Endogenous Sex Hormones and Incident Fracture Risk in Older Men

The Dubbo Osteoporosis Epidemiology Study

Christian Meier, MD; Tuan V. Nguyen, PhD; David J. Handelsman, MBBS, PhD, FRACP; Christian Schindler, PhD; Mark M. Kushnir, PhD; Alan L. Rockwood, PhD; A. Wayne Meikle, MD; Jacqueline R. Center, MBBS, FRACP, PhD; John A. Eisman, MBBS, PhD, FRACP; Markus J. Seibel, MD, PhD, FRACP

Background: Data on the influence of gonadal hormones on incident fracture risk in elderly men are limited. We prospectively examined the relationship between serum levels of testosterone and estradiol and future fracture risk in community-dwelling men.

Methods: A total of 609 men older than 60 years had been observed between January 1989 and December 2005, with the median duration being 5.8 years (up to 13 years). Clinical risk factors, including bone mineral density and lifestyle factors, were assessed at baseline. Serum testosterone and estradiol levels were measured by tandem mass spectrometry. The incidence of a low-trauma fracture was ascertained during follow-up.

Results: During follow-up, 113 men had at least 1 low-trauma fracture. The risk of fracture was significantly increased in men with reduced testosterone levels (hazard ratio [HR], 1.33; 95% confidence interval [CI], 1.09-1.62). After adjustment for sex hormone–binding globulin, serum testosterone (HR, 1.48; 95% CI, 1.22-1.78) and serum estradiol (HR, 1.21; 95% CI, 1.00-1.47) levels were associated with overall fracture risk. After further adjustment for major risk factors of fractures (age, weight or bone mineral density, fracture history, smoking status, calcium intake, and sex hormone–binding globulin), lower testosterone was still associated with increased risk of fracture, particularly with hip (HR, 1.88; 95% CI, 1.24-2.82) and nonvertebral (HR, 1.32; 95% CI, 1.03-1.68) fractures.

Conclusion: In community-dwelling men older than 60 years, serum testosterone is independently associated with the risk of osteoporotic fracture and its measurement may provide additional clinical information for the assessment of fracture risk in elderly men.

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One-third of all osteoporotic fractures occur in men. After the age of 60 years, the residual lifetime risk of hip or vertebral fracture in men is similar to that of prostate cancer.[1,2] Moreover, in men, a prior osteoporotic fracture increases the risk of subsequent fracture by 3- to 4-fold to at least that of a woman of the same age with a fracture.[3] Preventing the first such fracture may have major public health implications. Thus, understanding the determinants of fracture risk in men may reduce the burden of disease through facilitating better prevention strategies.

Male aging is associated with a gradual decrease in circulating testosterone,[4] which may be detrimental to bone.[5] However, the relationship between testosterone and incident fracture risk remains unclear. A recent study[6] from Sweden reported that free testosterone within the normal range was independently associated with prevalent osteoporotic fractures in elderly men. In contrast, a subset analysis from the Rotterdam Study[7] failed to confirm an association between testosterone and fracture risk. Data from the Framingham Study recently indicated a synergistic effect of sex hormones on fracture risk in that men with low serum testosterone and low estradiol (E_2) levels were at increased risk for incident hip fractures. Analyses restricted to either sex hormone alone, however, revealed that in elderly men, serum E_2 but not testosterone was associated with hip fracture risk.[8] In these earlier studies,[7,8] however, serum testosterone levels were measured using immunoassay-based methods that have been shown to be unreliable, particularly in the lower concentration range.[9]

The present study was aimed at determining whether endogenous sex hor-

Author Affiliations are listed at the end of this article.

mones, measured by tandem mass spectrometry, are associated with incident vertebral and nonvertebral fractures over a median of 5.8 years in a large cohort of ambulatory community-dwelling men older than 60 years and determining how such effects relate to known fracture risk factors.

METHODS

PARTICIPANTS

The present analysis is part of the ongoing Dubbo Osteoporosis Epidemiology Study.[1,3,10] Briefly, since 1989, all men and women 60 years or older living in Dubbo, a regional city of 32 000 predominantly white people in New South Wales, Australia, were invited to participate in the study. The age and sex distribution of the Dubbo population closely resembles that of the general Australian population.

By July 2004, 868 men were participating and followed up at approximately 2-year intervals and, of these men, 609 (70.2%) had a serum sample available. Baseline characteristics (age, weight, height, and bone mineral density [BMD]) were comparable between participants (men with serum samples available) and nonparticipants (men without samples [n=259]). Study enrollment was defined as the time point at which a first blood sample was available. Men with incident symptomatic minimal trauma fractures during the prospective follow-up until December 2005 formed the fracture group (n=113), whereas men without incident fractures during the same interval were defined as nonfracture controls (n=496). The study was approved by St Vincent's Hospital Ethics Review Committee; all subjects gave written informed consent.

CLINICAL DATA COLLECTION

Participants were interviewed by a nurse coordinator who obtained anthropometric variables and administered a questionnaire to collect data on lifestyle factors and calcium intake.[11] Bone mineral density was measured at the lumbar spine (LSBMD) and the femoral neck (FNBMD) by dual-energy x-ray absorptiometry (LUNAR DPX-L; GE-LUNAR, Madison, Wisconsin). The same densitometer was used throughout the study; the coefficient of variation for the BMD measurements was 1.3% at the lumbar spine and 3.5% at the femoral neck.[12]

ASCERTAINMENT OF FRACTURES

Dubbo has 3 radiological services to which the study has access. Therefore, all fractures that occurred within the city could be ascertained. Circumstances surrounding fracture were determined by telephone call after the fracture. All fractures included in the study were low-trauma fractures associated with a fall from standing height or less and were confirmed by radiograph. Because the first available blood sample may have been collected at the second or even third visit, some fractures had occurred before that sample. All fractures occurring before the first blood collection were defined as prevalent (ie, prior fracture history), whereas all fractures occurring thereafter were defined as incident. There was no systematic x-ray screening to identify asymptomatic vertebral fractures. All vertebral fractures in the present study were clinically symptomatic fractures, identified in individuals who underwent x-ray screening for back pain or symptoms that showed a vertebral deformity. Similar deformities identified in radiographs taken for other reasons were considered to be prevalent fractures.

LABORATORY MEASUREMENTS

Nonfasting blood samples were collected mostly in the morning. Serum samples were stored at –80°C until analysis. Samples were analyzed for serum testosterone and E_2 levels using a liquid chromatography tandem mass spectrometry method. The limit of quantitation for E_2 was 1.5 pg/mL (to convert to picomoles per liter, multiply by 3.671) and for testosterone it was 3.0 ng/dL (to convert to nanomoles per liter, multiply by 0.0347). Method imprecision was less than 10%.[13,14] Serum levels of sex hormone–binding globulin (SHBG) were measured by a commercial immunoassay (DELFIA; Wallac Oy, Turku, Finland). Coefficients of variation were 10.2% at high (14.6 µg/mL), 5.3% at midrange (6.4 µg/mL), and 8.3% at low (2.2 µg/mL) concentrations (to convert to nanomoles per liter, multiply by 8.896).

STATISTICAL ANALYSIS

The Cox proportional hazards model was used to characterize the association, and estimate the magnitude of association, between serum testosterone and/or serum E_2 and fracture risk. The association was further adjusted for known risk factors, such as advancing age, baseline weight, FNBMD, prior fracture, dietary calcium intake, and smoking. Femoral neck BMD, rather than LSBMD, was considered because in elderly men FNBMD measurement is less likely to be affected by degenerative changes than LSBMD measurement. However, if FNBMD was replaced by LSBMD, hazard ratios remained essentially unchanged. Because weight and BMD were significantly interrelated ($r=0.41$, $P<.001$) and the effect of sex hormones on fracture risk might be independently mediated through body mass and bone mass, the second model was fitted to the data by replacing weight in the first model with FNBMD. Because the distributions of testosterone, SHBG, E_2, and dietary calcium intake were skewed, these variables were subjected to a log $(x+c)$ transformation, making them normally distributed and stabilizing their variance (x indicates an original variable and c indicates a constant). Because analyses of subcategories of fractions were considered secondary, we did not adjust the respective P values for multiple comparisons. To quantify the impact of serum testosterone on fracture risk, the partial attributable risk fraction was estimated from the multivariate variables.[15] All analyses were done using the R statistical environment on a Windows platform.[16]

RESULTS

INCIDENCE OF FRACTURES

In total, 609 men (mean [SD] age, 72.6 [5.7] years) had been observed for a median of 5.8 years (range, 0-12.6 years). During the follow-up, 113 men sustained at least 1 low-trauma fracture. Twenty-five men experienced multiple incident fractures. A total of 149 incident fractures were reported, including 55 vertebral, 27 hip, 28 rib, 6 wrist, and 16 upper and 17 lower extremity fractures. The incidence of all new fractures was 3.4 per 100 person-years (95% confidence interval, 3.4-3.5 per 100 person-years); 79.3% of all fractures occurred in men aged 70 years or older, in whom the incidence was 4.7% (95% confidence interval, 4.6%-4.8%), representing a 2.7-fold higher incidence than that seen in those younger than 70 years.

SEX HORMONES AND CLINICAL VARIABLES

Serum testosterone levels were inversely related to age ($r=-0.13$, $P=.001$) and weight ($r=-0.13$, $P=.002$) and

Table 1. Clinical and Biochemical Characteristics of Men With Incident Low-Trauma Fractures and Individuals Without Fractures at Baseline and Association Between Individual Risk Factors and Fracture Risk

Variable	Men With Incident Fractures (n=113)[a]	Control Subjects Without Fractures (n=496)[a]	HR (95% CI)[b]	*P* Value
Age, y	74.5 (5.7)	72.2 (5.7)	1.76 (1.50-2.06)	<.001
Weight, kg	74.2 (12.6)	79.3 (12.9)	1.41 (1.20-1.66)	<.001
Height, cm	171.0 (6.6)	172.6 (6.2)	1.26 (1.09-1.47)	.002
Dietary calcium, mg/d	558 (356)	652 (336)	1.46 (1.19-1.78)	<.001
Past or present smokers, No. (%)	70 (61.9)	289 (58.3)	1.22 (0.83-1.78)	.31
LSBMD, g/cm^2	1.17 (0.20)	1.28 (0.23)	1.66 (1.36-2.01)	<.001
FNBMD, g/cm^2	0.85 (0.17)	0.92 (0.15)	1.99 (1.60-2.47)	<.001
Serum testosterone, ng/dL	400 (216)	435 (202)	1.33 (1.09-1.62)	.004
Serum estradiol, pg/mL	19.7 (10.2)	20.4 (9.1)	1.16 (0.95-1.40)	.14
Serum SHBG, µg/mL	6.6 (2.6)	5.9 (2.6)	1.36 (1.16-1.64)	<.001
Prior fracture, No. (%)	24 (21.2)	93 (18.8)	1.83 (1.16-2.87)	.009

Abbreviations: CI, confidence interval; FNBMD, femoral neck bone mineral density; HR, hazard ratio; LSBMD, lumbar spine bone mineral density; SHBG, sex hormone–binding globulin.

SI conversion factors: To convert estradiol to picomoles per liter, multiply by 3.671; to convert SHBG to nanamoles per liter, multiply by 8.896; and to convert testosterone to nanomoles per liter, multiply by 0.0347.

[a] Data are given as mean (SD) unless otherwise indicated.

[b] Data were estimated per SD or approximate SD.

positively related to serum E_2 ($r=0.45$, $P<.001$) and SHBG ($r=0.35$, $P<.001$) concentrations. There were no statistically significant associations between testosterone levels and BMD (LSBMD: $P=.43$; FNBMD: $P=.49$). Serum E_2 levels were positively related to BMD (LSBMD: $r=0.16$, $P<.001$; FNBMD: $r=0.11$, $P=.01$). Serum SHBG levels were positively correlated with age ($r=0.15$, $P<.001$) and inversely correlated with height ($r=-0.11$, $P=.007$), weight ($r=-0.30$, $P<.001$), LSBMD ($r=-0.20$, $P<.001$), and FNBMD ($r=-0.23$, $P<.001$).

BASELINE CHARACTERISTICS AND UNIVARIATE ASSOCIATIONS

At baseline and compared with men without fractures, men with incident fractures were older ($P<.01$), shorter ($P=.02$), and had lower body weight ($P<.01$) and had lower dietary calcium intake ($P=.002$) and a lower LSBMD and FNBMD ($P<.01$). Serum testosterone levels were significantly lower (median [interquartile range], 383 [282-519] ng/dL vs 403 [297-562] ng/dL; $P=.03$) and serum SHBG levels were significantly higher (6.2 [4.8-7.6] µg/mL vs 5.4 [4.1-7.1] µg/mL; $P=.003$) in men with incident fractures compared with men without fractures during follow-up. In contrast, baseline E_2 concentrations were comparable between both groups ($P=.20$).

In univariate analysis (**Table 1**), apart from established risk factors (eg, age, weight, prior fracture, and BMD), the hazard ratio of any osteoporotic fracture for each standard deviation decrease in serum testosterone was 1.33 and for each standard deviation increase in serum SHBG was 1.36. Serum E_2 levels were not significantly associated with fracture risk in bivariate analysis.

MULTIVARIATE ANALYSES

Low levels of serum testosterone were significantly associated with an increased risk of fracture after adjustment for SHBG, age, and weight; however, when weight was replaced by FNBMD, the association was similar but did not remain statistically significant (**Table 2**). On the other hand, low levels of serum E_2 were only significantly associated with fracture risk after adjustment for SHBG and not when age and weight or FNBMD were included in the model (Table 2).

Table 2. Association Between Testosterone and Estradiol and Fracture Risk: Unadjusted and Adjusted Analyses

Serum Analyte	HR (95% CI)[a]	*P* Value
Testosterone		
Unadjusted	1.33 (1.09-1.62)	.004
Adjusted for SHBG	1.48 (1.22-1.78)	<.001
Adjusted for SHBG and age	1.31 (1.08-1.58)	.005
Adjusted for SHBG, age, and weight	1.26 (1.03-1.55)	.02
Adjusted for SHBG, age, femoral neck, and BMD	1.20 (0.98-1.47)	.07
Estradiol		
Unadjusted	1.16 (0.95-1.40)	.14
Adjusted for SHBG	1.21 (1.00-1.47)	.046
Adjusted for SHBG and age	1.18 (0.98-1.43)	.09
Adjusted for SHBG, age, and weight	1.17 (0.96-1.43)	.11
Adjusted for SHBG, age, femoral neck, and BMD	1.12 (0.92-1.37)	.27

Abbreviations: BMD, bone mineral density; CI, confidence interval; HR, hazard ratio; SHBG, sex hormone–binding globulin.

[a] Data were estimated per SD of log testosterone and log estradiol. On back-transformation, the equivalent SD was 199 ng/dL (6.9 nmol/L) for serum testosterone and 2.53 pg/mL (9.3 pmol/L) for serum estradiol.

In a multivariate analysis with all known risk factors of fracture being considered simultaneously, baseline serum testosterone, SHBG, age, FNBMD, weight, and calcium intake were significantly and independently associated with any low-trauma fracture (**Table 3**). Each standard deviation decrease in serum testosterone was associated with an increased hazard of fracture by

Table 3. Association Between Individual Risk Factors and Fracture Risk: Multivariate Analysis

Risk Factor	Unit of Comparison	Model Including Weight		Model Including Femoral Neck BMD	
		HR (95% CI)[a]	*P* Value	HR (95% CI)[a]	*P* Value
Model With Testosterone					
Serum testosterone, ng/dL	−199	1.37 (1.11-1.68)	.003	1.28 (1.05-1.57)	.02
Serum SHBG, µg/mL	2.5	1.32 (1.07-1.64)	.01	1.29 (1.04-1.60)	.02
Age, y	5	1.56 (1.30-1.86)	<.001	1.56 (1.30-1.87)	<.001
Femoral neck BMD, g/cm^2	−0.15	NA	NA	1.43 (1.14-1.80)	.002
Weight, kg	−10	1.22 (1.02-1.47)	.03	NA	NA
Prior fracture	Yes	1.56 (0.95-2.58)	.08	1.51 (0.91-2.51)	.11
Smoking	Yes	1.62 (1.03-2.45)	.02	1.45 (0.96-2.21)	.08
Dietary calcium, mg/d	−322	1.53 (1.25-1.88)	<.001	1.43 (1.17-1.78)	<.001
Model With Estradiol					
Serum estradiol, pg/mL	−2.53	1.25 (1.02-1.54)	.03	1.19 (0.97-1.47)	.11
Serum SHBG, µg/mL	2.5	1.26 (1.02-1.56)	.03	1.22 (0.99-1.50)	.06
Age, y	5	1.61 (1.35-1.91)	<.001	1.58 (1.33-1.90)	<.001
Femoral neck BMD, g/cm^2	−0.15	NA	NA	1.43 (1.13-1.82)	.003
Weight, kg	−10	1.19 (0.99-1.42)	.07	NA	NA
Prior fracture	Yes	1.46 (0.90-2.42)	.14	1.43 (0.87-2.38)	.16
Smoking	Yes	1.59 (1.06-2.41)	.03	1.44 (0.95-2.19)	<.001
Dietary calcium, mg/d	−322	1.51 (1.23-1.85)	<.001	1.42 (1.15-1.74)	.001

Abbreviations: BMD, bone mineral density; CI, confidence interval; HR, hazard ratio; NA, data not applicable; SHBG, sex hormone–binding globulin.
SI conversion factors: To convert estradiol to picomoles per liter, multiply by 3.671; to convert SHBG to nanomoles per liter, multiply by 8.896; and to convert testosterone to nanomoles per liter, multiply by 0.0347.
[a] Data were estimated per SD or approximate SD.

Table 4. Association Between Individual Risk Factors and Nonvertebral and Vertebral Fracture Risk: Multivariate Analysis[a]

Risk Factor	Model Including Testosterone and Weight		Model Including Testosterone and Femoral Neck BMD	
	HR (95% CI)[b]	*P* Value	HR (95% CI)[b]	*P* Value
Nonvertebral Fracture				
Serum testosterone	1.32 (1.03-1.68)	.03	1.23 (0.97-1.57)	.09
Serum SHBG	1.25 (0.97-1.62)	.09	1.17 (0.90-1.51)	.24
Age	1.61 (1.31-2.00)	<.001	1.61 (1.31-2.00)	<.001
Femoral neck BMD	NA	NA	1.43 (1.09-1.90)	.01
Weight	1.11 (0.92-1.37)	.32	NA	NA
Prior fracture	1.35 (0.73-2.50)	.34	1.26 (0.68-2.34)	.47
Smoking	1.09 (0.68-1.74)	.71	1.02 (0.64-1.64)	.94
Dietary calcium	1.75 (1.38-2.23)	<.001	1.68 (1.31-2.14)	<.001
Hip Fracture				
Serum testosterone	1.88 (1.24-2.82)	.003	1.48 (0.98-2.23)	.06
Serum SHBG	1.39 (0.87-2.21)	.17	1.24 (0.79-1.93)	.35
Age	1.96 (1.32-2.89)	.007	1.79 (1.21-2.63)	.003
Femoral neck BMD	NA	NA	4.43 (2.47-7.93)	<.001
Weight	1.37 (0.90-2.09)	.14	NA	NA
Prior fracture	2.21 (0.79-6.18)	.13	1.69 (0.55-5.20)	.36
Smoking	1.38 (0.58-3.30)	.47	0.97 (0.40-2.32)	.94
Dietary calcium	1.58 (1.00-2.50)	.05	1.32 (0.81-2.16)	.26
Vertebral Fracture				
Serum testosterone	1.32 (0.95-1.84)	.09	1.23 (0.89-1.69)	.20
Serum SHBG	1.55 (1.09-2.20)	.02	1.61 (1.14-2.27)	.007
Age	1.44 (1.08-1.93)	.01	1.38 (1.02-1.87)	.04
Femoral neck BMD	NA	NA	1.79 (1.21-2.64)	.003
Weight	1.65 (1.19-2.28)	.002	NA	NA
Prior fracture	1.79 (0.80-3.98)	.16	1.75 (0.78-3.94)	.18
Smoking	6.16 (2.20-15.10)	<.001	4.89 (2.00-12.00)	<.001
Dietary calcium	1.27 (0.91-1.77)	.17	1.08 (0.77-1.51)	.66

Abbreviations: See Table 3.
[a] The units of comparison are the same as those given in Table 3 for testosterone.
[b] Data were estimated per SD or approximate SD.

1.37-fold with weight in the model and 1.28-fold after adjusting for FNBMD and covariates. Depending on whether BMD was taken into account, the fraction of fracture cases attributable to the variation in total testosterone was about either 3% or 6.7%.

The association between serum testosterone and fracture risk was further examined by fracture type (**Table 4**). After adjusting for covariates and weight, serum testosterone was significantly associated with an increased risk of nonvertebral fracture and hip fracture, but not with symptomatic vertebral fracture. However, in a model with adjustment for covariates and FNBMD, serum testosterone was only associated with hip fracture risk.

The association between serum E_2 and fracture risk was noted in the model with covariates and body weight: a decrease in E_2 by 1 SD was associated with an increased hazard of fracture. However, when weight was replaced by FNBMD, the relationship of serum E_2 with fracture risk was no longer significant (Table 3). Analysis stratified by fracture site did not reveal any significant association between serum testosterone and hip fracture, nonvertebral fracture, and symptomatic vertebral fracture after adjusting for covariates and weight or FNBMD.

QUARTILE ANALYSES

When the entire sample was analyzed by quartiles of baseline serum testosterone levels, adjusted for major independent covariables, the risk for any fracture tended to increase with decreasing concentrations of circulating testosterone. The hazard ratio was higher in men with testosterone levels in the lowest quartile (<294 ng/dL) compared with men with testosterone levels in the highest quartile (**Figure 1**A). When the entire sample was analyzed by quartiles of baseline serum SHBG levels (adjusted for covariates), a nonsignificant increase in fracture risk with increasing baseline SHBG levels was observed (Figure 1B). Quartile analyses for serum E_2, adjusted for covariates, showed no significant association with fracture risk (Figure 1C).

Based on their baseline serum testosterone levels, subjects were then grouped into 3 categories: low testosterone group (quartile 1, <294 ng/dL), intermediate testosterone group (quartiles 2 and 3, 294-559 ng/dL), and high testosterone group (quartile 4, >559 ng/dL). Time-to-event (time-to–first fracture) analyses demonstrated that men in the lowest testosterone group had a greater risk of low-trauma fracture during the follow-up than did men in the highest testosterone group. Fracture risk did not differ between the groups with intermediate and high serum testosterone levels (**Figure 2** and **Table 5**). After adjustment for age, weight, fracture history, smoking status, calcium intake, and serum SHBG levels, the risk of fracture was more than doubled in men with serum testosterone levels lower than 294 ng/dL compared with men with high serum testosterone levels. Similar time-to-event analyses for serum unadjusted or adjusted E_2 levels showed no difference in fracture risk between lower and higher E_2 groups (Table 5).

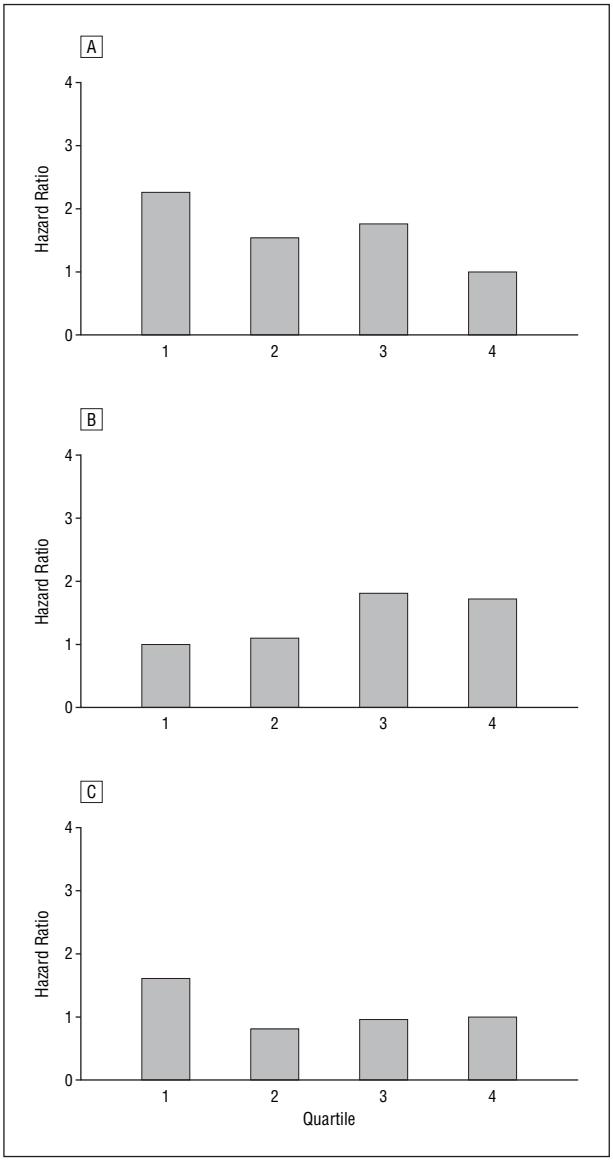

Figure 1. Serum levels of testosterone (A), sex hormone–binding globulin (SHBG) (B), and estradiol (E_2) (C) at baseline and risk of subsequent fracture in elderly men. In A, the hazard ratio (95% confidence interval) for quartile 1 was 2.26 (1.20-4.20); for quartile 2, 1.54 (0.80-2.80); for quartile 3, 1.76 (0.90-3.10); and for quartile 4, 1.00 (reference). The median (range) serum testosterone level for quartile 1 was 227 (3-291) ng/dL; for quartile 2, 343 (294-398) ng/dL; for quartile 3, 473 (401-559) ng/dL; and for quartile 4, 646 (559-1519) ng/dL. To convert serum testosterone to nanomoles per liter, multiply by 0.0347. In B, the hazard ratio (95% confidence interval) for quartile 1 was 1.00 (reference); for quartile 2, 1.10 (0.60-2.10); for quartile 3, 1.81 (1.00-3.20); and for quartile 4, 1.72 (0.90-3.20). The median (range) SHBG level for quartile 1 was 3.6 (0.4-4.3) µg/mL; for quartile 2, 5.0 (4.3-5.6) µg/mL; for quartile 3, 6.3 (5.6-7.2) µg/mL; and for quartile 4, 8.7 (7.2-30.0) µg/mL. To convert SHBG to nanomoles per liter, multiply by 8.896. In C, the hazard ratio (95% confidence interval) for quartile 1 was 1.61 (0.90-2.80); for quartile 2, 0.81 (0.40-1.50); for quartile 3, 0.96 (0.60-1.70); and for quartile 4, 1.00 (reference). The median (range) serum E_2 level for quartile 1 was 10.4 (0.8-13.9) pg/mL; for quartile 2, 16.9 (14.2-19.9) pg/mL; for quartile 3, 22.1 (20.2-25.3) pg/mL; and for quartile 4, 30.0 (25.6-69.7) pg/mL. To convert E_2 to picomoles per liter, multiply by 3.671. For calculations of hazard ratios, serum levels of testosterone and E_2 were adjusted for age, weight, calcium intake, prevalent fractures, smoking, and SHBG levels.

COMMENT

The present prospective study shows that men with lower serum testosterone levels had increased risk of osteopo-

rotic fracture, and this effect was independent of established risk factors, such as age and BMD. In contrast, there was no significant association between serum E_2 levels and fracture in the presence of BMD and age.

Observational studies[4,17,18] suggest that male aging is associated with a gradual decrease in circulating testosterone levels. However, the clinical significance of this change remains unclear, as does any justification for testosterone treatment.[19] Data relating serum testosterone levels to the prospective risk of osteoporotic fractures in community-dwelling older men are scarce and limited. A recent population-based study[7] of 178 elderly men from the Rotterdam Study detected no association between sex hormone levels and fracture risk in men; however, the statistical power of this study was inadequate for any firm conclusions. In the Framingham Study,[8] men with low serum testosterone levels tended to have a higher risk of hip fracture; however, the risk was only observed among a subset of men with low serum testosterone and low serum E_2 levels.

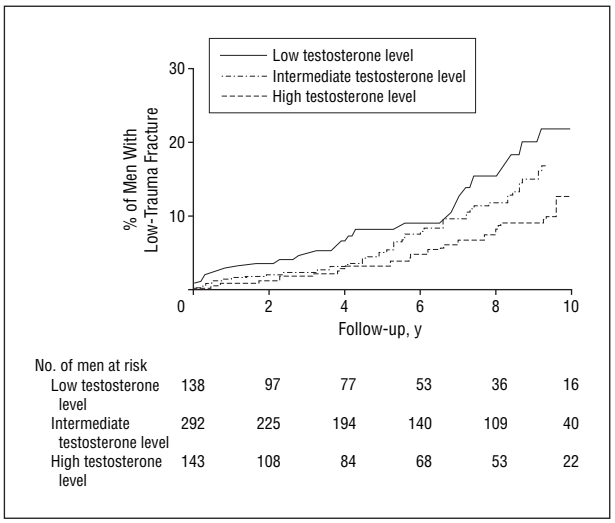

Figure 2. Proportion of study participants with low-trauma fractures during follow-up (time-to-event analysis) according to baseline sex hormone levels, grouped by adjusted baseline serum testosterone levels (low testosterone, <294 ng/dL; intermediate testosterone, 294-559 ng/dL; and high testosterone, >559 ng/dL) (to convert testosterone to nanomoles per liter, multiply by 0.0347). Group comparisons were performed using the Wald test (Cox proportional hazards regression); $P=.01$ for the low vs high testosterone group.

In the present prospective study, baseline serum testosterone was a risk factor for osteoporotic fractures independent of age and BMD. After adjusting for established risk factors, the risk of any fracture increased between 30% and 40% for each 1-SD lowering in serum testosterone levels. Circulating testosterone was associated with the risk of incident nonvertebral fractures, including hip fractures, and similarly, but not significantly, with the risk of vertebral fractures. This difference in the strength of statistical association might reflect the fact that vertebral fractures were clinically diagnosed without systematic x-ray screening to identify asymptomatic vertebral deformities. Therefore, the number of nontraumatic vertebral fractures is likely to be underestimated. Clinical[6,20] and histomorphometric[21] data suggest that testosterone may have a different influence on BMD at cortical than trabecular bone sites.

The association between circulating testosterone and fracture risk is only partially understood. Several community-based observational studies[20,22-25] suggest that the association between blood E_2 levels and BMD or bone turnover in men is stronger than the association with serum testosterone concentrations, perhaps reflecting the role of aromatization in the effects of testosterone on bone. Earlier longitudinal studies suggested that low serum E_2 levels are associated with an increased vertebral[26] and hip[8] fracture risk in elderly men. In these studies, measurements of sex hormones were performed using immunoassay-based methods, which are known to be particularly unreliable in the lower concentration range.[9] Based on a highly specific and sensitive tandem mass spectrometry technique, our results show only a weak relationship between serum E_2 levels and fracture risk in older men.[27] From these results, a role, albeit modest, of local aromatization of testosterone to E_2 in bone cannot be excluded.

Lower BMD and accelerated bone resorption have been shown to be independent determinants of fracture risk in elderly men. However, the 2 factors collectively accounted for only 20% of fracture cases in the general population.[10] Serum testosterone levels and BMD were independently associated with fracture risk in the present study; therefore, the effects of testosterone are only partially explained by its changes on BMD. Low serum testosterone levels may result in decreased bone strength by affecting determinants of bone quality, including bone

Table 5. Unadjusted and Adjusted Data for Time to First Fracture According to Baseline Sex Hormone Status

Group Comparisons by Sex Hormone[a]	Unadjusted Data		Adjusted Data	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Testosterone				
Quartile 1 vs 4	1.86 (1.08-3.20)	.03	2.26 (1.22-4.20)	.01
Quartiles 2 and 3 vs 4	1.32 (0.81-2.16)	.27	1.65 (0.97-2.82)	.06
Estradiol				
Quartile 1 vs 4	1.46 (0.89-2.39)	.14	1.61 (0.94-2.77)	.08
Quartiles 2 and 3 vs 4	0.83 (0.52-1.32)	.43	0.90 (0.55-1.46)	.67

Abbreviations: See Table 3.

[a] Serum testosterone levels were as follows: quartile 1, less than 294 ng/dL; quartiles 2 and 3, 294 to 559 ng/dL; and quartile 4, greater than 559 ng/dL (to convert to nanomoles per liter, multiply by 0.0347). Serum estradiol levels were as follows: quartile 1, less than 14.0 pg/mL; quartiles 2 and 3, 14.0 to 25.6 pg/mL; and quartile 4, greater than 25.6 pg/mL (to convert to picomoles per liter, multiply by 3.671).

geometry. Also, in addition to their skeletal effects, androgen actions affect body composition; thus, androgen deficiency results in decreased muscle mass,[28,29] thereby potentially contributing to fracture risk via impaired balance and decreased muscle strength, presumably through increased falls and less effective protective actions.[30,31]

Androgen deficiency in the aging male has become a topic of growing interest and has led to a marked increase in the prescribing of testosterone products in the United States, if not elsewhere.[32,33] Three randomized placebo-controlled studies[34-36] have evaluated the effects of testosterone on bone turnover and BMD in elderly nonosteoporotic men with low to normal testosterone concentrations. The effects varied according to testosterone dose and the degree of prior androgen insufficiency.[35] Our data suggest that if androgen therapy for the prevention of fragility fractures in healthy elderly men is justified on efficacy and safety grounds, it is most likely to be justifiable only in those with the most severe testosterone deficiency.

The present study's findings should be interpreted within the context of its strengths and limitations. Clearly, our findings should not be interpreted as an indication of any causal relationship between circulating testosterone and fracture risk. Although the homogeneity of ethnicity in the study is a strength, its results cannot be generalized to other populations. As is true for any long-term longitudinal study, serum samples were stored in the freezer for up to 13 years. While testosterone and E_2 are known to be stable over extended periods of storage at –80°C, SHBG levels may change slightly with time in storage. However, these latter changes are marginal and more likely because of changes in assay technique than in the analyte itself. Finally, serum was not collected consistently in the morning, which could introduce random measurement error. However, the circadian rhythmicity of testosterone synthesis is lost in older men[37]; therefore, diurnal variability was unlikely to have had a significant effect on these results.

We conclude that, while low levels of E_2 and testosterone, determined by tandem mass spectrometry, were associated with an increased fracture risk in community-dwelling men older than 60 years, only the effect of testosterone was independent of FNBMD and other established risk factors of osteoporotic fracture. While testosterone may affect fracture risk via skeletal and nonskeletal mechanisms, the present findings suggest that measurement of serum testosterone provides additional clinical information for the assessment of fracture risk in elderly men.

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Author Affiliations: Division of Endocrinology, Diabetes and Clinical Nutrition, University Hospital Basel (Dr Meier), and Institute of Social and Preventive Medicine, University of Basel (Dr Schindler), Basel, Switzerland; Bone Research Program (Drs Meier and Seibel) and Department of Andrology (Dr Handelsman), ANZAC Research Institute, University of Sydney, Bone and Mineral Research Program, Garvan Institute of Medical Research, St Vincent's Hospital and University of New South Wales (Drs Nguyen, Center, and Eisman), Sydney, Australia; ARUP Institute for Clinical and Experimental Pathology (Drs Kushnir and Rockwood) and Departments of Pathology (Drs Rockwood and Meikle) and Medicine, University of Utah, Salt Lake City.
Correspondence: Christian Meier, MD, Bone Research Program, ANZAC Research Institute, University of Sydney, Concord, New South Wales 2139, Australia (christian.meier@unibas.ch).
Author Contributions: *Study concept and design:* Meier, Nguyen, Handelsman, Center, Eisman, and Seibel. *Acquisition of data:* Nguyen, Handelsman, Kushnir, Center, Eisman, and Seibel. *Analysis and interpretation of data:* Meier, Nguyen, Handelsman, Schindler, Rockwood, Meikle, Eisman, and Seibel. *Drafting of the manuscript:* Meier, Nguyen, Handelsman, Eisman, and Seibel. *Critical revision of the manuscript for important intellectual content:* Meier, Nguyen, Handelsman, Schindler, Kushnir, Rockwood, Meikle, Center, Eisman, and Seibel. *Statistical analysis:* Meier, Nguyen, Handelsman, and Schindler. *Obtained funding:* Nguyen, Center, Eisman, and Seibel. *Administrative, technical, and material support:* Meier, Nguyen, Handelsman, Kushnir, Rockwood, Meikle, and Seibel. *Study supervision:* Nguyen, Eisman, and Seibel.
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