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Low androstenedione/sex hormone binding globulin ratio increases fracture risk in postmenopausal women. The Women's Health in the Lund Area study

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ABSTRACT

The Women's Health in the Lund Area (WHILA) project ($n = 6917$) is a cohort study that started in 1995 and includes a postal questionnaire, physical examination, bone density measurement and blood laboratory analyses. Fracture data have been added, and in this report fracture risk and its association with sex hormones was analysed in postmenopausal women without current hormone therapy (HT).

A total of 409 women (median age 56.8 years) with 489 fractures were identified from the postmenopausal women without HT during a median follow-up time of 8.4 years. Lower serum levels of androstenedione ($p < 0.001$), testosterone ($p = 0.008$), androstenedione/sex hormone binding globulin (SHBG) ratio ($p < 0.001$), testosterone/SHBG ratio ($p = 0.003$) and higher levels of SHBG ($p = 0.005$) were observed in women with fractures compared to no fracture. No difference in oestradiol levels was observed.

Androstenedione and androstenedione/SHBG ratio were further divided into percentiles. Increased fracture risk was found in postmenopausal women with androstenedione in 5th percentile compared to 11–89th percentile HR 1.51 (95% CI 1.02–2.24). The androstenedione/SHBG ratio (11–89th percentile as reference) showed increased fracture risk in women with low ratio 5th percentile HR 1.75 (95% CI 1.20–2.54) and decreased fracture risk with high ratio 95th percentile HR 0.52 (95% CI 0.28–0.98).

An increased fracture risk during follow-up was encountered in postmenopausal women with low serum androstenedione and androstenedione/SHBG ratio at baseline and a decreased fracture risk with high androstenedione/SHBG ratio. This study suggests that postmenopausal osteoporosis is influenced by lower levels of androgens.

1. Introduction

The WHILA project is a population-based study in middle-aged women and still ongoing. This follow-up is based on data from the original screening performed between 1995 and 2000 [1]. For this analysis, additional data were collected from study onset up to 2006 concerning occurrence of fractures.

The menopausal transition combines hormonal changes with adverse effects on bone mineral density (BMD) that are not fully understood. In bone tissue, aromatase is present to enable the peripheral conversion of androgens to oestrogens [2] and enable local effect on bone tissue. Few previous studies have compared androstenedione levels in women with and without fractures. Results are diverging with no difference in androstenedione comparing women without and with fractures [3–5], lower production rate of androstenedione in women with fracture [6] as well as lower levels of androstenedione in women with postmenopausal osteoporosis [7].

The effects of oestrogens and androgens in tissues are strongly correlated to their free fractions and hence influenced by the amount of circulating sex hormone binding globulin (SHBG) [8]. Some previous studies have found higher levels of SHBG in women with fractures [9–11] possibly due to the fact that higher levels of SHBG bind greater amounts of circulating sex steroids diminishing the free fraction that is biologically active.

The aim of this study was to describe the incidence of fractures and analyse the risk of fracture in relation to levels of sex steroid hormones in middle-aged postmenopausal women without HT.

Abbreviations: FTF, fragility type fractures; NFTF, non-fragility type fractures; PM, premenopausal with regular menstruations; PMO, postmenopausal where menstruations have ceased for more than 12 months ago; PMT, peri- and postmenopausal women with current hormone therapy.

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2. Subjects and methods

2.1. Participants

The WHILA project as detailed previously [1], invited all women (n = 10,766) born between December 2, 1935 and December 1, 1945 living in the area around Lund, Sweden on December 1, 1995, to participate in the study including a postal questionnaire, physical examination, bone density measurement and laboratory analyses at baseline inclusion in the study.

Women participating in the study filled out a generic questionnaire including 104 questions regarding general personal background, medical history as well as obstetric and gynaecological history.

Menopausal status was categorized as follows: premenopausal (PM) with regular menstruation, postmenopausal (PMO) in whom menstruations had ceased for more than 12 months previously, and peri- and postmenopausal women with current hormone therapy (HT) (PMT).

Body weight (kg) and height (cm) were measured and body mass index (BMI) (kg/m^2) was calculated. Venous blood was drawn, irrespective of time of the day, for future analysis of serum concentrations of steroidal hormones.

Bone mineral density (BMD) was measured at the wrist by dual X-ray absorptiometry (DXA) (Osteometer DTX 200; Medi-Tech A/S, Rodovre, Denmark) and T-score (BMD expressed as number of standard deviations (SD) compared to young adult values) and Z-score (BMD expressed as number of SDs compared to age-matched references) were stated [12]. A standardised phantom was used for daily calibration of the instrument. All measurements were performed by one and the same technician.

2.2. Ethical considerations

All women submitted their informed consent and the study was approved by the Ethics Committee at Lund University (LU 174-95) and the Swedish Data Inspection Board. Fracture data collection was approved in an additional further application by the Ethics Committee at Lund University (LU 505-03).

2.3. Fracture data collection

Using Sweden's unique personal identification number, study participants subject to fracture were identified from the computerised files at the Department of Orthopaedics at the University Hospital in Lund and the County Council (Region Skåne) Diagnostic Centre. Data were controlled for duplication of fracture data. The study period for fractures was between December 1st 1995 and August 31st 2006. Fracture dates were checked and included fractures occurred after the individual participant's inclusion in the study.

Fractures were divided according to fracture site into fragility type fracture (FTF) (including fractures of the vertebrae, hip, wrist–forearm, humerus, femur, rib, pelvis, clavicle, scapulae, tibia and fibula) [13]. The remaining fractures were considered non-fragility type fracture (NFTF).

2.4. Laboratory analyses

Determinations of serum androstenedione and SHBG levels were performed by enzyme-linked immunosorbent assay (ELISA) techniques (DRG Instrument GmbH, Marburg, Germany). KRYPTOR®-Testosterone and KRYPTOR®-Estradiol 17β (BRAHMS Ag, Heningsdorf, Germany) were used for automated immunofluorescent assays of testosterone and oestradiol in human serum.

For androstenedione intra- and interassay coefficient of variations (CV) were 6.3% and 8.1% respectively. The lower detection limit for androstenedione was 0.17 nmol/L and for androstenedione levels below this, a calculated theoretical median value of 0.13 nmol/L, based on the normal distribution, was set (n = 3). The intra- and interassay CV values for SHBG were 8.6% and 11.6% and detection limit 0.2 nmol/L. For SHBG levels below 4.00 nmol/L, a calculated theoretical median value of 3.00 nmol/L was set (n = 4). The intra- and interassay CV values for testosterone were 6.4% and 10.0% and lower detection limit for testosterone was 0.15 nmol/l and for testosterone levels below this, a calculated theoretical median value of 0.1125 nmol/l was set (n = 408). The intra- and interassay CV values for oestradiol were 7.1% and 6.0%. The lower detection limit for oestradiol was 3.5 pmol/L and for oestradiol levels below this, a calculated theoretical median value of 2.625 pmol/L was set (n = 841).

A testosterone/SHBG ratio was calculated as testosterone/SHBG × 100 [14]. An oestradiol ratio was calculated as oestradiol/SHBG × 100. These indices were calculated as to estimate free levels of testosterone and oestradiol respectively. An androstenedione/SHBG ratio was calculated as androstenedione/SHBG × 100.

2.5. Statistical analyses

Data distribution was analysed by the Kolmogorov–Smirnov test. Descriptive statistics were computed and results are expressed as mean (standard deviation) for parametric data and as median (minimum–maximum) for non-parametric data. Student's T-test was used for analysis of parametric data and the Mann–Whitney U-test was used for analysis of non-parametric data. Grouped data was analysed with crosstabs and the Pearson Chi-square test. Pearson's correlation test was used to analyse correlation between hormone levels. Hormone analyses were performed with logarithmic values of hormones where applicable.

Univariate and multivariate Cox proportional hazard models were used to analyse which hormones to use for further analysis. Overall fracture probability in PMO women was estimated using the Kaplan–Meier method. The Cox proportional hazards model was used for univariate analysis of percentiles' of androstenedione and androstenedione/SHBG levels and fracture risk. Assumptions of proportional hazards were verified graphically wherever applicable. Significant departures from proportionality were not observed. Adjustment was performed with age and BMI as continuous variables and current smoking as categorical variable (yes/no).

All comparisons were two-sided and P values less than 0.05 were considered statistically significant. The Holm–Bonferroni method was used for correction of multiple analyses, Statistical analysis was performed using SPSS (PASW) version 18.0 (SPSS Inc., Chicago, IL, USA).

3. Results

The WHILA project comprises a total of 6917 women participating in the baseline screening procedures. The main part of women in WHILA were born in Sweden (n = 6133; 88.7%) or nearby countries: Finland (n = 102; 1.5%), Germany (n = 89; 1.3%) and Denmark (n = 82; 1.2%).

A total of 3363 (48.6%) women were categorized as PMO. PMT women (n = 3053; 44.1%) and PM women (n = 492; 7.1%) were not included in the analyses to avoid interference of exogenous supplied hormones and endogenous fluctuating hormones.

Fractures occurred in 409 PMO women (12.2%) and some women had multiple fractures: two fractures (n = 65), three fractures (n = 12) and four fractures (n = 3). FTF had occurred in 253 PMO women and NFTF in 156 PMO women.