Low Serum DHEAS Predicts Increased Fracture Risk in Older Men: The MrOS Sweden Study

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ABSTRACT

The adrenal-derived hormones dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) are the most abundant circulating hormones and their levels decline substantially with age. DHEAS is considered an inactive precursor, which is converted into androgens and estrogens via local metabolism in peripheral target tissues. The predictive value of serum DHEAS for fracture risk is unknown. The aim of this study was, therefore, to assess the associations between baseline DHEAS levels and incident fractures in a large cohort of older men. Serum DHEAS levels were analyzed with mass spectrometry in the population-based Osteoporotic Fractures in Men study in Sweden ($n = 2568$, aged 69 to 81 years). Incident X-ray validated fractures (all, $n = 594$; non-vertebral major osteoporotic, $n = 255$; hip, $n = 175$; clinical vertebral, $n = 206$) were ascertained during a median follow-up of 10.6 years. DHEAS levels were inversely associated with the risk of any fracture (hazard ratio [HR] per SD decrease = 1.14, 95% confidence interval [CI] 1.05–1.24), non-vertebral major osteoporotic fractures (HR = 1.31, 95% CI 1.16–1.48), and hip fractures (HR = 1.18, 95% CI 1.02–1.37) but not clinical vertebral fractures (HR = 1.09, 95% CI 0.95–1.26) in Cox regression models adjusted for age, body mass index (BMI) and prevalent fractures. Further adjustment for traditional risk factors for fracture, bone mineral density (BMD), and/or physical performance variables as well as serum sex steroid levels only slightly attenuated the associations between serum DHEAS and fracture risk. Similarly, the point estimates were only marginally reduced after adjustment for FRAX estimates with BMD. The inverse association between serum DHEAS and all fractures or major osteoporotic fractures was nonlinear, with a substantial increase in fracture risk (all fractures 22%, major osteoporotic fractures 33%) for those participants with serum DHEAS levels below the median (0.60 μg/mL). In conclusion, low serum DHEAS levels are a risk marker of mainly non-vertebral fractures in older men, of whom those with DHEAS levels below 0.60 μg/mL are at highest risk. © The Authors. *Journal of Bone and Mineral Research* Published by Wiley Periodicals Inc.

KEY WORDS: SEX STEROIDS; GENERAL POPULATION STUDIES; FRACTURE RISK ASSESSMENT; DHEAS; MEN

Introduction

Dehydroepiandrosterone (DHEA) and its sulfated derivative (DHEAS) are the most abundant circulating steroids, secreted mainly by the adrenal glands. DHEAS acts as an inactive precursor, which is converted initially into DHEA and thereafter into active androgens and estrogens in peripheral target tissues. As a result, many of the actions of DHEAS are thought to be mediated by this downstream metabolism in target tissues.[1] Alternatively, DHEA action may be mediated via multiple signaling pathways involving a putative membrane receptor.[2] DHEA is the secreted form of the steroid precursor, but it is present in serum mainly as DHEAS. DHEAS has a longer half-life than DHEA and, unlike DHEA, shows little diurnal variation.[3] Therefore, DHEAS is considered the customary index of adrenal androgen secretion in clinical practice and epidemiological studies.

Circulating DHEAS levels decrease dramatically with age,[1,4] but the mechanism behind this decline and its implications for general health remain unclear. The age-related decline in DHEAS has led to speculation that a relative DHEAS deficiency

may contribute to the development of common age-related diseases, such as osteoporosis.[5] Conflicting results have been reported regarding the associations between serum DHEAS concentrations and bone mineral density (BMD) in men. Most studies reported no significant associations,[6–8] whereas Khosla and colleagues showed positive univariate correlations between serum DHEAS and several BMD sites in a population-based, age-stratified sample of 346 Rochester men.[4] Intervention studies, mostly in men with low baseline DHEAS levels (below ~1.50 μg/mL), showed no beneficial effect of DHEA treatment on BMD in men,[9–13] except for one study reporting a modest increase in femoral neck BMD after 2 years of treatment.[14] These data are in agreement with a recent meta-analysis concluding that DHEA supplementation in elderly men has no effect on BMD.[15] Modest positive effects of supplemental DHEA on BMD were reported in postmenopausal women,[10–14,16] but a meta-analysis of seven studies indicated no significant effect of DHEA treatment on BMD in women.[17] DHEA supplementation in adolescent and young women with anorexia nervosa, who characteristically have subnormal serum DHEA and estrogen levels, maintained BMD, but there was no significant increase after accounting for the accompanying weight gain.[18] On the other hand, combined adrenal and gonadal hormonal replacement not only improved bone density but also bone strength in young women with anorexia nervosa.[19]

To date, no data are available documenting the association between serum DHEAS levels and the risk of fractures in men. Therefore, the aim of this study was to investigate the predictive role of serum DHEAS levels for incident fracture risk in a large population-based cohort of older Swedish men.

Materials and Methods

Study sample

The Osteoporotic Fractures in Men (MrOS) study is an international multicenter, prospective study including older men in the United States ($n = 5994$), Sweden ($n = 3014$), and Hong Kong ($n = 2000$). In this study, associations between serum DHEAS and incident fractures were investigated in MrOS Sweden (Table 1).

The MrOS Sweden cohort consists of three subcohorts from three different Swedish cities ($n = 1005$ in Malmö, $n = 1010$ in Gothenburg, and $n = 999$ in Uppsala). Study participants (men aged 69 to 81 years) were randomly selected using national population registers, contacted, and asked to participate. To be eligible for the study, the participants had to be able to walk without assistance, provide self-reported data, and sign an informed consent.[20] The study was approved by the ethics committees at the Universities of Gothenburg, Lund, and Uppsala. Informed consent was obtained from all study participants. Data are presented for those participants with serum DHEAS levels available and excluding those with surgical or chemical castration, and androgen or anti-androgen treatment.

Assessment of covariates

A standardized questionnaire was used to gather information about self-reported previous fractures after age 50 years, falls (yes/no) during the last 12 months preceding the baseline visit, amount of physical activity, nutritional intake, smoking, alcohol use, prevalent major diseases (diabetes, stroke, chronic obstructive pulmonary disease, rheumatoid arthritis, and cancer), and use of glucocorticoids. Physical activity was the

Table 1. Characteristics of the Study Participants

	MrOS Sweden ($n = 2568$)
Age (years)	75.5 (3.2)
Height (cm)	174.7 (6.5)
Weight (kg)	80.4 (12.1)
Body mass index (kg/m^2)	26.3 (3.6)
Serum sex steroids	
DHEAS (μg/mL)	0.70 (0.46)
Estradiol (pg/mL)	21.2 (7.5)
Testosterone (ng/dL)	457 (176)
SHBG (nmol/L)	47.9 (22.1)
Serum IGF-1 (ng/mL)	120.7 (48.8)
Participants with validated incident fractures (n, %)	
All fractures	594 (23.1)
Major osteoporotic fractures	422 (16.4)
Non-vertebral major osteoporotic fractures	255 (9.9)
Hip fractures	175 (6.8)
Clinical vertebral fractures	206 (8.0)
Fractures after age 50 years (n, %)	168 (6.5)
Calcium intake (mg/d)	893 (425)
Physical activity (km/d)	3.9 (3.1)
Grip strength (kg)	39.9 (7.5)
Corticosteroid use (n, %)	48 (1.9)
Smoking (n, %)	217 (8.5)
Alcohol ≥3 units per day (n, %)	56 (2.2)
Falls during past 12 months (n, %)	419 (16.3)
Femoral neck BMD (g/cm^2)	0.83 (0.13)
Lumbar spine BMD (g/cm^2)	1.14 (0.20)
FRAX major osteoporotic fracture without BMD (%)	11.1 (4.4)
FRAX major osteoporotic fracture with BMD (%)	9.8 (5.5)
FRAX hip fracture without BMD	6.0 (4.1)
FRAX hip fracture with BMD	4.7 (5.0)
Prevalent diseases (n, %)	
Cancer	345 (13.4)
COPD	212 (8.3)
Diabetes	238 (9.3)
Stroke	163 (6.3)
Rheumatoid arthritis	40 (1.6)

BMD = bone mineral density; COPD = chronic obstructive pulmonary disease.

Values are given as mean (SD) or n (%). FRAX is the country-specific calculated estimate of the 10-year risk of a major osteoporotic fracture or a hip fracture. Data are presented for those subjects with serum DHEAS available and excluding those with surgical or chemical castration and androgen or antiandrogen treatment.

participant's average total daily walking distance, including both walking as a means of exercise and leisure and as a means of outdoor transportation in activities of daily life. Calcium intake was estimated from dairy product intake and does not include supplemental calcium. Alcohol use was expressed as three or more glasses of alcohol-containing drinks per day, calculated from the reported frequency and amount of alcohol use. Grip strength was analyzed using a dynamometer; two measurements were taken on each side and the average of right and left was used in the analyses. Height was measured using a

Harpender stadiometer, and weight was measured with an electric scale. Body mass index (BMI) was calculated by dividing the weight in kg by the height in meter squared.

Areal BMD (aBMD, g/cm^2) of the femoral neck and lumbar spine (L$_1$ to L$_4$) was assessed at baseline using the Lunar Prodigy dual-energy X-ray absorptiometry (DXA; GE Lunar Corp., Madison, WI, USA) in the Uppsala and Malmö cohorts and the Hologic QDR 4500/A-Delphi (Hologic, Inc., Bedford, MA, USA) in the Gothenburg cohort. The coefficients of variation (CVs) for the aBMD measurements ranged from 0.5% to 3% depending on the application. To be able to use DXA measurements performed with equipment from two different manufacturers, a standardized BMD was calculated, as described previously.[20]

The country-specific FRAX tool was used to assess a participant's calculated 10-year probability of a major osteoporotic fracture (clinical spine, distal radius, proximal humerus, or hip) or a hip fracture (www.shef.ac.uk/FRAX/). It integrates the fracture risks associated with clinical risk factors as well as femoral neck BMD.[21]

Assessment of incident fractures

Central registers covering all Swedish citizens were used to identify the participants, and at the time of fracture evaluation, computerized X-ray archives were searched for new fractures occurring after the baseline visit.[22] The follow-up time was recorded from the date of the baseline visit (2001 to 2004) until the date of the first fracture, death, or fracture data collection (December 31, 2013). Except for 3 participants who moved abroad, there was no loss of follow-up. When a participant sustained a first fracture at different sites during the follow-up, the various fractures and the corresponding follow-up times for each respective first fracture type were included in the analyses. All fractures were verified by X-ray. The median follow-up time was 10.6 years.

Serum analyses

A validated gas chromatography-tandem mass spectrometry system (Endoceutics, Québec, Canada)[23,24] was used to analyze serum testosterone (T, limit of detection 0.05 ng/mL, intra-assay CV 2.9%, interassay CV 3.4%) and estradiol (E2, limit of detection 2.00 pg/mL, intra-assay CV 1.5%, interassay CV 2.7%). Serum DHEAS (limit of detection 0.075 μg/mL, intra-assay CV 5.2%, interassay CV 6.3%) was measured by a validated liquid chromatography-tandem mass spectrometry method.[23] Serum was also assayed for sex hormone-binding globulin (SHBG) using an immunoradiometric assay (Spectria, Orion Diagnostica, Espoo, Finland) with a limit of detection of 1.3 nmol/L, an intra-assay CV of less than 5.5%, and an interassay CV of less than 6.9%. The majority of the serum samples (69%) were morning samples (drawn before 10.00 a.m.); the remaining were drawn around noon. Serum levels of T (471 ± 176 versus 424 ± 172 ng/dL, $p < 0.001$) and E2 (21.7 ± 7.5 versus 19.9 ± 7.4 pg/mL, $p < 0.001$) were higher in morning samples compared to non-morning samples, whereas serum DHEAS levels, as expected, showed no diurnal variation (0.70 ± 0.45 versus 0.71 ± 0.46 μg/mL, $p = 0.420$). All analyses were adjusted for time of serum sampling (morning sample yes/no).

Serum from the baseline visit was also assayed for insulin-like growth factor-1 (IGF-1) by a double-antibody IGF-binding protein blocked radioimmunoassay using a commercial kit (Mediagnost, Tubingen, Germany) with an intra-assay CV of less than 5% and an interassay CV of less than 8%.

Statistical analyses

The association between log-transformed DHEAS concentrations and age was tested using Pearson correlations. The associations between log-transformed DHEAS and femoral neck or lumbar spine BMD were examined used linear regression models controlling for age, MrOS Sweden site, and time of serum sampling (morning sample yes/no). Differences in serum DHEAS levels between participants with or without cancer or with or without prevalent diseases at baseline were examined using t tests. Differences in serum steroid levels (T, E2, and DHEAS) between participants with or without morning samples were also examined using t tests.

Cox proportional hazards models were used to analyze the associations between serum DHEAS and incident fractures. Hazard ratios (HRs) and 95% CIs were estimated from the models and expressed as a 1 SD increase (Z score) in log-transformed DHEAS levels. All estimates were adjusted for age, BMI, prevalent fractures, time of serum sampling (morning sample yes/no), and MrOS Sweden site. In predefined analyses, the following validated fracture types were analyzed: all fractures, major osteoporotic fractures (defined as hip, clinical vertebral, distal radius, and proximal humerus fractures), non-vertebral major osteoporotic fractures, hip fractures, and clinical vertebral fractures.

To investigate whether the associations between serum DHEAS and incident fractures were independent of the active sex steroids, the models were adjusted for baseline serum levels of T, E2, and SHBG. To test for further confounding, the following risk factors for fracture were examined in the model: smoking, alcohol use, calcium intake, falls during the past 12 months, prevalent diseases (diabetes, stroke, chronic obstructive pulmonary disease, rheumatoid arthritis, and cancer), and glucocorticoid use. Further adjustments for physical performance variables (grip strength and daily walking distance) and femoral neck BMD were performed to investigate the mechanism behind the associations between serum DHEAS and incident fractures. FRAX estimates with or without BMD were also included in the regression models to test the clinical utility of serum DHEAS as a risk marker of fractures.

Fracture risk discrimination was determined using C-statistics, where the statistical significance of change in the area under the ROC curve (AUC) between models was tested with the roc. test function in R using the pROC package.[25] Fracture risk reclassification analyses were performed by calculating the integrated discrimination improvement (IDI) and net reclassification improvement (NRI) according to Pencina and colleagues.[26] The improvements in risk prediction by serum DHEAS as a continuous parameter were evaluated over base models containing FRAX estimates with or without BMD. To identify the proportion of subjects correctly reclassified by adding serum DHEAS levels as extra predictors, the incremental discriminative ability of DHEAS levels compared with base models containing FRAX estimates with or without BMD was assessed using category-free IDI and NRI (after rounding the predicted values to two decimal places).

Quadratic models were used to test for possible nonlinearity in the relation between serum DHEAS and incident fractures. We also used a restricted cubic spline approach for a flexible nonlinear assessment of the HR in relation to serum DHEAS levels. The positions and the number of knots were selected using the Akaike Information Criterion (AIC). Three knots placed at the 5th, 50th, and 95th percentile of the Z score of

log-transformed DHEAS levels were found to give a small AIC and capture the average curve shape over a systematic assessment of different alternatives.

The restricted cubic spline models were fitted using the survival[27] and regression modeling strategies packages[28] in R. All other statistical analyses were performed using IBM SPSS software (version 21.0; Chicago, IL, USA).

Results

Characteristics of the study participants

The baseline characteristics of the MrOS Sweden study participants are shown in Table 1. At baseline, the mean age of the men was 75.5 years. As expected, serum DHEAS levels were significantly inversely associated with age ($r = -0.20$, $p < 0.001$). Marginal associations were also observed between serum DHEAS and either femoral neck BMD (standardized beta coefficient = 0.042, $p = 0.036$) or lumbar spine BMD (standardized beta coefficient = 0.046, $p = 0.024$) in regression models adjusted for age, time of serum sampling, and MrOS Sweden site. Participants with cancer at the baseline visit or those with one of the prevalent diseases (diabetes, stroke, chronic obstructive pulmonary disease, rheumatoid arthritis, or cancer) had lower serum DHEAS levels compared with those without cancer or without any prevalent disease (0.62 ± 0.43 versus $0.71 \pm 0.46 \, \mu g/mL$, $p < 0.001$ and 0.63 ± 0.44 versus $0.74 \pm 0.46 \, \mu g/mL$, $p < 0.001$, respectively). Accordingly, our associations between serum DHEAS and fractures were tested for confounding.

During a median follow-up time of 10.6 years, 594 men experienced at least one fracture (rate of 21.8 per 1000 person-years), 422 men had a major osteoporotic fracture (rate of 15.5 per 1000 person-years), and 255 had at least one non-vertebral major osteoporotic fracture (rate of 9.4 per 1000 person-years). Of the 2568 men, 6.8% experienced a hip fracture (rate of 6.4 per 1000 person-years), whereas 8.0% had at least one validated incident clinical vertebral fracture (rate of 7.6 per 1000 person-years).

Serum DHEAS levels are associated with incident fractures

Cox proportional hazards models adjusted for age, BMI, and prevalent fractures demonstrated that low DHEAS levels were associated with an increased risk of any fracture, major osteoporotic fractures, non-vertebral major osteoporotic fractures, and hip fractures when analyzed as continuous variables (Table 2). The association between serum DHEAS and clinical vertebral fractures did not reach statistical significance.

Because DHEA can act as a precursor for both T and E2, we investigated whether the associations between serum DHEAS and fracture risk were dependent on the circulating levels of sex steroids. To this end, we adjusted the base models of serum DHEAS and fractures for baseline levels of T, E2, and SHBG. This adjustment did not materially affect the significant associations between low DHEAS and the likelihood of any fracture (hazard ratio [HR] per SD decrease = 1.11, 95% confidence interval [CI] 1.02–1.20), major osteoporotic fractures (HR per SD decrease = 1.17, 95% CI 1.06–1.29), and non-vertebral major osteoporotic fractures (HR per SD decrease = 1.26, 95% CI 1.11–1.42). Because it was shown that older men with low serum IGF-1 levels have an increased fracture risk,[29] we also tested whether the observed associations between serum DHEAS and incident fracture were

Table 2. Serum DHEAS and the Risk of Incident Fractures

	DHEAS (per SD decrease)
All fractures	1.14 (1.05–1.24)
Major osteoporotic fractures	1.22 (1.11–1.34)
Non-vertebral major osteoporotic fractures	1.31 (1.16–1.48)
Hip fractures	1.18 (1.02–1.37)
Clinical vertebral fractures	1.09 (0.95–1.26)

Cox proportional hazards regression models adjusted for age, body mass index, prevalent fractures, morning sample yes/no, and MrOS Sweden site. Hazard ratios are given with 95% CI within parentheses.

mediated by circulating IGF-1 levels. Addition of serum IGF-1 to the base model did not, however, substantially change the association between serum DHEAS and any fracture (HR per SD decrease = 1.14, 95% CI 1.05–1.24).

Serum DHEAS levels are independent risk markers of fracture risk

To adjust for possible confounding in our analyses, we adjusted our models for traditional risk factors for fracture (smoking, alcohol use, calcium intake, falls during the past 12 months, prevalent diseases [diabetes, stroke, chronic obstructive pulmonary disease, rheumatoid arthritis, and cancer], and glucocorticoid use). The association between serum DHEAS and the risk of any fracture, major osteoporotic fractures, non-vertebral major osteoporotic fractures, and hip fractures did not materially change in these multivariate models (Table 3).

Next, we investigated whether the associations between serum DHEAS and fracture risk were mediated by BMD or physical performance variables (Table 3). Adjustment of the base models for femoral neck BMD only marginally attenuated the associations between serum DHEAS and all fractures, major osteoporotic fractures, and non-vertebral major osteoporotic fractures, whereas the association no longer reached statistical significance for hip fractures. Similarly, the associations between serum DHEAS and the risk of any fracture, major osteoporotic fractures, and non-vertebral major osteoporotic fractures were only slightly attenuated after adjustment for grip strength and daily walking distance. Finally, simultaneous adjustment for confounding variables, femoral neck BMD, and physical performance variables only partially attenuated the results for all fractures, major osteoporotic fractures, and non-vertebral major osteoporotic fractures (Table 3).

To further evaluate the robustness of our findings, we performed sensitivity analyses exploring the impact of subclinical/undiagnosed diseases. Exclusion of the first year of follow-up did not substantially alter the associations between DHEAS levels and the likelihood of any fracture, major osteoporotic fractures, and non-vertebral major osteoporotic fractures (Table 3).

Serum DHEAS levels are associated with incident fractures independently of FRAX

To evaluate the impact of other risk factors for fracture incorporated into the FRAX score on the observed associations between serum DHEAS and fractures, we performed additional analyses in which the base models were further adjusted for

Table 3. Serum DHEAS Is Independently Associated With Incident Fractures

DHEAS (per SD decrease)	All fractures	Major osteoporotic fractures	Non-vertebral major osteoporotic fractures	Hip fractures
Base model	1.14 (1.05–1.24)	1.22 (1.11–1.34)	1.31 (1.16–1.48)	1.18 (1.02–1.37)
Base model + confounding risk factors for fracture	1.13 (1.03–1.23)	1.20 (1.09–1.33)	1.31 (1.15–1.49)	1.20 (1.03–1.40)
Base model + BMD	1.13 (1.04–1.23)	1.20 (1.09–1.32)	1.28 (1.13–1.44)	1.15 (0.99–1.33)
Base model + physical performance variables	1.13 (1.03–1.23)	1.20 (1.09–1.33)	1.28 (1.13–1.45)	1.15 (0.98–1.34)
Base model + confounding risk factors + BMD + physical performance variables	1.12 (1.02–1.23)	1.20 (1.08–1.33)	1.30 (1.13–1.49)	1.17 (0.99–1.39)[a]
Base model, excluding first year of follow-up	1.10 (1.01–1.20)	1.17 (1.05–1.29)	1.26 (1.11–1.43)	1.16 (0.99–1.35)[b]

Cox proportional hazards regression models for a base model (adjusted for age, body mass index, prevalent fractures, morning sample yes/no, and MrOS Sweden site) and base models with additional adjustments for confounding risk factors for fracture (smoking, alcohol use, calcium intake, falls during the past 12 months, prevalent diseases [diabetes, stroke, chronic obstructive pulmonary disease, rheumatoid arthritis, and cancer], and glucocorticoid use), femoral neck BMD and/or physical performance variables (grip strength, daily physical activity) or exclusion of men with a follow-up time of 1 year or less. Hazard ratios are given with 95% CI within parentheses.
[a]$p = 0.059$.
[b]$p = 0.059$.

FRAX with or without BMD (Table 4). The inverse associations between serum DHEAS and the likelihood of fractures remained unchanged after adjustment for FRAX estimates without BMD, whereas the addition of FRAX with BMD only marginally reduced the point estimates for the risk of the various fractures. No significant improvements in fracture risk discrimination with serum DHEAS were observed over base models adjusted for FRAX with or without BMD. When evaluated with the more sensitive IDI and NRI, DHEAS levels moderately but significantly improved fracture risk reclassification compared with base models including FRAX estimates without BMD but not compared with base models in which FRAX probabilities were combined with BMD (Supplemental Table S1).

Serum DHEAS levels below the median are associated with increased fracture risk

Quadratic models supported a nonlinear association between serum DHEAS levels and the risk of any fracture ($p = 0.033$) and major osteoporotic fractures ($p = 0.016$) but not for the non-vertebral major osteoporotic fractures and hip fractures. To further explore this, we performed restricted cubic spline analyses, which confirmed a statistically significant nonlinear relationship between serum DHEAS and either all fractures (p for nonlinearity $= 0.03$, Fig. 1A) or major osteoporotic fractures (p for nonlinearity $= 0.02$, Fig. 1B) for a base model adjusted for age, BMI, and prevalent fractures. Based on the appearance of the curves, the 50th percentile represents a cut-off for serum DHEAS with respect to fracture risk: the increased risk of any fracture (Fig. 1A) and major osteoporotic fractures

(Fig. 1B) emerges most clearly for those participants with DHEAS levels below the median DHEAS concentration, ie, 0.60 μg/mL. Accordingly, participants with serum DHEAS levels below this cut-off had a significantly increased fracture risk (all fractures: HR = 1.22, 95% CI 1.04–1.44; major osteoporotic fractures: HR = 1.33, 95% CI 1.09–1.61) compared with participants with serum DHEAS above the median in models adjusted for age, BMI, and prevalent fractures.

Discussion

In this large, population-based cohort study of older men, we demonstrate that serum DHEAS levels are associated with incident fracture risk. The predictive role of serum DHEAS remained significant after adjustment for traditional risk factors for fracture. Interestingly, serum DHEAS added information beyond FRAX estimates with BMD for fracture risk prediction. The inverse association between serum DHEAS and the risk of any fracture or major osteoporotic fractures is nonlinear, with a significant increase in fracture risk for those participants with serum DHEAS levels below 0.60 μg/mL.

This study is, to the best of our knowledge, the first one to describe the associations between serum DHEAS levels and incident fracture risk in older men. Previous studies examining the associations between serum DHEAS and BMD[4,6–8] or the effect of DHEA treatment on BMD[9–14] reported conflicting findings, with the positive results, if any, being small, site-specific, and not reproducible. In our cohort, we found a marginal association between serum DHEAS levels and BMD

Table 4. Serum DHEAS as a FRAX-Independent Risk Marker of Fracture Risk

DHEAS (per SD decrease)	All fractures	Major osteoporotic fractures	Non-vertebral major osteoporotic fractures	Hip fractures
Base model	1.14 (1.05–1.24)	1.22 (1.11–1.34)	1.31 (1.16–1.48)	1.18 (1.02–1.37)
Base model + FRAX without BMD	1.14 (1.05–1.24)	1.21 (1.10–1.34)	1.31 (1.16–1.48)	1.18 (1.02–1.36)
Base model + FRAX with BMD	1.13 (1.04–1.23)	1.20 (1.09–1.32)	1.28 (1.14–1.44)	1.17 (1.01–1.35)

Cox proportional hazards regression models for a base model (adjusted for age, body mass index, prevalent fractures, morning sample yes/no, and MrOS Sweden site), and base models with additional adjustments for FRAX without BMD or FRAX with BMD. FRAX is the country-specific calculated estimate of the 10-year risk of a major osteoporotic fracture or hip fracture. Hazard ratios are given with 95% CI within parentheses.
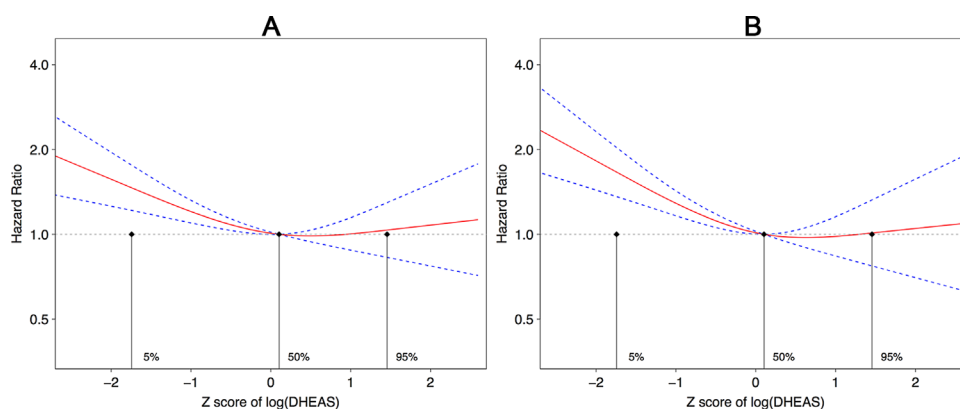
Fig. 1. Smoothed plots of the likelihood of fractures according to serum DHEAS concentrations. Hazard ratios (HRs) for all fractures (*A*) and major osteoporotic fractures (*B*) were estimated with Cox regression analyses using a restricted cubic spline approach and with the median DHEAS concentration (0.60 μg/mL) as reference value. Three knots were positioned at the 5th, 50th, and 95th percentiles of the serum DHEAS concentration (*Z* score of log-transformed values) (vertical lines). The models were adjusted for age, BMI, prevalent fractures, morning sampling (yes/no), and MrOS study site. Data are presented as HRs (red solid line) and 95% CIs (blue dashed lines). The horizontal dashed line corresponds to the reference (median 0.60 μg/mL) HR of 1.0.

measured either at the lumbar spine or femoral neck. We report a predictive role of serum DHEAS for incident fracture risk that is apparent not only for all fractures but also for different fracture types such as major osteoporotic fractures, non-vertebral major osteoporotic fractures, and hip fractures. Moreover, the point estimate of the association was most pronounced for non-vertebral major osteoporotic fractures (HR = 1.31) whereas it was substantially lower (HR = 1.09), and not statistically significant, for clinical vertebral fractures. This might indicate that serum DHEAS influences cortical bone more than trabecular bone, thereby predominantly impacting the risk of non-vertebral fractures.

It is generally assumed that DHEAS acts as a precursor for the active sex steroids and that its effects are mediated by local metabolism in peripheral tissues. The observed associations between serum DHEAS and fracture risk would then be explained by the local actions of E2 and T on the skeleton. We and others have previously shown that circulating E2, and to a lesser extent T, are inversely associated with fracture risk in men, whereas circulating SHBG is directly related to incident fractures.[22,30–36] In addition, several intervention studies have shown increases in serum T and/or E2 levels after DHEA treatment,[15] supporting this hypothesis. Nevertheless, in the present study, the associations between serum DHEAS and the likelihood of fractures persisted and were only slightly attenuated after adjustment for serum levels of E2, T, and SHBG, suggesting that circulating sex steroids are not crucial mediators of the inverse association between DHEAS and fracture risk. Yet, because DHEAS is converted to active sex steroids locally in peripheral tissues, which is not reflected by the circulating DHEAS levels, we believe it is plausible that intra-tissue sex steroid levels may be involved in the impact of DHEAS on fracture risk.

Adjustment for traditional risk factors for fracture did not materially change the associations between serum DHEAS and the risk of different types of fractures. Also, exclusion of the first year of follow-up did not alter the inverse association between serum DHEAS and fracture risk, arguing against comorbidities confounding the observed associations. The predictive value of

serum DHEAS for fracture risk was largely independent of BMD because the associations were not substantially altered in BMD-adjusted models. This is consistent with the marginal association between serum DHEAS and BMD observed in the present study and no or limited site-specific increases in BMD in DHEA intervention studies.[9–14] Also, our observed associations were only slightly attenuated by physical activity or muscle strength, which makes it unlikely that muscle mass mediates the impact of DHEAS on fracture risk. This is in agreement with the lack of effect of DHEA on muscle mass in treatment studies.[15]

Interestingly, the inverse associations between DHEAS and incident fracture risk remained largely unchanged after adjustment for FRAX estimates together with BMD, indicating that low serum DHEAS levels are a risk marker for fracture risk by adding information beyond the probability estimates from the FRAX tool in combination with BMD. With regard to fracture risk discrimination and reclassification, serum DHEAS levels showed limited clinical utility.

The association between serum DHEAS levels and incident fracture risk was nonlinear, with a 22% and 33% increased risk of any fracture and major osteoporotic fractures, respectively, for those men with serum DHEAS levels below the threshold of 0.60 μg/mL. This threshold is much lower than the DHEAS cut-off (around 1.50 μg/mL) that was used in several DHEA intervention studies,[11,14] possibly explaining the lack of or limited effect of DHEA treatment on BMD in those studies. In the case of a causal inverse association between serum DHEAS and fracture risk, the present study suggests that older men with serum DHEAS levels below 0.60 μg/mL would benefit from DHEA treatment, but previous treatment studies in men have not demonstrated any substantial beneficial effect on BMD.[9–14]

Our study has a number of strengths. It is population based and consists of a large data set with a high fracture incidence and well-characterized fracture phenotypes. Moreover, baseline serum sex steroid levels and their precursor are determined by mass spectrometry technology. Our study has, however, limitations. The results are based on single serum measurements of DHEAS and may thus underestimate true associations. Also, the inclusion of some non-morning serum samples might have

contributed to increased variability, but this was adjusted for in all analyses by time of sampling. In addition, no validation of the model was performed. A major drawback is also that no measurements of follicle-stimulating hormone (FSH) or luteinizing hormone (LH) are available. Finally, we have tried to adequately adjust for confounders in all our analyses but cannot rule out residual confounding.

In conclusion, low serum DHEAS levels are independent risk markers of mainly non-vertebral fractures in older men. The association between serum DHEAS and fracture risk is nonlinear, with men with DHEAS levels below 0.60 μg/mL at greatest risk of fracture.

Disclosures

Acknowledgments

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