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Dihydrotestosterone is a determinant of calcaneal bone mineral density in men

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ABSTRACT

Male osteoporosis is an increasingly important health problem worldwide. Though androgen deficiency leads to bone loss in men, information on the relative contribution of aromatizable and non-aromatizable androgens in maintaining bone mineral density (BMD) and the mechanisms involved are unclear. This cross-sectional study was designed to explore the same. Hundred osteoporotic men with age matched normal were studied for serum levels of sex steroids, PTH, IGF system components, cytokines and bone turnover markers. Our findings show that serum DHT, IGF-I, IGF-II and IGFBP-3 levels were significantly decreased while IL-1β and bone turnover markers were significantly increased in osteoporotic men compared to normal. Pearson correlation analysis revealed that serum DHT, IGF-I, IGF-II and IGFBP-3 levels were positively and strongly correlated with BMD, while serum IL-1β levels were negatively correlated with BMD. Serum PTH, testosterone, estradiol, IGFBP-4, TNF-α, IL-4 and IFN-γ levels were similar between the two groups. We observed that DHT levels significantly declined with age. However, the significant difference in DHT between the osteoporotic and normal groups is the same regardless of age. A multiple regression model adjusted for age demonstrated that DHT/BMD association is fairly stronger among those with osteoporosis than the normal. Our findings for the first time point out that DHT is an important determinant of BMD in men. Most importantly, the strong positive correlation of serum DHT with BMD offers new perspectives in understanding the role of non-aromatizable androgen in regulating bone metabolism in men, and might serve as a potential clinical marker in the diagnosis of male osteoporosis.
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1. Introduction

Male osteoporosis has become a formidable health problem worldwide. Despite the considerable public health burden attributable to male osteoporotic fractures, the causative factors of this metabolic bone disease are largely unknown. Sex steroids play key role in the construction and conservation of the adult skeleton [1]. Over the last three decades, it has become apparent that androgens play a significant role in the maintenance of bone mass [2]. Although androgens are crucial for both skeletal development and maintenance in men [3], several clinical studies have indicated a major role for estrogens in the regulation of male skeleton [4,5]. Accumulating evidences suggest that the actions of androgens may be mediated primarily by conversion to estrogens. Therefore, the relative contribution of estrogens *versus* androgens in regulating bone mass in men is yet to be clarified. In addition, the relative roles of testosterone (aromatizable androgen) and dihydrotestosterone (DHT, non-aromatizable androgen) in regulating bone mineral density (BMD) are also yet to be identified.

Parathyroid hormone (PTH) is a stimulator of bone resorption. However, it is also known to have anabolic effect on bone possibly by stimulating osteoblasts [6]. The role of growth factors and cytokines on bone metabolism has received much attention as bone cells could produce and respond to these factors [7,8]. The findings that serum levels of insulin-like growth factor (IGF) system components are altered during physiological and pathological states when bone metabolism is distorted provide evidence for an important role for IGF system components in bone remodeling [9]. There is now an increasing body of evidence that bone-resorbing cytokines, such as interleukin (IL)-1, IL-6, tumor necrosis factor-α

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(TNF-α) and macrophage-colony stimulating factor (M-CSF) may be potential candidates for mediating the bone loss following estrogen deficiency [10]. In contrast, IL-4, interferon-γ (IFN-γ) and transforming growth factor-β (TGF-β) inhibit both osteoclast formation and its activity [7]. Nevertheless, the relationship between systemic cytokine levels and BMD is still unclear.

Therefore, to understand the mechanisms that underlie in maintaining BMD in men by sex steroids and other factors, the present study was designed to estimate the serum levels of sex steroids, PTH, IGF system components, cytokines and bone turnover markers, and correlate them with BMD.

2. Subjects and methods

2.1. Study subjects

All men participated in this study were living in and around the Chennai city, India. Bone density screening camps were conducted with the help of orthopaedicians and the subjects were chosen by the convenience sampling method in a cross-sectional population. A standardized questionnaire was completed which included informations about cigarette smoking, alcohol use, physical activity and medication use of the participants. All the subjects who attended the camps were initially enrolled in the study and were categorized into normal and osteoporotic based on the calcaneal BMD *T*-scores. Later, we excluded the subjects who were smokers and/or alcoholics as these factors influence the hormonal profiles and BMD. Based on the questionnaire, very few subjects reported low or high physical activities. Due to the low number of subjects in these categories, they were not included in this study. Therefore, all the subjects in this study had moderate physical activity. In the present study, 100 osteoporotic men (age range 40–70 years) and 100 healthy volunteers who served as normal (age range 40–70 years) were investigated for various hormonal and biochemical parameters. None of the subjects selected for this study were receiving any treatments known to interfere with bone metabolism. This study was approved by institutional human ethical committee and all participants gave written informed consent.

2.2. Anthropometric measurements

Body weight and height were recorded, and body mass index (BMI) was calculated as the body weight in kilograms divided by the square of the height in meters (kg/m^2).

2.3. BMD measurements

BMD was measured at the calcaneum using peripheral dual energy X-ray absorptiometry (DEXA). The current World Health Organization (WHO) definition of osteoporosis applied to postmenopausal women was used to identify osteoporotic men as the International Society for Clinical Densitometry (ISCD) recommended and recently reaffirmed use of a BMD *T*-score of −2.5 or below to diagnose osteoporosis in men [11].

2.4. Laboratory methods

Fasting blood samples were collected from the antecubital vein of the normal and osteoporotic men between 08:00 and 09:00 h. Samples were immediately centrifuged, sera separated and then stored at −86 °C until assayed. Serum total DHT (DSL, USA; sensitivity, 4 pg/ml; intra- and inter-assay coefficient of variations (CV), 3.1–6.2% and 2.3–8.5%, respectively) and estradiol (DSL, USA; sensitivity, 4.7 pg/ml; intra- and inter-assay CV, 3.2–5.3% and 8.1–9.3%, respectively) were measured by radioimmunoassay (RIA) kits. Testosterone was quantified by liquid phase RIA (sensitivity, 0.3 ng/ml; intra- and inter-assay CV, 6–8% and 4–6%, respectively) adopting the WHO procedure [12]. Intact human PTH (1–84) was measured by immunoradiometric assay (IRMA) kit (DiaSorin, USA; sensitivity, 0.7 ng/ml; intra- and inter-assay CV, 2.0–3.6% and 3.4–4.9%, respectively).

Serum IGF-I (DSL, USA; sensitivity, 2.06 ng/ml; intra- and inter-assay CV, 3.9–7% and 3.8–7.4%, respectively) and IGF-II (DSL, USA; sensitivity, 12 ng/ml; intra- and inter-assay CV, 3.4–6.5% and 4.5–6.3%, respectively) were measured using non-extraction IRMA kits. IGF binding protein-3 (IGFBP-3) (DSL, USA; sensitivity, 0.5 ng/ml; intra- and inter-assay CV, 1.8–3.9% and 0.5–1.9%, respectively) and IGFBP-4 (DSL, USA; sensitivity, 1 ng/ml; intra- and inter-assay CV, 2.8–6.4% and 2.3–6.7%, respectively) were determined using IRMA and enzyme-linked immunosorbent assay (ELISA) kits, respectively.

Serum concentrations of TNF-α, IL-1β, IL-4 and IFN-γ were measured using the sandwich ELISA kits, according to the manufacturer's instruction (R&D Systems, Minneapolis, USA). Human osteocalcin (OCN) was measured using IRMA kits (DSL, USA; sensitivity, 0.3 ng/ml; intra- and inter-assay CV, 1.4–3.4% and 3.3–5.3%, respectively). Serum concentrations of total alkaline phosphatase (ALP) [13] and tartrate-resistant acid phosphatase (TRAP) [14] were estimated by the standard biochemical methods using *p*-nitrophenyl phosphate as substrate. Serum calcium (Crest Biosystems, Goa, India) and phosphorus (Agappe Diagnostics, Maharashtra, India) were determined by using commercial kits according to the manufacturer's instruction.

2.5. Statistical analysis

Values are represented as mean ± SEM. Univariate analysis was performed by Student's '*t*' test using SPSS statistical software (Version 7.5). Correlation analyses were performed using Pearson correlation and the independent predictors of the various parameters were tested using linear regression analysis using SAS (Version 9.1.3). Correlations were only calculated for the variables of interest and possible associations were indicated by scatter plots. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of the population

In the present study, 100 osteoporotic men (mean age 62 ± 1.1 years, range 40–70 years) and 100 healthy volunteers who served as normal (mean age 60 ± 1.3 years, range 40–70 years) were investigated for various hormonal and biochemical parameters. Clinical and biochemical data from the normal and osteoporotic men are shown in Tables 1 and 2. There was no significant difference in BMI between normal and osteoporotic men. BMD *T*-score was obviously decreased ($P < 0.05$) in osteoporotic men when compared with normal (Table 1). There was no significant difference in the serum level of PTH between the normal and osteoporotic men (Table 2),

Table 1
Values of BMD *T*-score and BMI in normal and osteoporotic men.

Parameters	Normal ($n = 100$)	Osteoporotic ($n = 100$)
BMD *T*-score	−0.17 ± 0.09	−3.02 ± 0.06*
BMI (kg/m^2)	24.8 ± 1.2	24.1 ± 1.1

Each value is mean ± SEM.
The study subjects were assigned as normal or osteoporotic based on the BMD *T*-score values.
* Statistical significance at $P < 0.05$.

Table 2
Serum PTH and bone turnover markers in normal and osteoporotic men.

Parameters	Normal ($n = 100$)	Osteoporotic ($n = 100$)
PTH (pg/ml)	19 ± 1.5	21 ± 1.6
OCN (ng/ml)	5.7 ± 0.4	7.1 ± 0.5*
Total ALP (IU/l)	38.18 ± 2.9	53.12 ± 3.32*
TRAP (IU/l)	9.96 ± 0.99	18.26 ± 1.33*
Calcium (mg/dl)	8.94 ± 0.4	9.02 ± 0.5
Phosphorus (mg/dl)	3.5 ± 0.2	3.4 ± 0.2

Each value is mean ± SEM.
* Statistical significance at $P < 0.05$.

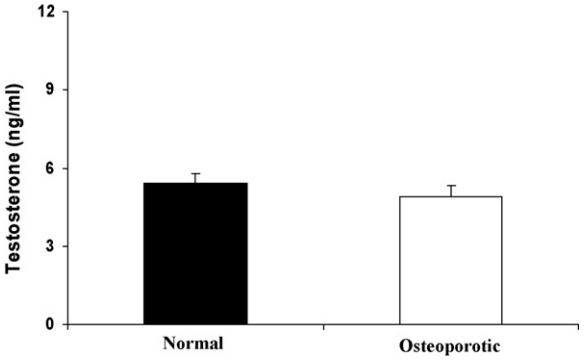

Fig. 1. Serum levels of testosterone in normal and osteoporotic men. Each value is mean ± SEM. Normal ($n = 100$) and osteoporotic ($n = 100$).

ruling out the possibility of hyperparathyroidism (a risk factor of osteoporosis). The bone turnover was assessed by measuring the markers of bone formation and bone resorption. Increased levels of bone formation markers (OCN, ALP) with concomitant increase in bone resorption marker (TRAP) in osteoporotic men reflect high bone turnover. However, there was no significant change in the serum levels of total calcium and phosphorus between these groups (Table 2).

3.2. Serum sex steroids in normal and osteoporotic men

Sex steroids are essential for the skeletal development and maintenance of bone health throughout adult life. In this study, there was no significant difference in the serum levels of total testosterone and estradiol between normal and osteoporotic men (Figs. 1 and 2). Interestingly, a significant decrease ($P < 0.0001$) in the level of non-aromatizable androgen (DHT) was observed in osteoporotic men when compared with normal (Fig. 3).

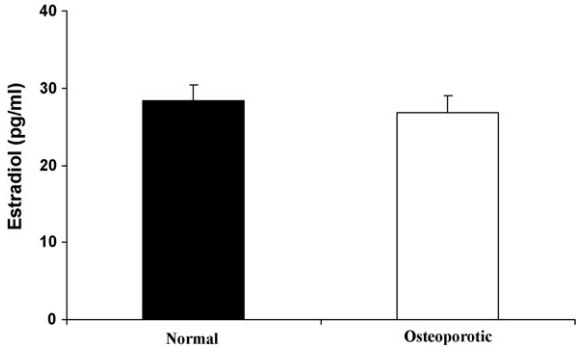

Fig. 2. Serum levels of estradiol in normal and osteoporotic men. Each value is mean ± SEM. Normal ($n = 100$) and osteoporotic ($n = 100$).

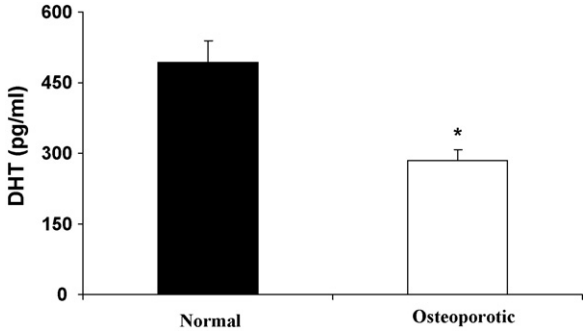

Fig. 3. Serum levels of DHT in normal and osteoporotic men. Each value is mean ± SEM. *Statistical significance at $P < 0.05$ compared with normal. Normal ($n = 100$) and osteoporotic ($n = 100$).

3.3. Serum IGF system components in normal and osteoporotic men

IGF system plays an important role in the maintenance of bone mass in adult as well as longitudinal growth of bone in childhood. We observed a decrease of 23% for IGF-I, 24% for IGF-II and 16% for IGFBP-3 in osteoporotic men compared with normal. This decrease was statistically significant ($P < 0.05$) for IGFs and IGFBP-3. However, there was no significant difference in the IGFBP-4 levels between the two groups (Table 3).

3.4. Serum cytokines in normal and osteoporotic men

Among the cytokines studied, we observed a 29% increase ($P < 0.05$) in IL-1β in osteoporotic men when compared with normal. Although an increase (16%) in TNF-α was observed in osteoporotic men, it was not statistically significant. Also, there was no significant change in the levels of IL-4 and IFN-γ between the two groups. Thus, the cytokines involved in bone resorption were increased, but the cytokines known to inhibit bone resorption were unaltered (Table 4).

3.5. Relationship of sex steroids, IGF system components and cytokines with BMD

Correlation analyses were performed using Pearson correlation to assess the relationship between the sex steroids, IGF system components and cytokines with BMD. Among the sex

Table 3
Serum levels of IGF system components in normal and osteoporotic men.

Serum IGF system components (ng/ml)	Normal ($n = 100$)	Osteoporotic ($n = 100$)
IGF-I	174 ± 10	135 ± 8*
IGF-II	955 ± 45	722 ± 35*
IGFBP-3	3651 ± 155	3055 ± 145*
IGFBP-4	542 ± 39	608 ± 48

Each value is mean ± SEM.
* Statistical significance at $P < 0.05$.

Table 4
Serum levels of cytokines in normal and osteoporotic men.

Serum cytokines (pg/ml)	Normal ($n = 100$)	Osteoporotic ($n = 100$)
IL-1β	11.75 ± 0.9	15.1 ± 0.9*
TNF-α	70 ± 6	81 ± 6
IL-4	97.0 ± 8	95.8 ± 7
IFN-γ	38.8 ± 2	40.1 ± 2.5

Each value is mean ± SEM.
* Statistical significance at $P < 0.05$.

steroids, serum DHT correlated positively with the BMD ($r = 0.691$, $P < 0.0001$). However, no such correlation exists for serum testosterone ($r = 0.133$) and estradiol ($r = 0.112$) with BMD. Pearson correlation analysis also showed a strong positive correlation between the levels of testosterone and DHT ($r = 0.444$, $P < 0.001$).

We compared the strength of the DHT/BMD association using a multiple regression model that included two groups (osteoporotic and normal), BMD, and their interaction. Presence of a group-BMD interaction ($P < 0.01$) is evident that the positive association between DHT and BMD is not the same in the two groups. Within-group correlations confirm that the association is fairly stronger ($r = 0.661$, $P < 0.0001$) among those with osteoporosis than normal ($r = 0.397$, $P < 0.0001$).

Relationship between IGF system components and BMD revealed that serum total IGF-I ($r = 0.528$, $P < 0.01$), IGF-II ($r = 0.303$, $P < 0.01$) and IGFBP-3 ($r = 0.281$, $P < 0.01$) were positively correlated with BMD, whereas serum IGFBP-4 ($r = -0.227$, $P < 0.05$) was negatively correlated with BMD. Interestingly, IL-1β ($r = -0.537$, $P < 0.01$) and TNF-α ($r = -0.389$, $P < 0.05$) were negatively correlated with BMD in men. However, no correlation was observed for IL-4 ($r = 0.102$) and IFN-γ ($r = -0.189$) with BMD.

3.6. Relationship of the sex steroids with age and BMI

We plotted testosterone, DHT and estradiol *versus* age in the two groups (osteoporotic vs. normal) and assessed whether there are age-specific differences by scatter plot. We used multiple regression analysis to verify that, for each of the three steroids studied, no interaction exists between age and group (for testosterone $P = 0.244$; for DHT $P = 0.907$ and for estradiol $P = 0.673$). Associations between the steroids and age, whether or not they exist, do not differ between the groups; differences between the groups are consistent across the age. The scatter plot graphs and our statistical analyses further show that no differences exist in testosterone (Fig. 4A) and estradiol (Fig. 4B) between the osteoporotic and normal groups whether or not we adjusted the analysis for age. However, DHT declines significantly with age; linear regression estimates that DHT decreases about 11.8 points for an additional year of age in our sample ($P < 0.0001$). Accordingly, we adjusted the analysis for the association between DHT and age. Fig. 4C shows that DHT levels in normal and osteoporotic subjects decline with age at the same rate, but the difference in DHT between the two groups is the same regardless of age.

We assessed the association between DHT and BMD using a multiple regression model that estimated between group differences in DHT adjusted for BMI and age. Interaction terms were investigated in the model and discarded when no interactions were detected. Because information on BMI did not contribute to the estimation of DHT in a no-interaction model ($P = 0.220$), we report differences in between the two groups for DHT that are adjusted only for age.

4. Discussion

Our findings demonstrate for the first time that DHT is an important determinant of calcaneal BMD in men. The determination of BMD in the calcaneum in this study is validated by various prospective studies that have demonstrated and confirmed that the correlation between reduced calcaneal quantitative ultrasound

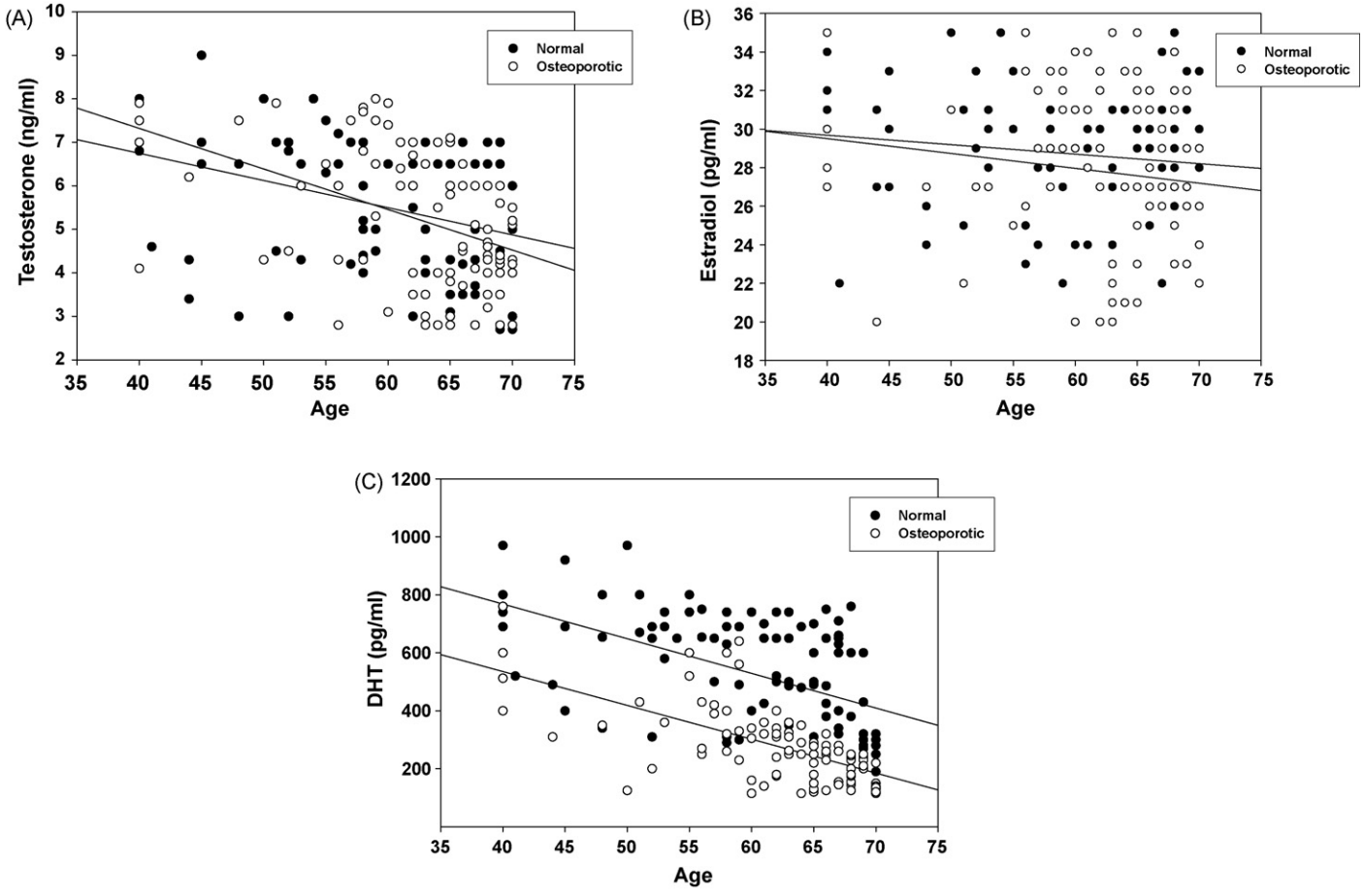

Fig. 4. Relationship of (A) testosterone vs. age, (B) estradiol vs. age, and (C) DHT vs. age by scatter plot. Normal ($n = 100$) and osteoporotic ($n = 100$).

(QUS) values and the risk of humeral and hip fractures is similar to that obtained with DEXA [15,16].

In the present study, we observed a significant decrease in DHT levels in osteoporotic men when compared with normal. Also, DHT is strongly and positively correlated with BMD. Indeed, DHT is the parameter that best correlated with BMD. These results are somewhat surprising. Amory et al. [17] demonstrated that testosterone therapy in older men increased the BMD when administered alone and when combined with finasteride suggesting that DHT is not essential for the beneficial effects of testosterone on BMD. The lack of a significant change in BMD in the presence of low DHT in their study could be explained by two distinct but non-exclusive possibilities. First, finasteride is a selective 5α-reductase type 2 inhibitor and it incompletely blocks the conversion of testosterone to DHT [18]. The finding that type 1, but not type 2, is the predominantly active form of the 5α-reductase in human osteoblastic cells [19] might explain the lack of differences in BMD observed between men treated with testosterone alone or testosterone combined with finasteride. Therefore, the possibility of testosterone being converted to DHT and its action on bone cannot be completely ruled out. Secondly, our data clearly show that there is a significant difference in DHT values between normal and osteoporotic subjects at any given age. Furthermore, in our study, the mean DHT value of osteoporotic subjects was almost half of the mean DHT value of normal. In their study, Amory et al. [17] observed decrease in DHT that is of low magnitude when compared to our study, and therefore might failed to observe a major impact on BMD. In contrast with the report of Amory et al. [17], and in support of our data, Hanada et al. [20] demonstrated a significant increase in BMD when orchidectomized and ovariectomized rats were treated with DHT, emphasizing the importance of DHT in regulating BMD.

Anderson et al. [21] demonstrated a significant decrease in BMD at the lumbar spine when hypogonadal men were administered with 7α-methyl-19-nortestosterone (MENT), while testosterone treatment demonstrated no significant change but maintained the lumbar spine BMD. MENT is a potent synthetic androgen that is resistant to 5α-reduction, but can be converted by the aromatase to an active estrogen [22]. It is reasonable to speculate that testosterone effects in maintaining the BMD might be mediated by DHT. This suggests that androgens have definite role on BMD rather than being converted to estrogen as MENT treatment was not able to maintain lumbar BMD.

In support of the role of androgens in maintaining skeletal mass in adults, clinical studies have shown that castration and decreased testicular function result in osteoporosis in men and treatment with androgens prevents its occurrence [23]. Moreover, androgen treatment of osteoporotic women demonstrated that androgen therapy is effective in increasing BMD [23]. In men, androgen deficiency is associated with premature bone loss and testosterone substitution increases or stabilizes bone mass in hypogonadal men [24]. Thus, the role for androgens in skeletal regulation is substantiated by numerous studies in humans [25] and rodents [26], demonstrating that chemical or surgical castration, as well as untreated hypogonadism in men, leads to accelerated bone loss. Importantly, the deleterious effects of these conditions on bone can be reversed by treatment with androgens. The maintenance of BMD in men is testosterone dependent, and it is lower in hypogonadal men and increased by exogenous testosterone administration [24]. Recent study by Basurto et al. [27] illustrated that testosterone treatment in healthy elderly men with low testosterone resulted in a significant increase in lumbar BMD. Studies have demonstrated the direct effects of testosterone and 5α-reductase mediated effects on bone [28,19].

In our study, there was no association between testosterone and BMD. This is in apparent contrast with other reports [29]. Nevertheless, our study shows a positive correlation between the levels of testosterone and DHT. Also our multivariate analysis shows the DHT is significantly associated with BMD. Since testosterone can be converted into DHT, we cannot rule out the possibility of DHT mediated bone protective effects in those studies.

The present finding that DHT is more correlated with BMD than testosterone is strongly supported as DHT binds more tightly to the androgen receptor (AR), and the DHT-AR complex is more readily transferred to the DNA-binding site and activates a receptor reporter gene more efficiently than testosterone [30]. In addition, DHT amplifies a weak androgen signal and also regulates specific genes that do not respond to testosterone [19]. Both *in vitro* and *in vivo*, non-aromatizable androgens have similar or more effects on bone compared with testosterone. These data clearly indicate that androgens can act directly on bone and suggest that these actions may be physiologically relevant [31].

Our result is corroborated by the observations that administration of non-aromatizable androgens prevented castration-induced bone loss in adult rats [32]. Vandenput et al. [3] pointed out that administration of testosterone prevented orchidectomy-induced bone loss in estrogen receptor-α knock out (ERαKO) male mice. This suggests that androgen has direct effect on bone and need not be converted to estrogen by aromatase for its action, ruling out the possibility of ERα mediated action of androgen. The stimulatory role of testosterone and DHT on periosteal bone formation in male rats strengthens the importance of androgens in bone mass accretion [33]. Taken together, it is obvious that DHT plays an important role in bone metabolism and the decrease in DHT might contribute to diminished BMD in osteoporotic men. The possible reasons for the decrease in serum DHT might be due to defective expression and activity of 5α-reductase or increased metabolic clearance of DHT in osteoporotic men.

In our study, there was no association between estradiol and BMD, which is consistent with the previous studies from other groups [34,35]. However, contrary to this, others have reported that BMD seems to correlate more with estrogen than androgen [36,37]. Amin et al. [38] demonstrated higher incidence of hip fracture in the group of men with low estradiol (2–18.1 pg/ml) when compared with those men in the middle (18.2–34.2 pg/ml) and high estradiol (≥34.3 pg/ml) groups. Curiously, in our study the estradiol levels range between 20 and 35 pg/ml. So, it is also possible that the comparatively high range of estradiol levels in osteoporotic subjects in the present study might contribute for no detectable association between estradiol and BMD.

Fink et al. [39] demonstrated that older men with both low testosterone and estradiol were more likely to be osteoporotic. In our population, there was no relationship between testosterone and estradiol with BMD. However, our findings show that DHT is significantly decreased in osteoporotic men. There are several differences between these two studies. In their study, the subjects were Caucasian with the mean age of 73 (range 65–99) and BMD is measured at hip, femoral neck and lumbar spine, whereas our study subjects are Asian with mean age of 62 (range 40–70) and BMD is measured at calcaneum. Thus the discrepancy can be attributed to the differences in race, age and the site of BMD assessed.

In the present study, osteoporotic men showed a reduced levels of IGF-I, IGF-II and IGFBP-3 with no change in IGFBP-4 when compared with normal. Boonen et al. [40] suggested that decrease in serum levels of IGF system components could lead to a decrease in the endocrine actions of IGFs. The finding that systemic administration of IGF-I caused a marked increase in bone formation in both animals and humans [41,42] provides evidence for endocrine actions of IGFs. Further Mohan et al. [43] reported that the age-related decrease in serum levels of stimulatory IGF system components may in part, reflect decreased bone cell production of stimulatory IGF system components. Thus the decrease in both local and endocrine actions of IGFs might result in decreased osteoblast

proliferation and differentiation and a subsequent reduction in bone formation [40]. This suggests the possibility that the decrease in stimulatory IGF system components may be the other important factors in the etiology of male osteoporosis. Therefore, the decreased level of IGF system components recorded in osteoporotic men might also be responsible for the decreased BMD. The positive correlation of IGFs and IGFBP-3 with BMD in this study is in accordance with the earlier reports [44,45].

Gori et al. [46] reported that DHT increased the levels of IGF-I and IGFBP-3 in human osteoblastic cell line (hFOB/AR-6). Further, Kasperk et al. [47] demonstrated that DHT enhances the mitogenic effect of IGF-II in bone cells. These reports suggest the stimulatory effect of DHT on IGF production and actions in bone cells. Thus, the decreased DHT might result in impaired stimulatory IGF system components. Therefore, it appears that the decreased DHT and the associated decrease in IGFs and IGFBP-3 production and/or actions might collectively contribute for the decreased bone formation and ultimately cause decreased BMD in osteoporotic men.

Secondary hyperparathyroidism contributes to osteoporosis in the elderly population [40]. The normal levels of PTH in osteoporotic men rule out the possibility of hyperparathyroidism in these subjects. Increase in bone resorption and bone formation markers recorded in this study reflects high bone turnover in osteoporotic men. IL-1β and TNF-α are potent stimulators of bone resorption, but also inhibit collagen synthesis although they increase DNA synthesis in bone [48]. These cytokines are also potent stimulators of prostaglandin production in bone [49] and such stimulation may result in a secondary increase in bone formation [48]. Since increased resorption, even when accompanied by increased formation, can cause bone loss [50], an increased bone turnover might result in decreased BMD.

The increase in IL-1β in osteoporotic men could be due to decreased DHT as our *in vitro* study demonstrated that DHT significantly decreased the secretion of IL-1β by SaOS-2 cells (unpublished data). Khalkhali-Ellis et al. [51] also reported that DHT exerts a suppressive effect on IL-1β induced IL-6 production by synoviocytes. In addition, IL-1- or PTH-stimulated PGE_2 production in mouse calvarial cells was suppressed by 5α-DHT [52]. Therefore, it is reasonable to suggest that decreased DHT might contribute to increased IL-1β in osteoporotic men. Our results demonstrated that decreased DHT in osteoporotic men was not associated with a significant increase in TNF-α. Takei et al. [53] reported that the treatment of human leukemia T cell line, Jurkat with 5α-DHT resulted in a 50% decrease in the level of TNF-α mRNA. Our recent *in vitro* study also demonstrated that DHT significantly decreased the secretion of TNF-α in the conditioned media of SaOS-2 cells (unpublished data). A significant increase in serum IL-1β might result in decreased BMD in osteoporotic men. Pearson correlation coefficients strongly support our results as both IL-1β and TNF-α are negatively associated with BMD. Our data on serum IL-4 and IFN-γ suggest that these cytokines are not associated with BMD in men. However, many *in vitro* and *in vivo* studies have shown that IL-4 and IFN-γ inhibit bone resorption [7,54]. Thus, it appears that the availability of cytokines at the bone microenvironment than at systemic level may determine its influence on bone function and regulate BMD.

The present investigation has revealed some important findings. In addition to the serum levels of total sex steroids, further studies on measurement of bioavailable hormones in osteoporotic subjects might reveal some additional information on sex steroids and their relation to BMD. The subjects participated in this study were chosen by convenience sampling method in a cross-sectional population. Probably, a longitudinal study of cohort population might provide stronger correlation between serum hormone profiles and BMD.

In conclusion, we demonstrate for the first time that DHT is an important determinant of BMD in men and suggests that non-aromatizable androgen has distinct effect from aromatizable androgen in bone. A strong positive correlation of serum DHT with BMD offers new perspectives in understanding the role of non-aromatizable androgen in the regulation of bone metabolism in men and might serve as a potential clinical marker in the diagnosis of male osteoporosis.

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References

[1] B.L. Riggs, S. Khosla, L.J. Melton 3rd, Sex steroids and the construction and conservation of the adult skeleton, Endocr. Rev. 23 (3) (2002) 279–302.
[2] F. Saad, L. Gooren, The role of testosterone in the metabolic syndrome: a review, J. Steroid Biochem. Mol. Biol. 114 (1–2) (2009) 40–43.
[3] L. Vandenput, A.G. Ederveen, R.G. Erben, K. Stahr, J.V. Swinnen, E. Van Herck, A. Verstuyf, S. Boonen, R. Bouillon, D. Vanderschueren, Testosterone prevents orchidectomy-induced bone loss in estrogen receptor-alpha knockout mice, Biochem. Biophys. Res. Commun. 285 (1) (2001) 70–76.
[4] P. Szulc, F. Munoz, B. Claustrat, P. Garnero, F. Marchand, F. Duboeuf, P.D. Delmas, Bioavailable estradiol may be an important determinant of osteoporosis in men: the MINOS study, J. Clin. Endocrinol. Metab. 86 (1) (2001) 192–199.
[5] L. Gennari, D. Merlotti, G. Martini, S. Gonnelli, B. Franci, S. Campagna, B. Lucani, N. Dal Canto, R. Valenti, C. Gennari, R. Nuti, Longitudinal association between sex hormone levels, bone loss, and bone turnover in elderly men, J. Clin. Endocrinol. Metab. 88 (11) (2003) 5327–5333.
[6] D. Goltzman, Studies on the mechanisms of the skeletal anabolic action of endogenous and exogenous parathyroid hormone, Arch. Biochem. Biophys. 473 (2) (2008) 218–224.
[7] G.D. Roodman, Role of cytokines in the regulation of bone resorption, Calcif. Tissue Int. 53 (Suppl. 1) (1993) S94–S98.
[8] J.E. Compston, Sex steroids and bone, Physiol. Rev. 81 (1) (2001) 419–447.
[9] P.M. Jehle, K. Schulten, W. Schulz, D.R. Jehle, S. Stracke, B. Manfras, B.O. Boehm, D.J. Baylink, S. Mohan, Serum levels of insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-1 to -6 and their relationship to bone metabolism in osteoporosis patients, Eur. J. Intern. Med. 14 (1) (2003) 32–38.
[10] F. Syed, S. Khosla, Mechanisms of sex steroid effects on bone, Biochem. Biophys. Res. Commun. 328 (3) (2005) 688–696.
[11] W.D. Leslie, R.A. Adler, G. El-Hajj Fuleihan, A.B. Hodsman, D.L. Kendler, M. McClung, P.D. Miller, N.B. Watts, Application of the 1994 WHO classification to populations other than postmenopausal Caucasian women: the 2005 ISCD Official Positions, J. Clin. Densitom. 9 (1) (2006) 22–30.
[12] S.B. Sufi, A. Donaldson, S.L. Jeffcoate, Testosterone Assay: World Health Organization Collaborating Center for Research and Reference Services in the Immunoassay of Hormones in Human Reproduction, 10th edition, 1986, pp. 57–69.
[13] M.A. Andersch, A.J. Szecypinski, Use of *p*-nitrophenyl phosphate as the substrate for determination of serum acid phosphatase, Am. J. Clin. Pathol. 17 (1947) 571–574.
[14] M. Tenniswood, C.E. Bird, A.F. Clark, Acid phosphatases: androgen dependent markers of rat prostate, Can. J. Biochem. 54 (4) (1976) 350–357.
[15] P.W. Thompson, J. Taylor, R. Oliver, A. Fisher, Quantitative ultrasound (QUS) of the heel predicts wrist and osteoporosis-related fractures in women age 45–75 years, J. Clin. Densitom. 1 (3) (1998) 219–225.
[16] P.D. Miller, E.S. Siris, E. Barrett-Connor, K.G. Faulkner, L.E. Wehren, T.A. Abbott, Y.T. Chen, M.L. Berger, A.C. Santora, L.M. Sherwood, Prediction of fracture risk in postmenopausal white women with peripheral bone densitometry: evidence from the National Osteoporosis Risk Assessment, J. Bone Miner. Res. 17 (12) (2002) 2222–2230.
[17] J.K. Amory, N.B. Watts, K.A. Easley, P.R. Sutton, B.D. Anawalt, A.M. Matsumoto, W.J. Bremner, J.L. Tenover, Exogenous testosterone or testosterone with finasteride increases bone mineral density in older men with low serum testosterone, J. Clin. Endocrinol. Metab. 89 (2) (2004) 503–510.

[18] J. Li, J. Kim, Molecular profiles of finasteride effects on prostate carcinogenesis, Cancer Prev. Res. (Phila Pa) 2 (6) (2009) 518–524.
[19] S. Issa, D. Schnabel, M. Feix, L. Wolf, H.E. Schaefer, D.W. Russell, H.U. Schweikert, Human osteoblast-like cells express predominantly steroid 5alpha-reductase type 1, J. Clin. Endocrinol. Metab. 87 (12) (2002) 5401–5407.
[20] K. Hanada, K. Furuya, N. Yamamoto, H. Nejishima, K. Ichikawa, T. Nakamura, M. Miyakawa, S. Amano, Y. Sumita, N. Oguro, Bone anabolic effects of S-40503, a novel nonsteroidal selective androgen receptor modulator (SARM), in rat models of osteoporosis, Biol. Pharm. Bull. 26 (11) (2003) 1563–1569.
[21] R.A. Anderson, A.M. Wallace, N. Sattar, N. Kumar, K. Sundaram, Evidence for tissue selectivity of the synthetic androgen 7 alpha-methyl-19-nortestosterone in hypogonadal men, J. Clin. Endocrinol. Metab. 88 (6) (2003) 2784–2793.
[22] A. LaMorte, N. Kumar, C.W. Bardin, K. Sundaram, Aromatization of 7 alpha-methyl-19-nortestosterone by human placental microsomes in vitro, J. Steroid Biochem. Mol. Biol. 48 (2–3) (1994) 297–304.
[23] J. Dequeker, P. Geusens, Anabolic steroids and osteoporosis, Acta Endocrinol. Suppl. (Copenh.) 271 (1985) 45–52.
[24] H.M. Behre, S. Kliesch, E. Leifke, T.M. Link, E. Nieschlag, Long-term effect of testosterone therapy on bone mineral density in hypogonadal men, J. Clin. Endocrinol. Metab. 82 (8) (1997) 2386–2390.
[25] H.W. Daniell, S.R. Dunn, D.W. Ferguson, G. Lomas, Z. Niazi, P.T. Stratte, Progressive osteoporosis during androgen deprivation therapy for prostate cancer, J. Urol. 163 (1) (2000) 181–186.
[26] D. Vanderschueren, L. Vandenput, S. Boonen, E. Van Herck, J.V. Swinnen, R. Bouillon, An aged rat model of partial androgen deficiency: prevention of both loss of bone and lean body mass by low-dose androgen replacement, Endocrinology 141 (5) (2000) 1642–1647.
[27] L. Basurto, A. Zarate, R. Gomez, C. Vargas, R. Saucedo, R. Galvan, Effect of testosterone therapy on lumbar spine and hip mineral density in elderly men, Aging Male 11 (3) (2008) 140–145.
[28] L.C. Hofbauer, S. Khosla, Androgen effects on bone metabolism: recent progress and controversies, Eur. J. Endocrinol. 140 (4) (1999) 271–286.
[29] D. Mellstrom, O. Johnell, O. Ljunggren, A.L. Eriksson, M. Lorentzon, H. Mallmin, A. Holmberg, I. Redlund-Johnell, E. Orwoll, C. Ohlsson, Free testosterone is an independent predictor of BMD and prevalent fractures in elderly men: MrOS Sweden, J. Bone Miner. Res. 21 (4) (2006) 529–535.
[30] M.J. McPhaul, M. Young, Complexities of androgen action, J. Am. Acad. Dermatol. 45 (3 Suppl.) (2001) S87–S94.
[31] J.E. Compston, Bone marrow and bone: a functional unit, J. Endocrinol. 173 (3) (2002) 387–394.
[32] D. Vanderschueren, E. Van Herck, A.M. Suiker, W.J. Visser, L.P. Schot, K. Chung, R.S. Lucas, T.A. Einhorn, R. Bouillon, Bone and mineral metabolism in the androgen-resistant (testicular feminized) male rat, J. Bone Miner. Res. 8 (7) (1993) 801–809.
[33] R.T. Turner, G.K. Wakley, K.S. Hannon, Differential effects of androgens on cortical bone histomorphometry in gonadectomized male and female rats, J. Orthop. Res. 8 (4) (1990) 612–617.
[34] J.R. Center, T.V. Nguyen, P.N. Sambrook, J.A. Eisman, Hormonal and biochemical parameters and osteoporotic fractures in elderly men, J. Bone Miner. Res. 15 (7) (2000) 1405–1411.
[35] E. Legrand, C. Hedde, Y. Gallois, I. Degasne, F. Boux de Casson, E. Mathieu, M.F. Basle, D. Chappard, M. Audran, Osteoporosis in men: a potential role for the sex hormone binding globulin, Bone 29 (1) (2001) 90–95.
[36] C.W. Slemenda, C. Longcope, L. Zhou, S.L. Hui, M. Peacock, C.C. Johnston, Sex steroids and bone mass in older men. Positive associations with serum estrogens and negative associations with androgens, J. Clin. Invest. 100 (7) (1997) 1755–1759.
[37] J.R. Center, T.V. Nguyen, P.N. Sambrook, J.A. Eisman, Hormonal and biochemical parameters in the determination of osteoporosis in elderly men, J. Clin. Endocrinol. Metab. 84 (10) (1999) 3626–3635.
[38] S. Amin, Y. Zhang, D.T. Felson, C.T. Sawin, M.T. Hannan, P.W. Wilson, D.P. Kiel, Estradiol, testosterone, and the risk for hip fractures in elderly men from the Framingham Study, Am. J. Med. 119 (5) (2006) 426–433.
[39] H.A. Fink, S.K. Ewing, K.E. Ensrud, E. Barrett-Connor, B.C. Taylor, J.A. Cauley, E.S. Orwoll, Association of testosterone and estradiol deficiency with osteoporosis and rapid bone loss in older men, J. Clin. Endocrinol. Metab. 91 (10) (2006) 3908–3915.
[40] S. Boonen, S. Mohan, J. Dequeker, J. Aerssens, D. Vanderschueren, G. Verbeke, P. Broos, R. Bouillon, D.J. Baylink, Down-regulation of the serum stimulatory components of the insulin-like growth factor (IGF) system (IGF-I, IGF-II, IGF binding protein [BP]-3, and IGFBP-5) in age-related (type II) femoral neck osteoporosis, J. Bone Miner. Res. 14 (12) (1999) 2150–2158.
[41] H.P. Guler, J. Zapf, E. Scheiwiller, E.R. Froesch, Recombinant human insulin-like growth factor I stimulates growth and has distinct effects on organ size in hypophysectomized rats, Proc. Natl. Acad. Sci. U.S.A. 85 (13) (1988) 4889–4893.
[42] L.J. Ghiron, J.L. Thompson, L. Holloway, R.L. Hintz, G.E. Butterfield, A.R. Hoffman, R. Marcus, Effects of recombinant insulin-like growth factor-I and growth hormone on bone turnover in elderly women, J. Bone Miner. Res. 10 (12) (1995) 1844–1852.
[43] S. Mohan, J.R. Farley, D.J. Baylink, Age-related changes in IGFBP-4 and IGFBP-5 levels in human serum and bone: implications for bone loss with aging, Prog. Growth Factor Res. 6 (2–4) (1995) 465–473.
[44] J.A. Janssen, P. Uitterlinden, L.J. Hofland, S.W. Lamberts, Insulin-like growth factor I receptors on blood cells: their relationship to circulating total and "free" IGF-I, IGFBP-1, IGFBP-3 and insulin levels in healthy subjects, Growth Horm. IGF Res. 8 (1) (1998) 47–54.
[45] E.S. Kurland, F.K. Chan, C.J. Rosen, J.P. Bilezikian, Normal growth hormone secretory reserve in men with idiopathic osteoporosis and reduced circulating levels of insulin-like growth factor-I, J. Clin. Endocrinol. Metab. 83 (7) (1998) 2576–2579.
[46] F. Gori, L.C. Hofbauer, C.A. Conover, S. Khosla, Effects of androgens on the insulin-like growth factor system in an androgen-responsive human osteoblastic cell line, Endocrinology 140 (12) (1999) 5579–5586.
[47] C. Kasperk, R. Fitzsimmons, D. Strong, S. Mohan, J. Jennings, J. Wergedal, D. Baylink, Studies of the mechanism by which androgens enhance mitogenesis and differentiation in bone cells, J. Clin. Endocrinol. Metab. 71 (5) (1990) 1322–1329.
[48] E. Canalis, Interleukin-1 has independent effects on deoxyribonucleic acid and collagen synthesis in cultures of rat calvariae, Endocrinology 118 (1) (1986) 74–81.
[49] A.H. Tashjian Jr., E.F. Voelkel, M. Lazzaro, D. Goad, T. Bosma, L. Levine, Tumor necrosis factor-alpha (cachectin) stimulates bone resorption in mouse calvaria via a prostaglandin-mediated mechanism, Endocrinology 120 (5) (1987) 2029–2036.
[50] S. Harada, G.A. Rodan, Control of osteoblast function and regulation of bone mass, Nature 423 (6937) (2003) 349–355.
[51] Z. Khalkhali-Ellis, R.J. Handa, R.H. Price Jr., B.D. Adams, J.J. Callaghan, M.J. Hendrix, Androgen receptors in human synoviocytes and androgen regulation of interleukin 1beta (IL-1beta) induced IL-6 production: a link between hypoandrogenicity and rheumatoid arthritis? J. Rheumatol. 29 (9) (2002) 1843–1846.
[52] C.C. Pilbeam, L.G. Raisz, Effects of androgens on parathyroid hormone and interleukin-1-stimulated prostaglandin production in cultured neonatal mouse calvariae, J. Bone Miner. Res. 5 (11) (1990) 1183–1188.
[53] S. Takei, A. Redford, S. Katayama, H. Toyoda, Destabilization of tumor necrosis factor-alpha mRNA by 5-alpha dihydrotestosterone in Jurkat cells, Life. Sci. 66 (20) (2000) PL277–282.
[54] D.L. Lacey, J.M. Erdmann, S.L. Teitelbaum, H.L. Tan, J. Ohara, A. Shioi, Interleukin 4, interferon-gamma, and prostaglandin E impact the osteoclastic cell-forming potential of murine bone marrow macrophages, Endocrinology 136 (6) (1995) 2367–2376.