromatase efficiency [5, 14]. Such differences in aromatase activity, and, hence, estrogen levels, might become particularly important in elderly males in whom age-related declines in testicular and adrenal androgen precursors are common.

4.1. Inherited Variation in Aromatase Activity. Several polymorphic regions have been detected in the human *CYP19A1* gene that could be responsible for qualitative and/or quantitative differences in gene expression and aromatase activity (Figure 2). The most widely studied include a silent polymorphism (G/A at Val80) in exon 3, a tetranucleotide (TTTA)n tandem repeat polymorphism in intron 4, a Arg264Cys (C/T) substitution in exon 7, and a single nucleotide change (C/T) in exon 10. These polymorphisms were investigated in postmenopausal women or elderly men. In particular, several studies evidenced an association between the number of TTTA repeats and estrogen levels, breast cancer, or osteoporotic risk [77–83]. More recently other polymorphic variants within the promoter region of the *CYP19A1* gene have been widely investigated and associated with BMD in both genders as well as with susceptibility to breast or uterine cancer in females [84–86]. Most of these evidences were confirmed in subsequent meta-analyses and large scale studies [87–91]. Thus, it is possible that the presence of particular *CYP19A1* variants could be responsible of higher aromatase activity and increased estrogen production. If so, these polymorphisms should be protective for bone loss in elderly men or postmenopausal women while potentially also increasing the risk of estrogen-related cancer.

Of interest, the skeletal consequences of genetic variation in *CYP19A1* appear to be modulated by fat mass, particularly in men. In fact, BMD differences associated with *CYP19A1* genotypes were greater in male subjects with a normal BMI, while the association progressively decreased when overweight and obese men were analyzed [81] (Figure 3). This point suggests that fat mass may be a mitigating factor in the expression of *CYP19A1* genotypes on bone. It is possible that with more adipose tissue, the associated increase in adipose aromatase activity dominates any effect of the polymorphisms on intrinsic aromatase activity.

Given the importance of estrogen in bone growth, it is likely that genetic variation in *CYP19A1* may be also relevant for young individuals, before the attainment of peak bone

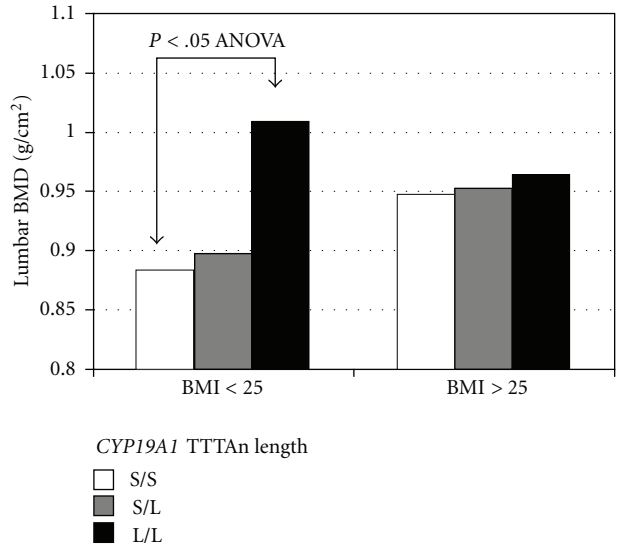

Figure 3: Lumbar BMD values according to *CYP19A1* (TTTA)n repeat genotype in subjects divided by BMI in normal (BMI ≤ 25) and overweight or obese groups (BMI > 25), respectively. Subjects were grouped according to short (S, TTTA ≤ 9) and long (L, TTTA > 9) repeats number (adapted from [81]).

mass. Despite an initial study in 140 middle-aged Finnish men evidenced an association between the number of TTTA repeat sequences and height or BMI but not with BMD [92], a larger analysis in younger individuals confirmed that *CYP19A1* polymorphisms significantly affect the attainment of peak bone mass [93]. Thus, in a well-characterized cohort of 1068 men at the age of peak bone mass (18.9 ± 0.6 years), both the TTTA repeat variation and a silent G/A polymorphism at Val80 of the *CYP19A1* gene were predictors of areal BMD, lumbar spine, total body, and cortical bone size (cortical cross-sectional area and thickness) at 2 peripheral sites (radius and tibia).

To date, the molecular mechanisms through which the different *CYP19A1* variants affect aromatase activity, and bone metabolism remain in great part unknown. In a preliminary analysis, higher in vitro aromatase efficiency and greater estrogen production were observed in fibroblasts from men with a high TTTA repeat genotype than in men bearing a low TTTA repeat genotype [81]. Even though, it is unlikely that this polymorphism might have a direct effect on aromatase expression and activity (due to its intronic location within the *CYP19A1* gene), a different study described a strong degree of linkage disequilibrium between the TTTA repeat variation and the C-T substitution in exon 10, just 19 base-pairs downstream of the termination site of translation [78]. Interestingly, in that study, the T allele was associated with a higher number of TTTA repeats and showed a high-activity phenotype, with increased aromatase activity, increased aromatase mRNA levels, and with a switch in promoter usage from adipose tissue promoter to the more active ovary promoter. More recent studies also evidenced a functional role of other polymorphisms located within the complex promoter region of the *CYP19A1* gene. In particular, a C/G polymorphisms in promoter

I.2 (rs1062033) were associated with differences in gene transcription by interacting with the transcription factor CEBPβ [94], which affects aromatase expression in different tissues [95, 96]. In fact, the expression of the reporter luciferase gene in osteoblast cell lines was significantly higher in constructs bearing the G allele (which was also associated with higher BMD in population studies) than in those with the C allele, and this difference was particularly evident after cotransfection with CEBPβ. Consistent with these results, differential allelic expression was also evidenced in bone tissue samples, again indicating the G allele as the more overexpressed. Although these studies, in the aggregate, provide data to argue for the importance of *CYP19A1* polymorphisms as determinants of estrogen production and bone strength, larger and more definitive studies are needed before any firm conclusion can be drawn.

Interestingly, epigenetic effects on aromatase transcription and activity have been also evidenced in recent studies. In fact, CpG methylation has been described as an important epigenetic mechanism for the regulation of *CYP19A1* expression. To this regard, an in vitro study performed in skin fibroblast indicated that differences in methylation patterns may be responsible for the observed interindividual variation in promoter-driven expression of aromatase [97]. In particular, in this study, unmethylated constructs showed consistently higher promoter activity than methylated constructs.

4.2. Acquired Variation in Aromatase Activity. Besides the above genetic and epigenetic considerations, several additional mechanisms have been proposed in which aromatase activity could be modulated under certain circumstances in different tissues. It is known, for example, that aromatase is a specific marker of the undifferentiated adipose mesenchymal cell phenotype while it is less expressed in mature adipocytes. Thus, factors that stimulate adipocyte differentiation, such as PPARγ agonists could also lead to the downregulation of aromatase gene and a reduction in aromatase activity [98–100]. Of course, if there are more adipocytes, there could be more aromatase activity even with reduced production of estrogen per fat cell. Moreover, the skeletal effects of PPARγ agonists are more complex and mainly involve direct negative effects on bone cells [101]. Other agents acting on PPARγ and PPARα pathways, such as the phthalates (ubiquitous environmental toxins found in plasticizers) have been associated with a decrease in aromatase mRNA and aromatase activity in ovarian granulosa cells [102]. Moreover, the activation of PPARα pathway by fenofibrate in female mice significantly reduced aromatase mRNA and activity and was associated with a decrease in femoral BMD and uterine size [103]. Despite these different experimental evidences, the clinical relevance of these environmental agents on global aromatase activity and estrogen production in man remains unknown.

Several other contaminants may affect aromatase activity and estrogen production. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines that are widely used across the world. Their residues are frequent

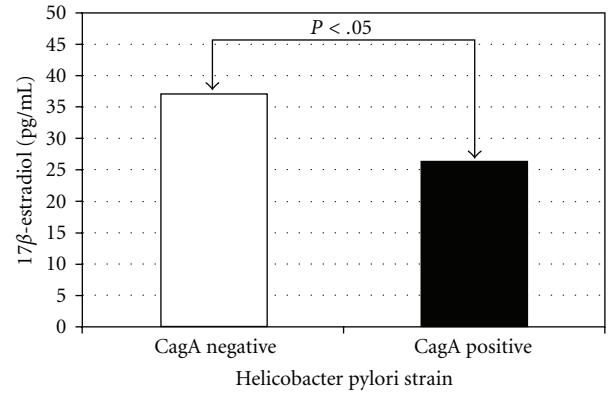

Figure 4: Variations in estrogens levels in elderly men affected by Helicobacter Pylori CagA positive or negative strains (adapted from [104]).

pollutants in the environment and are spread on most eaten transgenic plants, modified to tolerate high levels of these compounds in their cells. While up to 400 ppm of these residues are accepted in some feed, recent experimental studies demonstrated that aromatase transcription and activity were disrupted with subagricultural doses and with residues from 10 ppm [105]. In addition, a different study indicated that phytochemicals such as procyanidin B dimers contained in red wine and grape seeds inhibits aromatase activity *in vitro* and suppress aromatase-mediated breast tumor formation *in vivo* [106]. To this regard, it has been assumed that daily consumption of 125 mL of red wine would provide adequate amounts of procyanidin B dimers to suppress in situ aromatase in an average postmenopausal woman. By a different mechanism myosmine, a minor tobacco alkaloid widely occurring in food products of plant and animal origin inhibits the conversion of testosterone to estradiol by human aromatase with potential implications for sex hormone homoeostasis [107]. Another important and well-recognized modulator of aromatase efficiency in bone cells is vitamin D that has been shown to stimulate glucocorticoid-induced aromatase activity in cultured osteoblasts [35]. The magnitude of this effect varies largely among individuals, depending on the level of the vitamin D receptor [107]. Of interest, vitamin D receptor knock-out mice showed reduced aromatase activity with respect to wild-type animals [108].

Importantly aromatase activity may be also affected by pathological conditions. In this respect, it is known that increased androgen aromatization can occur in case of hepatocellular carcinoma [109], adrenocortical tumors [110], and testicular tumors [111, 112]. In all these neoplastic conditions, inappropriate amounts of aromatase enzyme are expressed and estrogen levels are increased. Elevated plasma estradiol concentrations also have been described in men with liver cirrhosis together with decreased plasma testosterone [112, 113]. In these patients, the metabolic clearance rate of estrogens seems to be unaltered, suggesting that the observed hyperestrogenism could be caused solely by an increase in aromatization of androgen precursors. Conversely, other pathological conditions may negatively affect aromatase activity and estrogen levels in males. In a preliminary study on elderly men Figura et al. described

significant differences in estradiol levels in relation to Helicobacter pylori infection, independently from circulating testosterone levels [105]. Serum concentrations of estradiol were significantly lower in infected CagA-positive patients than CagA-negative patients (Figure 4), and this variation was associated with differences in bone turnover. The mechanism underlying this association is unknown and deserves further investigations. Indeed, aromatase activity and production of estradiol were recently demonstrated in gastric parietal cells [114]. Finally, more recent observations suggested that diabetes may negatively affect expression levels of aromatase at least in the ovary and the testis [115, 116]. The effects of this disorder on major extragonadal sites of aromatase activity including bone remains to be determined. Of interest, experimental studies also evidenced that oral antidiabetic agents such as metformin can decrease aromatase expression in both granulose-luteal cells and breast adipose cells while, on the opposite, insulin has been associated with enhanced aromatase expression in different cell lines [117, 118]. Importantly, since a recent study evidenced that metformin-induced inhibition of aromatase expression occurs via the downregulation of promoter II, I.3, and 1.4 [118], its potential negative effects on skeletal estrogen production and bone health should be investigated.

5. Conclusions

Extraglandular aromatization of circulating androgen precursors is the major source of estrogen in men. Several lines of clinical and experimental evidence now clearly indicate that aromatase activity and estrogen production are necessary in men (as well as in women) for longitudinal bone growth, the pubertal growth spurt, epiphyseal closure, normal bone remodeling, and the attainment of peak bone mass. Moreover, like in women, estrogen production from androgen precursors by peripheral aromatase activity (even within the bone) is also important for the maintenance of bone mass and the prevention of bone loss in aging men. Further studies are required to better understand how genetic, epigenetic, environmental, pathologic, and pharmacological influences might modulate aromatase activity, increasing or reducing estrogen production in ageing individuals, and thereby affecting skeletal health.

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Review Article

Osteoporosis in Men with Diabetes Mellitus

Claire Issa, Mira S. Zantout, and Sami T. Azar

Department of Internal Medicine, Division of Endocrinology, American University of Beirut-Medical Center, P.O Box 11-0236, Riad El Solh, Beirut 1107 2020, Lebanon

Correspondence should be addressed to Sami T. Azar, sazar@aub.edu.lb

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Osteoporosis is more common in women than in men. The prevalence in men is not defined yet; however it is becoming much more recognized as its prevalence and impact have become explicable. It is estimated that around 1% of bone mineral density is lost in men every year. Studies show that secondary osteoporosis is the major cause thus, making it important to define the disorders associated with male osteoporosis. Diabetes is a risk factor for bone fractures. In male patients with diabetes measures should be undertaken such as encouraging exercise, assuring adequate calcium and vitamin D intake, and treating diabetic complications.

1. Introduction

Although it is the most common cause in women, primary osteoporosis is less common in men, but it is a disease that is being more and more recognized. Prevalence has always been a challenge to determine, mainly because of the lack of consensus on the definition of osteoporosis in men. With age, men are estimated to lose around 1% of bone mineral density per year [1, 2]. Despite that, osteoporosis in men should not be assumed to be primary because some studies have shown that more than 50% of osteoporosis in men has a secondary cause [3]. The definition of secondary osteoporosis is bone loss resulting from a specific, well-defined disease. Since this form of osteoporosis can respond to the treatment of the underlying disease, and with the presence of many treatment options now available, it is imperative to recognize the disorders that are associated with male osteoporosis. More evidence is evolving about the association between diabetes and osteoporosis in both men and women. Both conditions affect a large proportion of men, thus, it is very important to assess whether there is a causal relationship that might orient further screening and management of male osteoporosis. Men and women with diabetes were found to have higher risk of fractures compared to nondiabetics [4–9]. The risk seems to be multifactorial, with osteoporosis gaining more and more interest recently. This association seems to be race, sex, and type dependent.

2. Type 2 DM

BMD changes in males with type 2 diabetes are very controversial, with both tendencies toward higher, normal, or lower values. In studies assessing osteoporosis in diabetic patients, osteoporosis was defined according to the WHO definition with T-score > -1 as normal, between -1 and -2.5 as osteopenia, and < -2.5 as osteoporosis. The Rotterdam study [10] was a cross-sectional study that measured BMD at lumbar spine and proximal femur using DXA in 243 DM men and 2238 healthy men. It is one of the largest studies on BMD in type 2 DM. The study showed around 3% higher BMD at both sites in DM vs non-DM subjects that remained significant even after adjustment for confounders, mainly BMI and age.

Another study showing higher BMD in diabetic men was the EVOS study [11] that is a population-based prevalence study evaluating the effects of diabetes on bone density (measured using DXA at lumbar spine, femoral neck and femoral trochanter) and bone deformity prevalence in DM men versus non-DM. The study demonstrated that men with DM not treated with insulin had an increase in BMD only at the spine that was significant even after adjustment for body weight.

In the Health, Aging, and Body composition study [12] by Strotmeyer et al. 323 both white (38%) and black (62%) men with type 2 DM were evaluated. Fat mass and lean

body mass were measured using DXA and CT. The study reported higher BMD (4-5%) at the hip in both races that was independent of body mass and composition, and the results were in concordance with older studies that also showed higher BMD in type 2 DM [13, 14].

Krakauer et al. [15] evaluated 109 diabetic patients (46 type 1 and 63 type 2). In this study, radial bone density, bone markers, and bone biopsy (in 8 patients) were assessed. It was shown that there was lower radial bone density in both groups relatively to nondiabetic controls, with no difference between patients with either type of diabetes. Transiliac bone biopsy results showed decreased bone formation and mean adjusted apposition by 75% and 70%, respectively. Some of these patients were followed up after 2.5 years (41 patients) and 12.5 years (35 patients) showing that bone loss continued at an expected rate in type 1 with maintenance of the same deficit, whereas in type 2 there was a slower than expected loss such that the initial deficit was completely corrected.

In contrast to the above studies finding higher BMD in type 2 diabetic men, other studies showed no difference in BMD [15–17]. In one of them, Tuominen et al. [18] showed no significant difference in BMD between men with type 2 diabetes and controls at the femoral neck and trochanter. The study involved 56 patients with type 1 DM and 68 patients with type 2 DM from both sexes along with 498 non-DM controls. Similar findings were shown in a study by Shwartz et al. [19] evaluating bone loss at the hip over 4 years (measuring BMD at baseline and at the end of 4 years) in 480 DM men and women, 439 with impaired glucose metabolism, and 1172 healthy controls. It was found that despite having higher baseline BMD, only diabetic white women, but not black women nor men with DM and impaired glucose metabolism, demonstrated significant bone loss.

Other studies, on the other hand, showed lower BMD in patients with type 2 DM [20, 21]. In a cross-sectional study [22] involving 735 type 2 DM and 3458 nondiabetic men, BMD at the hip and spine was measured. This study showed lower BMD at the hip and higher incidence of osteoporosis in diabetic men that was significant even after adjustment for age and BMI. BMD at the spine was significantly higher in diabetics when compared to controls, but when adjusted for BMI, it became similar.

In another study by Petit et al. [23], using peripheral quantitative CT (pQCT) this time instead of assessing BMD by DXA to measure tibial and radial bone volumetric density, bone geometry, and bone strength. Bone strength was determined by measuring estimates of bone compressive and bending strength. Calculated bone strength index was used as an index of bone compressive strength, and calculated strength strain index was used as an index of bone bending strength. It was shown that older men with type 2 DM have bone strength that is low relative to body weight at the cortical-rich midshaft of the radius despite no difference in cortical bone volumetric density. This can account for the increased risk of fractures despite higher BMD in type 2 DM patients which might incriminate DXA as being a weak tool to assess bone in type 2 DM males.

A conclusion is very hard to draw after all the controversies shown in those studies, and to make things even more complicated, all those trials neglected to study the cortical bone which is a major limitation since bone is heterogenous (cortical and trabecular) and since diabetes, as many other endocrinological disorders such as hyperparathyroidism and hyperthyroidism, might affect cortical bone more than trabecular bone.

In order to correct for this limitation, other studies tried to study the BMD at the cortical bone showing that diabetes can actually affect bone heterogeneously by affecting cortical more than the trabecular bone [24, 25]. One of them [25] was conducted on 64 diabetic and 41 healthy Japanese men. BMD was measured using DXA at the lumbar spine, femoral neck, and distal radius, and it showed a significantly lower BMD at the distal radius in type 2 DM patients versus controls, that was even lower than their own BMD at the spine and femur. In type 2 DM, there was a negative correlation between BMD at the distal radius and mean HBA1C during the past 2 years. These findings demonstrate the importance of measuring 3 sites in patients with type 2 DM because of the possible selective cortical involvement.

Since diabetes is preceded by several years of pre-diabetic stage, it is worth to study the effect of impaired fasting glucose (defined as fasting glucose between 100–125 mg/dL) or impaired glucose tolerance (defined as a 2 hr glucose level between 140–200 mg/dL post 75 gr oral glucose load) on the BMD, in order to try to find a pathophysiology behind osteoporosis in diabetes and to try to find when does the effect on BMD start and whether there is a way to prevent it or stop it. Unfortunately, few studies evaluated BMD in prediabetic men. One of them [26] compared BMD in 272 men with prediabetes and 406 normal men. The study showed no difference in BMD between the 2 groups. However, when the prediabetic men were divided into quartiles based on fasting insulin and insulin levels 2 hrs after-75 gr glucose, it was noted that the BMD T-score increased with the increase in fasting insulin ($P = .004$). Additionally, the subjects with the highest concentrations of fasting insulin belonged to the groups with higher BMD T- scores ($P < .001$).

3. Type 1 DM

As in type 2 DM, there is also some controversy as to the association between type 1 DM and osteoporosis, but in contrast to type 2 DM most [27–31] but not all [32, 33] studies showed decreased BMD. In contrast to type 2 DM, there seems to be an important gender difference with more marked bone loss in men versus women when compared to matched controls [27–30].

The same study by Tuominen et al. [18] on both type 1 and type 2 DM patients of both genders, measuring BMD using DXA at the proximal femur, revealed that among both sexes, BMD values are significantly lower in type 1 versus type 2 DM or controls. The difference between type 1 DM and controls remained significant in both sexes even after adjustment for age and BMI, whereas the difference between type 1 and type 2 remained only significant in men. The latter

difference remained unaltered after further adjustment for duration of diabetes, but was slightly reduced when additionally adjusted for duration of insulin treatment and dose.

In another study [28] conducted on 30 type 1 DM men and 30 type 1 DM women versus 60 healthy controls, followed retrospectively, it was shown that male patients with type 1 diabetes has a significantly lower BMD values and lower Z-scores at the spine and femoral neck when compared with healthy men ($P < .05$). This difference remained the same after adjustment for age. The percentages of both osteoporosis and osteopenia were higher in DM men when compared to both normal men and diabetic women. There was no significant correlation between age-adjusted BMD values, and either diabetes duration, HBA1C values or age of onset of diabetes. Femoral neck BMD values were positively correlated with BMI in both female groups but only in healthy men. In conclusion, this study showed low BMD values in type 1 DM men and showed the gender difference on the effect of diabetes on BMD where diabetic men had lower BMD values when compared with diabetic women.

Few studies have assessed bone markers in diabetic men. In one of them [31], both BMD (measured by DXA) and serum bone markers (osteocalcin, C-terminal telopeptide of type 1 collagen (CTX), leptin and osteoprotegerin (OP)) were measured in 42 adult type 1 DM men and 24 nondiabetic controls. It was shown that 40% of type 1 DM patients had osteopenia at the spine and/or hip and 7% met criteria for osteoporosis. BMD z-score was correlated with age, negatively correlated with CTX, and osteocalcin. Osteocalcin, CTX and leptin concentrations were comparable in both groups, while OPG concentrations tended to be higher in DM. Despite the fact that there was not an increase in bone resorption markers in this study, this does not exclude a previous state of increased bone resorption. This is favored by the increase in OPG observed in this study that can be a protective mechanism of the skeleton to compensate for the possible previous increased bone resorption and bone loss.

In another study, where more bone markers and hormonal markers, especially testosterone, were measured, Hamilton et al. [33] conducted a cross-sectional trial involving 50 type 1 DM men, and 50 healthy controls, aged 30–71 years, assessing biochemical/hormonal markers of bone metabolism (25-hydroxy vitamin D3, PTH, CTX, osteocalcin, procollagen type 1 N-terminal propeptide (P1NP), total and free serum testosterone, sex hormone-binding globulin (SHBG), luteinizing hormone (LH), and follicule stimulating hormone (FSH)). BMD values at forearm, spine, and hip were recorded. It was shown, after adjustment for age and BMI, that BMD values, T and Z-scores were lower in DM men versus controls ($P < .048$). Prevalence of osteoporosis and osteopenia was higher only at the spine in DM men ($P = .03$). After adjusting for age and BMI, the investigators found that BMD was not significantly associated with HBA1C, smoking, nephropathy, retinopathy, or neuropathy. Only higher BMD at the spine was associated with diabetes duration. On multiple linear regression analysis, which adjusted for the natural logarithm (Ln) of the sex hormone-binding globulin concentration, smoking status, and alcohol consumption, it was shown that serum alkaline phosphatase was significantly negatively associated with BMD at 3 sites. There was a positive association between Ln (free testosterone) and BMD at the forearm and a negative association between Ln (osteocalcin) and BMD at the forearm.

In a Dutch study [34], osteopenia was found in as many as 67% and osteoporosis was seen in as many as 14% of 21 type 1 DM men compared to healthy controls. It was shown also that osteopenia was associated with low serum IGF-1 levels and bone formation markers.

In the study that was mentioned previously, conducted by Krakauer et al. [15], again it was shown that radial bone density was lower in type 1 DM relatively to nondiabetic controls, but this low BMD seems to be stable over time, with expected stable bone loss, according to this study.

In another study [35], trying to follow type 1 DM patients over time, to study for the changes in bone density, 41 type 1 DM (19 female, 22 males), mean age 9 years, were followed for around 5 years. Two sites of the nondominant radius were analyzed by pQCT. At the distal radius (metaphyseal site) total and trabecular BMD and at the proximal radius (diaphyseal site) total and cortical BMD, total cross-sectional area (CSA), cortical CSA, medullary CSA, muscle CSA, and strength strain index as measure of bone stability were calculated. It was shown in this study that at the 1st evaluation, mean SD value of trabecular BMD was even higher in type 1 diabetic patients than in controls, irrespective of age, sex, and Tanner stage. At the diaphysis, patients with type 1 DM had significantly reduced mean SD values for total, cortical, and medullary SCA as well as cortical BMD which had normalized at the 2nd measurement. The younger the patients were at the disease manifestation and at the 1st evaluation, the more the increase in total CSA was detectable. As a conclusion, this study suggests a defect in bone accretion early in the course of type 1 DM, which then ameliorates with time. A limitation of this study was the small number, but the strength is using pQCT as a tool for bone measurements and the 5-year followup.

Moreover, in another study [36] using both pQCT and DXA to measure bone mass and structure in 48 adolescents with type 1 DM (26 girls and 22 boys), pQCT measurements were performed at the distal and shaft sites of the dominant radius and the right tibia. BMC, total cross-sectional area and trabecular density were determined for the distal sites, BMC, cortical density, and cortical cross-sectional area were determined for the shaft sites. It was shown that diabetes was associated with reduced bone mineral content (BMC) and smaller bone cross-sectional size, with boys being more affected than girls with a mean deficit in BMC of all measured skeletal sites of >10% in boys and <5% in girls.

4. Type 1 and Type 2 DM

Because most of the studies involved a small number of patients, in order to correct for this limitation and increase the power, a meta-analysis [30] including 80 papers on BMD and fracture risk in patients with type 1 and 2 DM was done and showed that in both genders there was an increased

risk of fractures in both types of diabetes mellitus compared to non-diabetes mellitus. Z-score in hip and spine was decreased in type 1 and increased in type 2 DM. A meta-regression showed that mainly BMI was the major determinant for BMD, whereas HBA1C was not linked to BMD. The increase in fracture risk was higher and BMD was lower in patients with complications of DM.

4.1. In Conclusion. The relationship between diabetes, both types, and osteoporosis seems complex. Mainly because the studies were limited by the small number, patients werenot followed up for a long period of time, studies were heteregenous with different diabetes control and duration, different complications, which makes it hard to draw a unique conclusion. But the trend seems to be a low BMD in type 1 DM and a high BMD in type 2 DM. More studies correcting for the mentioned limitations need to be performed.

5. Pathophysiology of Altered BMD in Diabetic Patients

Several hypotheses have been raised to explain the altered BMD in diabetic patients. Although the most popular one was the higher BMI in type 2 DM that can protect from osteoporosis, most of the studies discussed previously corrected for BMI, yet despite this correction there was still a higher BMD in patients with type 2 diabetes relatively to type 1 and healthy controls.

6. Insulin and Insulin Growth Factors

One popular hypothesis to the effect of diabetes on bone is through insulin, acting as a bone anabolic factor. The importance of this hypothesis is that it can also explain the pathophysiology behind the difference between type 1 and type 2 diabetic men. Insulin seems to have both direct and indirect effects on bone.

Support for a direct role of insulin in bone comes mainly from animal studies, specifically rats, where streptozocin-induced diabetes led to defects of bone mineralization. Moreover, rats lacking insulin receptors had impaired bone formation and low bone turnover [37–39]. In a recent study conducted by Fulzele et al. [40], to try and directly examine the function of insulin signaling in bone, they engineered mice lacking insulin receptor (IR) specifically in osteoblasts. It was shown that osteoblasts lacking IR had severely impaired differentiation, with increased apoptosis. They showed a 79% decrease in the number of osteoblasts per bone perimeter at 3 weeks of age. This led to a dramatic impairement in postnatal trabecular bone acquisition. Administration of IGF-1 did not correct for the abnormalities seen, showing the direct effect of insulin, independently of IGF-1 on the bone. A high level of expression of insulin receptors on osteoblasts was reported [41], and insulin binding to these receptors led to cell proliferation, production of alkaline phosphatase, collagen synthesis, and glucose uptake [42–45]. The effect of insulin seems not only limited to osteoblasts, because in vitro studies showed that osteoclasts as well have insulin receptors where insulin can act to inhibit their action [46].

Human data support this hypothesis. A large study comprising of over 100 subjects with type 1 diabetes mellitus showed lower IGF-1 levels as well as bone formation markers when compared to healthy controls [47, 48]. Moreover, low levels of IGF-1 were found to be associated with osteopenia in type 1 DM patients [48].

In contrast to type 1 diabetes mellitus where there is insulin deficiency, in type 2 diabetes mellitus there is insulin resistance and hyperinsulinemia which can explain the higher BMD in type 2 diabetes mellitus. Since the insulin resistance is selective and only restricted to the effect of insulin on glucose transport [49], the high insulin levels can still act on the osteoblast to increase BMD. Indeed some investigators found a positive correlation between insulin levels and BMD [50, 51], yet others did not [52, 53].

In addition to a direct effect of insulin on osteoblast and osteoclast, insulin can indirectly act on the bone by decreasing sex-hormone binding globulin [54–57] leading to higher levels of free estrogen and testosterone, acting positively on the bone to increase BMD [58]. It can act indirectly by suppressing IGFBP-1 thus increasing the sensitivity of osteoblasts to IGF-1, then IGF-1 will modulate the actions of PTH on bone leading to a synergistic effect between insulin and PTH as well an indirect synergistic effect with other substances that mediate anabolic effects on bone [59, 60]. These are all theories, and more studies are needed to assess the effect of insulin on bone in male patients with diabetes.

7. Diabetic Complications

Uncontrolled diabetes with hyperglycemia has been suggested as a possible mechanism for osteoporosis in both type 1 and type 2. This can occur by the formation of nonenzymatic glycosylation of various bone proteins, including type 1 collagen, leading to impaired bone quality [61]. There are also some studies [62] associating high levels of pentosidine to higher risk of fractures in diabetic patients. Moreover, glucose is the principle source of energy for osteoclasts and is able to increase avian osteoclast activity in vitro in a dose-dependent manner [63]. Another indirect effect of hyperglycemia on bone can be through hypercalciuria secondary to glycosuria and other interactions with PTH and vitamin D metabolism [64]. Some studies have shown that type 2 diabetes mellitus is associated with lower vitamin D levels compared to healthy controls [65, 66]. Despite these theories, only some [67] but not all [33, 68] studies have demonstrated that glycemic control and HBA1C levels were associated with osteoporosis in diabetic patients.

Other than glycemic control, diabetic complications were incriminated in osteoporosis, mainly retinopathy [69–71] by decreasing exercise thus leading to decreased muscle mass and poor vision leading to increased incidence of falls, nephropathy [70, 72, 73] by affecting bone metabolism, microangiopathy by directly affecting bone vascularisation, and neuropathy [74] by decreasing exercise. Yet again other

studies have shown contradictory results [28, 33] with no association between either complication and BMD.

8. Bone Turnover and Bone Stiffness

Most studies assessing bone turnover in diabetic patients were limited by the small number of patients included. In general, it was suggested that there is an imbalance between bone formation and bone resorption in diabetes. Kemink et al. reported a lower BMD in diabetic patients who concomitantly had lower alkaline phosphatase levels [34]. Dobnig et al. [75] showed that subjects with type 2 diabetes mellitus had lower levels of PTH and osteocalcin. The same was shown by Achemelal et al. [76]. Thus, it seems that in type 2 DM, there is a decreased bone formation. This was also shown by the study, previously mentioned above, by Krakauer et al. [15], where they came up with a conclusion that can explain the discrepancy between type 1 and type 2 DM, that diabetes is accompanied by low bone formation rate, that can lead to osteopenia in the growing skeleton, whereas in type 2 DM, there is low bone turnover that will retard bone loss, explaining the increased bone density in type 2 DM. Another contradictory study, done by Alexopulou et al. [27], showed that there was no difference in osteocalcin levels or C-terminal telopeptide type 1 collagen between males with type 1 diabetes mellitus and controls, but osteoprotegerin was increased, showing contrary to previous studies that bone formation was normal. In contrast to bone formation, most studies [27, 75, 76] have shown normal bone resorption markers in diabetic patients, suggesting with the associated low bone formation a state of low bone turnover or adynamic bone in diabetes. Again this is just a theory and more advanced studies are needed with more assessment of bone markers for both bone formation and resorption.

9. Hormonal Imbalance

Since hypogonadism in men was associated with low BMD and fractures, and since most diabetic men have lower testosterone levels when compared to nondiabetics, it was suggested that diabetes can cause low BMD and higher risk of fractures through causing hypogonadism. Studies on this association are very limited, with one conducted by Asano et al. [68] showing a weak but a significantly positive correlation between serum bioavailable testosterone and bone stiffness in type 2 DM.

10. Conclusion

Both osteoporosis and diabetes are increasing in men. Evidence of an association between both diseases is increasing, but in face of the controversy and the limitation of the studies done (small studies, cross-sectional, no clear assessment of the pathophysiology), more studies correcting for these limitations (with a longer followup, assessing bone markers, hormonal factors, complications, shifting to a better imaging, as CT&MRI instead of DXA assessment of BMD) are still needed. Despite the fact that specific evidence-based recommendations based on the present data are not available and cannot be made, there must be awareness about the fact that diabetes, especially type 1, might be a risk factor for osteoporosis, that it is multifactorial, possibly affecting cortical and trabecular bone differently. Specific measures should be undertaken as encouraging exercise, assuring adequate calcium and vitamin D intake, and treating diabetic complications.

Evidence Acquisition

The major source of data acquisition included Medline search strategies, using the words "type 1 diabetes mellitus," "type 2 diabetes mellitus," "osteoporosis," and "men." Articles published in the last 13 years were screened.

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