Title

U-shaped association of plasma testosterone, and no association of plasma estradiol, with incidence of fractures in men

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Key words

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Disclosure summary

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Abstract

Purpose

Whether androgens, distinct from oestrogen, maintain bone health during male ageing has implications for understanding osteoporosis. We assessed associations of different sex hormones with incidence of any bone fracture or hip fracture in older men.

Participants and methods

Analysis of 3,307 community-dwelling men aged 76.8±3.5 years, median follow-up period of 10.6 years. Plasma testosterone (T), dihydrotestosterone (DHT) and estradiol (E2) assayed by mass spectrometry, sex hormone-binding globulin (SHBG) and luteinising hormone (LH) using immunoassay. Incident fractures determined via data linkage. We analysed probability of fracture and performed Cox regression adjusted for age, medical comorbidities and frailty.

Results

Incident fractures occurred in 330 men, including 144 hip fractures. Probability plots suggested non-linear relationships between hormones and risk of any fracture and hip fracture, with higher risk at lower and higher plasma T, lower E2, higher SHBG and higher LH. In fully-adjusted models, there was a U-shaped association of plasma T with incidence of any fracture (Quartile 2 [Q2] vs Q1: fully-adjusted hazard ratio [HR]=0.69, 95% confidence interval [CI]=0.51-0.94, p=0.020; Q3: HR=0.59, CI=0.42-0.83, p=0.002) and hip fracture (Q2 vs Q1: HR=0.60, CI=0.37-0.93, p=0.043; Q3: HR=0.52, CI=0.31-0.88, p=0.015). DHT, E2 and LH were not associated with fracture. Higher SHBG was associated with hip fracture (Q4 vs Q1: HR=1.76, CI=1.05-2.96, p=0.033).

Conclusions

Mid-range plasma T was associated with lower incidence of any fracture and hip fracture, and higher SHBG with increased risk of hip fracture. Circulating androgen rather than estrogen represents a biomarker for hormone effects on bone driving fracture risk.

Precis

In 3,307 community-dwelling older men, mid-range plasma testosterone was associated with lower incidence of any fracture, and hip fracture. Plasma estradiol did not predict fracture risk.

Introduction

Osteoporosis affects both men and women, imposing a large burden of morbidity and increasing mortality risk due to weakened bone microarchitecture predisposing to fractures. In men, hypogonadism resulting from hypothalamic, pituitary or testicular diseases is a recognised cause of osteoporosis [1]. Pituitary luteinising hormone (LH) stimulates testicular production of testosterone (T), and the major portion of T in the circulation is bound to sex hormone-binding globulin (SHBG). Actions of T are mediated via its binding to the androgen receptor, and by conversion of T to dihydrotestosterone (DHT, a potent ligand for androgen receptors) and to estradiol (E2, a ligand for estrogen receptors). In androgen deficient men T treatment relieves symptoms and signs of androgen deficiency [1] and in older men with lower T concentrations without recognisable reproductive pathology it improves bone strength and bone mineral density [2].

The influence of sex hormones on bone extends beyond men with organic hypogonadism, to the wider male population. There have been several studies examining the relationship between sex hormone concentrations and bone turnover markers. In young men, circulating T concentrations correlated with the bone turnover marker total osteocalcin, while E2 correlated inversely with total osteocalcin, type I procollagen aminoterminal propeptide (P1NP) and urinary type 1 collagen aminoterminal telopeptide [3]. In older men, T did not correlate with bone turnover markers, while E2 correlated inversely with total and undercarboxylated osteocalcin and with collagen type 1 C-terminal cross-linked telopeptide CTX [4]. SHBG correlated with bone turnover markers in both young and older men [3,4], and older men with either low T or low E2 were more likely to lose bone mineral density at the hip [5].

Several studies have also examined the relationship between sex steroids and SHBG with fracture risk. In a study of 609 men aged >60 years, the risk of low trauma fracture was higher in men with low T or low E2 concentrations [6]. However, in 1,882 men aged ≥65 years, those with lower bioavailable E2 or higher SHBG had higher risk of fracture [7]. In 2,639 men with mean age 75.4 years, lower T, lower E2 or higher SHBG were associated with increased fracture risk in age-adjusted analyses, but only lower free E2 and higher SHBG remained associated with fracture risk in the fully-adjusted model [8]. More recently in a study of 1,657 men aged ≥70 years, neither T nor E2 were associated with fracture risk [9]. Furthermore, in that study the temporal increase in SHBG, but not T or E2, was predictive of incident fracture [10]. Therefore, the results of existing studies have not been consistent and the relative importance of circulating androgens compared to oestrogens for fracture risk in men remains uncertain.

In this study, we tested the hypothesis that exposure to circulating sex hormones predicts incidence of fractures in older men most at risk of osteoporosis.

Participants and methods

Study population

The Health In Men Study (HIMS) is a cohort of community-dwelling men from Perth, Western Australia [11]. Men aged 65 years or more were randomly selected from the electoral roll and invited to participate (voting is compulsory in Western Australia). There were 12,203 participating men assessed in 1996-99. Of these, 4,248 attended for re-assessment and had blood samples collected in 2001-04, comprising the cohort for this analysis. The date of blood sampling in 2001-04 represents the baseline time-point. Participants were predominantly white. The University of Western Australia Human

Research Ethics Committee approved the study, and all men provided written informed consent.

Assessment of medical comorbidities

Medical data collected in 2001-04 were used to identify men with a history of cardiovascular disease (CVD), orchidectomy, prostate cancer, osteoporosis or bone fracture, and Paget's disease of the bone. Questionnaire data were analysed to identify men receiving androgens or antiandrogen therapy, bisphosphonates, or glucocorticoids. Height, weight and blood pressure were measured using standard procedures, and body mass index (BMI) calculated as weight divided by the square of height (kg/m^2). Men were considered to have hypertension if they reported this diagnosis or the use of antihypertensive medication or had blood pressure ≥140/90 mm Hg. Dyslipidemia was defined as having fasting high-density lipoprotein cholesterol <0.9 mmol/L, low-density lipoprotein cholesterol ≥3.4 mmol/L, triglycerides ≥1.8 mmol/L, total cholesterol ≥5.5 mmol/L or receiving lipid-lowering therapy. Men diagnosed with diabetes, reporting the use of blood glucose–lowering medication, or with fasting or non-fasting blood glucose ≥7.0 mmol/L or ≥11.1 mmol/L, respectively, were considered to have diabetes [12]. Prevalent CVD was defined as a self-reported history of angina, acute myocardial infarction, stroke, or abdominal aortic aneurysm by questionnaire responses or diagnoses of these conditions prior to baseline. Prevalent depression was defined according self-reported history of depression by questionnaire, or diagnosis of this condition prior to baseline.

Assessment of frailty

We assessed frailty in 2001-04 with the FRAIL scale, as previously described [13]. Briefly, this assesses five domains: 1) fatigue, 2) resistance (ability to climb a single flight of stairs),

3) ambulation (ability to walk one block), 4) illnesses (more than five), and 5) loss of weight (more than 5%). Participants scored positive for loss of weight if their weight decreased by more than 5% between assessments at 1996-99 and 2001-04. We considered participants to be frail if they presented problems in three or more of these five domains. The FRAIL scale has been validated against the outcome of incident disability and mortality in older men [13].

Ascertainment of incident fractures

Incident fractures occurring after the baseline date were ascertained using the Western Australian Data Linkage System (WADLS), which provides electronic linkage to records from death, hospital, emergency department and cancer registries [14]. WADLS captures emergency department presentations and admissions to all public and private hospitals in Western Australia. WADLS was used to identify emergency department presentations and hospital admissions resulting from fractures (Table 1) which were associated with falls (International Statistical Classification of Diseases and Related Health Problems, 9th revision [ICD-9]: E880 to E886, E888; or ICD-10-Australian Modification [AM]: W00 to W19), after excluding pathological fractures (ICD-9: 733.1 and 198.5 or ICD-10: M84.4 and M90.7). There were two main outcomes of interest. One was the composite outcome of all incident fractures reflecting the full dataset. The other was incident hip fracture, a major cause of osteoporosis-related morbidity and mortality which is routinely managed in hospital and therefore accurately tracked via WADLS. Follow-up was from baseline in 2001-04 until 31 December 2016.

TABLE 1

Laboratory assays

Blood samples were collected between 0800 and 1030 h at baseline (2001-04), and serum and plasma prepared immediately after phlebotomy and stored at -80^{o}C until assayed. T, DHT, E2, SHBG and LH were assayed as previously described [15]. Briefly, plasma T, DHT and E2 were quantified using liquid chromatography-tandem mass spectrometry (LC-MS) in a single run without derivatisation using atmospheric pressure photoionization in positive mode for androgens and negative mode for estrogens [16]. Calibration standards, including the certified reference material M914b for testosterone, were obtained from the National Measurement Institute (North Ryde, New South Wales, Australia). M914b was approved by the Centers for Disease Control and Prevention as an appropriate standard for testosterone LC-MS assays [17]. Interassay coefficients of variation (CVs; percentage) were as follows: T 3.9% at 29.8 nmol/L, 6.8% at 5.9 nmol/L, and 6.5% at 2.0 nmol/L; DHT 6.7% at 29.5 nmol/L, 9.1% at 5.7 nmol/L and 13.4% at 1.9 nmol/L; and E2 4.8% at 1,568 pmol/L, 8.1% at 308 pmol/L and 8.6% at 103 pmol/L [15]. SHBG and LH were determined by chemiluminescent immunoassay on an Immulite 2000 analyser (Diagnostic Products Corp., Biomediq, Doncaster, Australia). The interassay CV for SHBG was 6.7% at 5.2 nmol/L and 6.2% at 81 nmol/L, and for LH 6.4% at 2.3 IU/L and 5.8% at 19 IU/L. Free T was calculated using an empirical algorithm (FTZ) as previously described [15,18].

Statistical analysis

The statistical package Stata (version 12.1; StataCorp) was used to analyse the data. Baseline characteristics are shown as mean ± SD or numbers and percentages for the cohort as a whole, and stratified according to whether or not men experienced any incident fracture during follow-up. Means comparisons were performed using two-sample *t*-tests with equal variances, and categorical variables were compared using Chi-square tests. Associations of sex hormones with fracture were graphically studied by estimating the probability of

experiencing any fracture, or hip fracture, according to plasma concentrations of T, calculated free T (cFT), DHT, E2, SHBG and LH modelled by logistic regression using a restricted cubic spline with 3 knots. Cox proportional hazards regression was performed to assess the independent associations of quartiles of T, cFT, DHT, E2, SHBG and LH with incidence of any fracture, and of hip fracture, adjusting for variables that might possibly confound the results, including age, smoking, alcohol, hypertension, dyslipidemia, prevalent diabetes, prevalent cardiovascular disease, depression, frailty, body mass index (BMI), waist, creatinine and vitamin D. To ensure results were not influenced by high outliers each analysis was trimmed to exclude men who had a value for that specific hormone in the lowest and highest 1%. A two-tailed P value <0.05 was considered significant.

Results

Characteristics of the study population

From the 12,203 men who took part in the first wave of the study, 5,570 returned for assessment in 2001-04 (45.6% of the original cohort), of whom 4,248 provided a blood sample (see Supplementary Figure S1 in [19]). Of these 4,248 men, 4,230 had plasma levels of total T, DHT, and E2 assayed by LC-MS. From these, men receiving androgens or antiandrogen therapy (103), or with a history of orchidectomy (51) or prostate cancer (386) were excluded. Of the remaining 3,690 men, those with known osteoporosis, previous bone fracture, Paget's disease of the bone, or receiving bisphosphonates or glucocorticoids were excluded, leaving 3,307 men for analysis. Mean age (±SD) was 76.8 ± 3.5 years.

Over a median follow-up period of 10.6 years, 330 men experienced an incident fracture of any type, including 144 men who experienced an incident hip fracture. In 30,355 participant-years of follow-up, the incidence of any fracture was 1.1% per participant per year. The

incidence of hip fracture was 0.5% per participant per year. Baseline characteristics of the study cohort as a whole, and stratified according to whether or not men experienced an incident fracture, are shown (Table 2). Men who experienced any incident fracture were older (77.7 vs 76.8 years) and more likely to be frail (22.0 vs 12.7%), have depression (14.6 vs 9.2%) or have diabetes (17.6 vs 14.9%). Men who experienced any incident fracture had lower baseline T and cFT, and higher LH, compared with men who did not experience a fracture. Men were vitamin D replete and there was no difference in vitamin D concentrations between groups.

TABLE 2

Probability of any fracture according to baseline sex hormone concentrations

Men with lower T concentrations had a higher risk of any fracture but the risk was not progressively lower with higher T concentrations (Figure 1A). A similar relationship was seen with cFT (Figure 1B). DHT was not associated with fracture risk (Figure 1C). There were equivocal trends for men with lower E2 appearing to have a higher risk of any fracture (Figure 1D), and for men with higher SHBG or higher LH appearing to have a higher risk of any fracture (Figures 1E and F).

FIGURE 1

Probability of hip fracture according to baseline sex hormone concentrations

There was a U-shaped relationship between T concentrations and risk of hip fracture (Figure 2A). A similar pattern was seen with cFT (Figure 2B). There was no association of DHT with risk of hip fracture (Figure 2C). There were equivocal trends for men with lower E2

appearing to have a higher risk of hip fracture (Figure 2D), and men with higher SHBG or higher LH appearing to have a higher risk of hip fracture (Figure 2E and F).

FIGURE 2

Multivariable analysis of baseline sex hormone concentrations with incidence of any fracture

In regression models adjusting for age and other covariates including frailty (Table 3), men with T concentrations in the middle of the range had the lowest hazard ratio (HR) for incidence of any fracture (fully-adjusted HR: quartile 2 [Q2] vs Q1 0.69, p=0.020; Q3 vs Q1 0.59, p=0.002). Risk of any fracture was not different in men with T concentrations in Q4 vs Q1 (HR 0.85, p=0.335). Risk of any fracture was lowest in men with cFT in the third quartile (fully-adjusted HR Q3 vs Q1 0.67, p=0.018), with no difference for Q2 or Q4 vs Q1. Of note, DHT, E2, SHBG and LH were not associated with risk of any fracture. Exclusion of finger fractures did not alter the results (data not shown).

TABLE 3

Multivariable analysis of baseline sex hormone concentrations with incidence of hip fracture

In regression models adjusting for age and other covariates including frailty (Table 4), men with T concentrations in the middle of the range had the lowest HR for incidence of hip fracture (fully-adjusted HR: Q2 vs Q1: 0.60, p=0.43; Q3 vs Q1 0.52, p=0.015). Risk of hip fracture was not different in men with T concentrations in Q4 vs Q1 (HR 1.04, p=0.866). Men with SHBG in the highest quartile had an increased risk of hip fracture (fully-adjusted HR: Q4 vs Q1 1.76, p=0.033), with no difference in risk for Q2 or Q3 vs Q1. cFT, DHT, E2 and LH were not associated with risk of hip fracture.

TABLE 4

Supplementary analyses

The U-shaped association of total T with incidence of any fracture, and hip fracture, was robust to inclusion of either SHBG or E2 into the fully-adjusted model (Supplementary Table S1 in [19]). No association of E2 with incidence of any fracture or hip fracture was found when either SHBG or T was included in the fully-adjusted model (Supplementary Table S2 in [19]). There was no association of E2 analysed as a continuous variable, with incidence of any fracture, or of hip fracture (per 1 SD increase in E2: HR=0.94, 95% confidence interval 0.83-1.06, p=0.325; and 0.92, 0.76-1.10, p=0.353, respectively, both remaining non-significant in age-adjusted and fully-adjusted models). The association of higher SHBG with incidence of hip fracture was largely unchanged when T was included in the fully-adjusted model, but was attenuated when E2 was included in the model; while HR was similar the confidence interval was wider (Supplementary Table S3 in [19]).

Exploratory analyses

When the composite outcome of vertebral, hip, humerus and forearm fractures was analysed, there was a U-shaped association of T (men with values of T in Q3 had the lowest incidence of these fractures) and no association of E2 with this outcome (Supplementary Table S4 in [19]). There were relatively fewer incident fractures in the first 5 years of follow-up, and more occurred after 5 years (Supplementary Tables S5 and S6 in [19]). The U-shaped association of T with incident any fracture, and hip fracture, remained when fractures occurring after 5 years were assessed with the lowest risk in men with T in Q3 (Supplementary Table S5 in [19]). The association of higher SHBG with incident hip fracture

was attenuated when fractures occurring within and after 5 years were analysed separately (Supplementary Table S6 in [19]).

Discussion

There was a non-linear association of baseline T concentrations with incidence of any fracture, and of hip fracture, in our cohort of community-dwelling older men. Men with mid-range plasma T concentrations had the lowest risk of any fracture and of hip fracture, consistent with a U-shaped association. Men with high SHBG had an increased risk of hip fracture. Results for cFT mirrored those of T for any fracture, but not for hip fracture, while DHT, E2 and LH were not predictive of fracture risk.

These results differ from previous studies, in the nature of the non-linear association of T with incidence of fracture, and the lack of an association of E2 with this outcome. In the Dubbo study of 609 men aged ≥60 years, 113 men had a low-trauma fracture and lower T was associated independently with risk of hip and of non-vertebral fractures [6]. In that study, lower E2 was associated with fracture risk in unadjusted analysis, but not after adjustment for age and weight. In the US Osteoporotic Fractures in Men Study (MrOS) analysis of 1,978 men aged ≥65 years, in whom 102 incident fractures occurred, lower calculated bioavailable E2 was associated with non-vertebral fracture risk, and higher SHBG with risk of both non-vertebral and hip fractures [7]. Men with calculated bioavailable T in the lowest quartile had an increased risk of non-vertebral fracture after adjusting for age, race and BMI, but not after further adjustment for bioavailable E2 [7]. Men with both low bioavailable T and high SHBG had increased risk of non-vertebral fracture. A more recent analysis from US MrOS found no association of total T with loss of hip bone mineral density, while the rate of hip bone mineral density loss increased in men with higher SHBG [20].

In the MrOS Sweden study of 2,639 men aged ≥75 years, 209 men had an incident fracture including 38 hip fractures [8]. In multivariable models both lower calculated free E2 and higher SHBG were associated independently with risk of any fracture, and lower free E2 with risk of hip fracture. Another analysis from MrOS Sweden and MrOS Hong Kong associated higher SHBG, but not T or E2, with incidence of clinical vertebral fractures [21]. In the EPIC-Oxford prospective study of 155 men who had incident fracture and 309 controls, lower calculated free E2 at baseline was associated with higher relative risk of fracture on follow-up [22]. In the Concord Health and Aging in Men Project (CHAMP) of 1,705 men aged ≥70 years, 171 men had an incident fracture including 44 hip fracture [9]. Neither T, DHT nor E2, nor SHBG, were associated with any fracture or hip fracture in multivariable-adjusted models. Progressive longitudinal increases in SHBG (but not T or E2) were associated with incidence of any fracture, and of hip fracture in CHAMP [10].

Therefore, our findings add to the evidence that circulating T is independently associated with the incidence of any fracture and with hip fracture in older men, while E2 was not associated with fracture risk. When the composite outcome of vertebral, hip, humerus or forearm fractures was analysed, the U-shaped association of T with fracture outcomes remained. It is possible that our findings differ from other studies due to the larger size of the HIMS cohort of older men, with extended follow up yielding larger numbers of outcome events (any fractures N=330, and hip fractures N=144). Larger numbers of outcome events facilitate longitudinal analyses of this type, allowing associations of hormones with fractures to be defined more precisely. In contrast to the Dubbo study, we found that the risk of any fracture and of hip fracture was lowest in men with T in the middle two quartiles. Men with T in the highest quartile had similar risk of any fracture, and of hip fracture, as men with T in

the lowest quartile. Thus men with T concentrations in the middle of the normal range, rather than at the upper end of the range, had the lowest risk of fracture.

Hypogonadism is a recognised cause of osteoporosis in men [23]. Both androgen and oestrogen receptors are expressed in human osteoblast-like cells, and oestrogen receptors in osteoclast-like cells [24,25]. Of note, men with genetic mutations of the estrogen receptor or the aromatase gene have low bone mass [26,27]. Furthermore, the beneficial effects of T treatment on bone density in hypogonadal men are mediated substantively via aromatisation of T into E2 [28]. In one study men treated with the non-aromatisable androgen DHT showed a reduction in spine bone mineral density [29], and men treated with aromatase inhibitors are at risk of bone loss [30]. The role of E2 is supported by Mendelian randomisation analyses in which genetically determined higher E2 is associated with higher bone mineral density, and with lower fracture risk in men [31,32]. However, in our study circulating E2 was not associated with incidence of any fracture or hip fracture. This is contrary to previous studies and the reasons for this are unclear. We postulate that in older men, circulating E2 may not be fully indicative of E2 exposure in specific tissues. Nevertheless, older men with lower circulating T concentrations were at risk of fracture, likely reflecting reduced substrate for aromatisation to E2 and hence reduced exposure to E2 at a tissue level. The finding that older men with higher circulating T concentrations were also at risk of fracture is intriguing. It is possible to speculate that these men might have reduced conversion of T to E2 at a tissue level, hence be vulnerable to fracture. Mechanistic studies at different levels of T (beyond the scope of the present analysis) would be needed to explore this result.

Our findings with regard to SHBG are consistent with other studies in men, where higher SHBG or an increase in SHBG are associated with greater risk of fractures including hip

fractures [7,8,10]. In the analyses from MrOS US and MrOS Sweden, both lower E2 and higher SHBG were associated with fracture risk [7,8]. In the analysis from CHAMP, higher SHBG was associated with incident hip fracture, while T and E2 were not associated [10]. Higher SHBG was also associated with vertebral fracture in analyses from MrOS US and MrOS Sweden, in those analyses neither T nor E2 were associated with vertebral fracture risk [21,33]. As we had a limited number of vertebral fractures in out cohort, we did not conduct a separate analysis of this outcome. Causality is supported by a Mendelian randomisation study in which polymorphisms in the SHBG gene promoter were associated with SHBG concentrations and with bone mineral density [34]. Overall, our results suggest that T and SHBG are the main hormonal factors associated with incidence of fracture. SHBG might impact on fracture risk via its role as a carrier protein influencing androgen concentrations both in the circulation and in peripheral target tissues [35].

Of note, results for cFT resembled those for total T for incidence of any fracture, but there was no association of cFT with hip fracture. Therefore, total T appears to be the more informative measure of circulating androgen. The finding that associations of total T with incidence of any fracture and of hip fracture were robust to adjustment for SHBG support the concept of androgen having an influence on these outcomes. In the HIMS cohort, LH was inversely associated with total T concentration [15]. However, in our regression analyses there was no association of LH with incidence of any fracture, or hip fracture. Therefore, circulating T not LH appears to influence fracture risk in older men. We opted not to calculate free or bioavailable E2, as there is limited validation data available for the accuracy of these calculations in relation to free E2 measured by gold standard methodology such as equilibrium dialysis, particularly in men [36]. Instead, we analysed total E2 measured using LC-MS, including in the analyses adjustment for SHBG.

We noted there was a higher baseline prevalence of depression and diabetes in men who experienced an incident fracture, compared to men who did not. Both diabetes and depression have been associated with lower circulating T concentrations in men [37,38]. There is a recognised association of diabetes with increased fracture risk [39]. Depression is also associated with increased fracture risk [40]. We adjusted for both diabetes and depression in our analyses, thus the observed associations of total T and SHBG with fracture outcomes were independent of these factors.

This study has several limitations. This is an observational study, therefore causality cannot be established. Men who provided blood samples in 2001-04, had participated in the earlier wave of the study in 1996-99, therefore a "healthy survivor" effect may be present making our results more applicable to generally healthier older men. Hormone assays were performed on a single baseline blood sample, so serial measurements of hormones over time were not available. Nevertheless, a single blood sample can be an informative marker of hormonal status [41]. Our rate of any fracture, and of hip fracture, was relatively low. Although X-rays were not obtained for adjudication, misdiagnosis rates are likely to be very low as all imaging would have been assessed by specialist radiologists, and their reports used as the basis for the diagnosis and coding of fractures. Men who experienced fractures but neither presented to an emergency department nor required hospital admission, would probably have been missed. Many vertebral fractures may remain asymptomatic or may present with back pain not requiring an emergency visit or hospitalisation, so vertebral fractures are likely under-counted. Thus our endpoints likely reflect more clinically significant fracture events, and may also reflect a lower rate in generally healthier men. While the ascertainment of outcomes was based on emergency department presentations and hospital admissions resulting from

fractures associated with falls, we did not obtain a history of preceding falls. Bone mineral densitometry data were not collected in HIMS. Men in HIMS were largely Caucasian, thus we cannot extrapolate our findings to men of other ethnicities, nor to women.

Strengths of the study include the large sample size and duration of follow-up, resulting in substantial number of outcome events for analysis of incidence of any fracture and hip fractures. We measured sex hormones accurately using mass spectrometry, that has advantages over immunoassay particularly for assessment of lower hormone concentrations found in older men [42-44]. To reduce the risk of confounding, men with prevalent bone fractures or known osteoporosis were excluded. Additionally, we adjusted for age and medical comorbidities, and also for presence of frailty in our analyses. WADLS covers all emergency department presentations and hospital admissions to both public and private hospitals in Western Australia, and few men of this age emigrate from the state thus enabling comprehensive coverage for outcomes [45].

In conclusion, we found that mid-range plasma T is associated with lower incidence of any fracture and hip fracture, and higher SHBG with increased risk of hip fracture. Thus exposure to circulating androgen rather than oestrogen predicted fracture risk in this cohort of older men. In older men with low-normal circulating T in the absence of organic hypogonadism, treatment with T increases bone mineral density [2], but a randomised controlled trial adequately powered for the outcome of fracture is lacking [46]. In the light of our findings, such future studies should recruit men with baseline T in the lowest quartile of values, and explore whether the degree to which exogenous T is aromatised at the tissue level predicts its beneficial effect on bone.

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Data availability

Restrictions apply to the availability of data analysed during this study to preserve participant confidentiality. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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Figure legends

Figure 1

Probability of experiencing any fracture according to plasma concentrations of testosterone (A), calculated free testosterone (B), dihydrotestosterone (C), estradiol (D), sex hormone-binding globulin (E) and luteinising hormone (F) in 3,307 community-dwelling older men. Shaded areas represent 95% confidence intervals. Data have not been adjusted for other variables.

Figure 2

Probability of experiencing a hip fracture according to plasma concentrations of testosterone (A), calculated free testosterone (B), dihydrotestosterone (C), estradiol (D), sex hormone-binding globulin (E) and luteinising hormone (F) in 3,307 community-dwelling older men. Shaded areas represent 95% confidence intervals. Data have not been adjusted for other variables.

Table 1

Types and numbers of incident fractures with their International Statistical Classification of Diseases and Related Health Problems, 9th revision (ICD-9) and 10th revision (ICD-10) codes.

Fractures	**N***	**ICD-9**	**ICD-10**
Finger	6	816	S62.0, S62.1, S62.5, S62.6, S62.9
Hand	1	815,817	S62.2, S62.3
Wrist	0	814	S62.0, S62.1, S62.9
Forearm	13	813.00, 813.03, 813.04, 813.05, 813.06, 813.07, 813.08, 813.1, 813.2, 813.3, 813.4, 813.5, 813.8, 813.9	S52.09, S52.2, S52.3, S52.9, S52.1, S52.5, S52.6
Elbow	2	812.4, 812.5	S52.02, S52.03, S52.04, S42.4
Humerus	24	812.0, 812.1, 812.2, 812.3	S42.2, S42.3, S42.9
Clavicle	6	810	S42.0
Pelvis	34	808	S32.3, S32.4, S32.5, S32.6, S32.8
Hip	144	820	S72.0, S72.1, S72.2
Femur	13	821	S72.3, S72.4, S72.9, S72.8
Knee	10	822	S82.0
Tibia	17	822, 823	S82.0, S82.86, S82.1, S82.2, S82.4
Ankle	4	824	S82.5, S82.6, S82.3, S82.82, S82.84, S82.87, S82.89
Foot	5	825	S92.0, S92.2, S92.3, S92.9
Vertebrae	57	805	S12, S22.0, S32.2, S32.1, S32.0
Rib	43	807.0, 807.1	S22.3, S22.4

*There were 379 incident fractures, experienced by 330 men (some men experienced more than one fracture).

Table 2

Baseline characteristics and distributions of demographic, biochemical and hormone variables in the study population. The P-value shown is for the comparison between men with no incident fracture and men with any incident fracture. Data are shown as mean ± SD and N (%).

Variable	Whole cohort (N=3,307)	No incident fracture (N=2,978)	Incident fracture (N=329)	P value
Age, years	76.8 ± 3.5	76.8 ± 3.5	77.7 ± 3.6	<0.001
Smoking: Never	1,119 (33.8)	1,017 (34.2)	102 (31.0)	0.503
Past	2,015 (60.9)	1,805 (60.6)	210 (63.8)	
Current	173 (5.2)	156 (5.2)	17 (5.2)	
Alcohol (>2 std drinks/d)	806 (24.4)	722 (24.3)	84 (25.6)	0.587
Hypertension	2,559 (77.4)	2,305 (77.4)	254 (77.2)	0.935
Dyslipidemia	2,425 (73.3)	2,178 (73.1)	247 (75.1)	0.45
Frailty	449 (13.7)	377 (12.7)	72 (22.0)	<0.001
Depression	321 (9.7)	273 (9.2)	48 (14.6)	0.002
Diabetes	502 (15.2)	444 (14.9)	58 (17.6)	0.192
Cardiovascular disease	1,188 (37.3)	1,056 (36.8)	132 (41.9)	0.073
BMI, kg/m^2	26.5 (3.6)	26.5 (3.6)	26.5 (4.0)	0.856
Waist, cm	98.9 (9.8)	98.8 (9.7)	99.4 (10.5)	0.29
Creatinine, umol/L	93.6 (31.8)	93.4 (31.7)	95.8 (32.9)	0.187
Vitamin D, nmol/L	68.6 (23.4)	68.6 (23.5)	68.6 (22.3)	0.982
Testosterone, nmol/L	13.2 (4.9)	13.3 (4.9)	12.7 (4.8)	0.033
Calculated free testosterone, pmol/L	186.6 (54.4)	187.4 (54.4)	179.5 (54.5)	0.014
Dihydrotestosterone, nmol/L	1.5 (0.7)	1.5 (0.7)	1.4 (0.7)	0.423
Estradiol, pmol/L	74.1 (29.0)	74.4 (29.0)	71.2 (28.3)	0.061
Sex hormone-binding globulin, nmol/L	42.3 (16.6)	42.2 (16.6)	43.3 (17.0)	0.244
Luteinising hormone, IU/L	5.6 (4.9)	5.6 (4.8)	6.4 (6.2)	0.004

Table 3

Sex hormones, SHBG and LH (in quartiles) as predictors of any fracture in older men. Cox proportional hazards regression with the outcome of incident (any) fracture according to T, calculated free T (cFT), DHT, E2, SHBG and LH modelled as quartiles. Data are shown as hazard ratios (95% confidence intervals) for each quartile with men in the lowest quartile (Q1) as the reference group. Models are shown unadjusted, adjusted for age, and adjusted for age, smoking, alcohol, hypertension, dyslipidemia, prevalent diabetes, prevalent cardiovascular disease, depression, frailty, body mass index (BMI)/waist, creatinine, and vitamin D.

				Hazard ratio (95% confidence interval) and p-value		
Variable	Quartile	Range	Incident any fracture, N (%)	Unadjusted	Age-adjusted	Fully adjusted
T (nmol/L)	1	3.61-9.96	105 (13.1)			
T	2	9.99-12.6	76 (9.3)	0.67 (0.50-0.90) 0.008	0.70 (0.52-0.93) 0.016	0.69 (0.51-0.94) 0.020
T	3	12.7-15.9	64 (7.7)	0.56 (0.41-0.76) <0.001	0.57 (0.42-0.78) <0.001	0.59 (0.42-0.83) 0.002
T	4	16.0-27.6	79 (10.1)	0.78 (0.58-1.05) 0.104	0.81 (0.60-1.08) 0.152	0.85 (0.61-1.17) 0.321
cFT (pmol/L)	1	53.5-151	96 (12.3)			
cFT	2	152-183	81 (10.0)	0.81 (0.60-1.09) 0.167	0.85 (0.63-1.14) 0.283	0.89 (0.65-1.21) 0.464
cFT	3	184-218	67 (8.3)	0.62 (0.45-0.85) 0.003	0.65 (0.47-0.89) 0.006	0.67 (0.47-0.93) 0.018
cFT	4	219-324	71 (9.1)	0.75 (0.55-1.02) 0.071	0.80 (0.59-1.09) 0.164	0.88 (0.63-1.24) 0.463
DHT (nmol/L)	1	0.23-0.94	83 (10.5)			
DHT	2	0.95-1.36	65 (8.1)	0.81 (0.58-1.12) 0.199	0.81 (0.58-1.12) 0.208	0.87 (0.62-1.22) 0.419
DHT	3	1.36-1.85	94 (11.5)	1.17 (0.87-1.58) 0.305	1.20 (0.89-1.61) 0.236	1.31 (0.95-1.80) 0.096
DHT	4	1.86-3.72	71 (9.1)	0.94 (0.68-1.29) 0.694	0.94 (0.69-1.30) 0.728	1.03 (0.72-1.46) 0.880
E2 (pmol/L)	1	17.4-54.3	89 (11.2)			
E2	2	54.7-71.2	79 (9.5)	0.90 (0.67-1.22) 0.511	0.90 (0.67-1.22) 0.508	0.88 (0.64-1.21) 0.441
E2	3	71.6-90.7	80 (9.8)	0.98 (0.73-1.33) 0.915	1.00 (0.74-1.35) 0.991	1.03 (0.75-1.40) 0.858
E2	4	90.9-157	74 (9.5)	0.94 (0.69-1.28) 0.679	0.98 (0.72-1.34) 0.897	1.02 (0.74-1.41) 0.908
SHBG (nmol/L)	1	15.7-31.4	73 (9.3)			
SHBG	2	31.5-39.6	86 (10.7)	1.12 (0.82-1.53) 0.479	1.07 (0.78-1.47) 0.657	1.18 (0.85-1.64) 0.319
SHBG	3	39.7-50.3	68 (8.3)	0.87 (0.63-1.22) 0.426	0.81 (0.58-1.13) 0.222	0.87 (0.61-1.23) 0.427

SHBG	4	50.4-99.5	90 (11.7)	1.31 (0.96-1.79) 0.086	1.17 (0.85-1.60) 0.327	1.39 (0.99-1.96) 0.058
LH (IU/L)	1	1.14-2.99	73 (9.3)			
LH	2	3.00-4.31	74 (9.1)	0.96 (0.70-1.33) 0.826	0.94 (0.68-1.29) 0.688	0.98 (0.70-1.36) 0.890
LH	3	4.32-6.45	82 (10.1)	1.02 (0.74-1.40) 0.899	0.97 (0.70-1.33) 0.834	0.99 (0.71-1.37) 0.931
LH	4	6.47-26.5	86 (11.0)	1.28 (0.94-1.75) 0.121	1.19 (0.87-1.64) 0.267	1.13 (0.81-1.57) 0.473

Table 4

Sex hormones, SHBG and LH (in quartiles) as predictors of hip fracture in older men. Cox proportional hazards regression with the outcome of incident hip fracture according to T, calculated free T (cFT), DHT, E2, SHBG and LH modelled as quartiles. Data are shown as hazard ratios (95% confidence intervals) for each quartile with men in the lowest quartile (Q1) as the reference group. Models are shown unadjusted, adjusted for age, and adjusted for age, smoking, alcohol, hypertension, dyslipidemia, prevalent diabetes, prevalent cardiovascular disease, depression, frailty, body mass index (BMI)/waist, creatinine, and vitamin D.

				Hazard ratio (95% confidence interval) and p-value		
Variable	Quartile	Range	Incident hip fracture, N (%)	Unadjusted	Age-adjusted	Fully adjusted
T (nmol/L)	1	3.61-9.96	45 (5.6)			
T	2	9.99-12.6	29 (3.5)	0.59 (0.37-0.95) 0.029	0.62 (0.39-0.99) 0.047	0.60 (0.37-0.98) 0.043
T	3	12.7-15.9	25 (3.0)	0.52 (0.32-0.85) 0.008	0.54 (0.33-0.88) 0.013	0.52 (0.31-0.88) 0.015
T	4	16.0-27.6	41 (5.2)	0.94 (0.62-1.44) 0.786	0.98 (0.64-1.49) 0.909	1.04 (0.65-1.67) 0.875
cFT (pmol/L)	1	53.5-151	41 (5.3)			
cFT	2	152-183	31 (3.8)	0.72 (0.45-1.15) 0.174	0.77 (0.48-1.22) 0.265	0.78 (0.48-1.28) 0.328
cFT	3	184-218	30 (3.7)	0.67 (0.42-1.07) 0.094	0.71 (0.45-1.14) 0.154	0.69 (0.42-1.15) 0.153
cFT	4	219-324	34 (4.4)	0.84 (0.53-1.33) 0.455	0.91 (0.57-1.44) 0.684	1.02 (0.62-1.69) 0.936
DHT (nmol/L)	1	0.23-0.94	38 (4.8)			
DHT	2	0.95-1.36	21 (2.6)	0.56 (0.33-0.95) 0.033	0.56 (0.33-0.96) 0.035	0.65 (0.37-1.13) 0.127
DHT	3	1.36-1.85	41 (5.0)	1.12 (0.72-1.74) 0.619	1.15 (0.74-1.79) 0.531	1.32 (0.82-2.11) 0.253
DHT	4	1.86-3.72	37 (4.7)	1.05 (0.66-1.65) 0.841	1.06 (0.67-1.66) 0.817	1.24 (0.75-2.05) 0.409
E2 (pmol/L)	1	17.4-54.3	38 (4.8)			
E2	2	54.7-71.2	38 (4.6)	0.99 (0.63-1.56) 0.977	0.99 (0.63-1.55) 0.970	0.96 (0.60-1.54) 0.880
E2	3	71.6-90.7	34 (4.2)	0.96 (0.60-1.52) 0.857	0.97 (0.61-1.55) 0.909	1.00 (0.62-1.61) 0.989
E2	4	90.9-157	29 (3.7)	0.87 (0.54-1.41) 0.571	0.92 (0.57-1.48) 0.721	0.94 (0.57-1.55) 0.809
SHBG (nmol/L)	1	15.7-31.4	28 (3.6)			
SHBG	2	31.5-39.6	40 (5.0)	1.37 (0.84-2.21) 0.204	1.30 (0.80-2.11) 0.285	1.35 (0.82-2.24) 0.239
SHBG	3	39.7-50.3	24 (2.9)	0.80 (0.46-1.37) 0.413	0.73 (0.42-1.26) 0.260	0.72 (0.40-1.28) 0.262

SHBG	4	50.4-99.5	46 (6.0)	1.72 (1.08-2.76) 0.023	1.51 (0.93-2.43) 0.093	1.76 (1.05-2.97) 0.033
LH (IU/L)	1	1.14-2.99	36 (4.6)			
LH	2	3.00-4.31	32 (3.9)	0.84 (0.52-1.36) 0.479	0.81 (0.50-1.30) 0.383	0.80 (0.49-1.31) 0.370
LH	3	4.32-6.45	30 (3.7)	0.78 (0.48-1.27) 0.319	0.73 (0.45-1.18) 0.201	0.71 (0.43-1.17) 0.185
LH	4	6.47-26.5	40 (5.1)	1.21 (0.77-1.89) 0.413	1.10 (0.70-1.73) 0.678	0.96 (0.60-1.55) 0.871

Figure 1

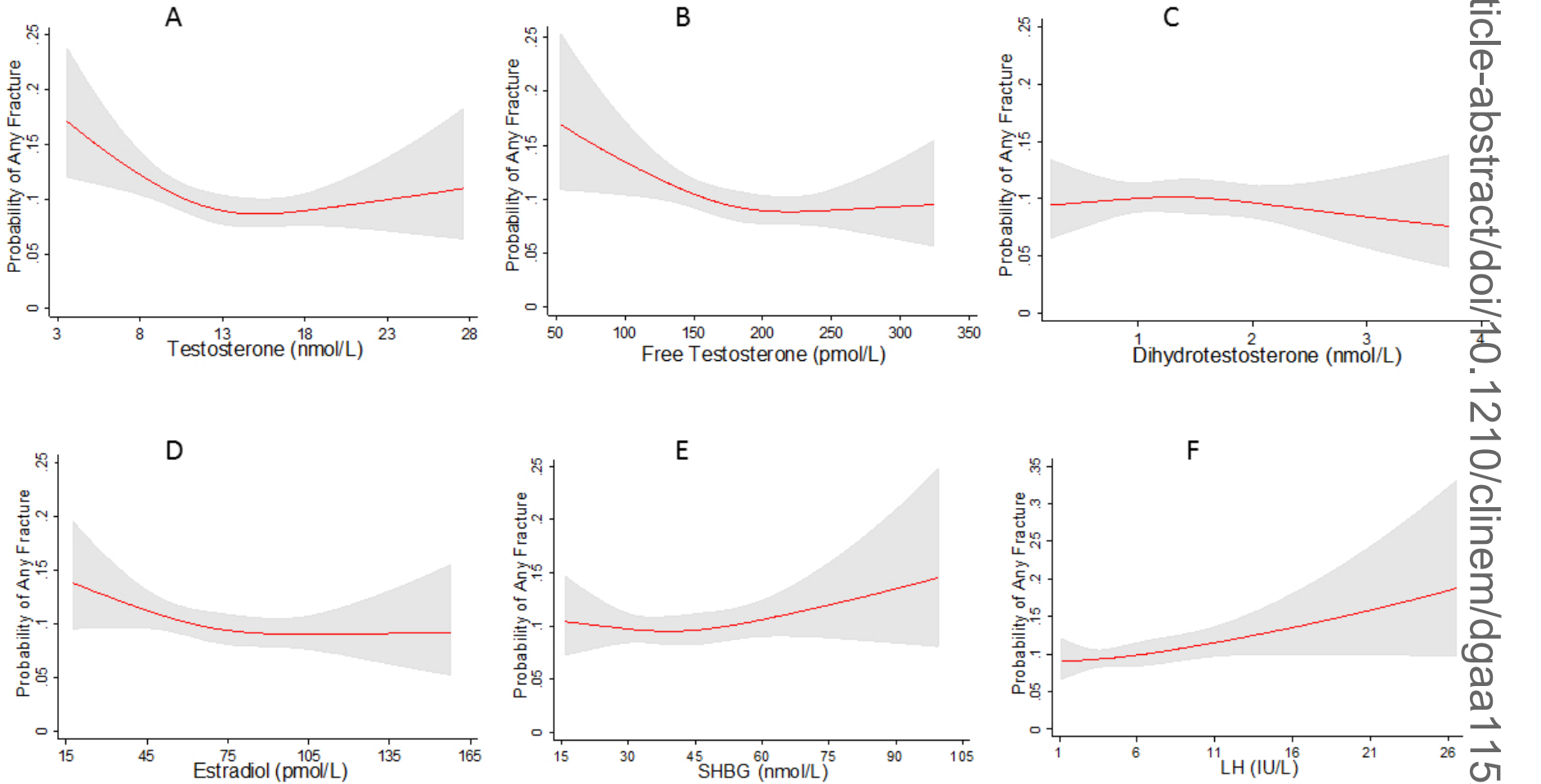

Figure 2