Circulating sclerostin levels in relation to nutritional status, sex hormones and selected bone turnover biochemical markers levels in peri- and postmenopausal women

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ABSTRACT

Objectives: Hormonal changes during the peri- and postmenopausal age, especially decreasing estradiol levels as the result of the expired ovarian function, are an established link of the pathogenesis of postmenopausal osteoporosis. The objective of the study was to examine the association between the circulating sclerostin levels and nutritional status, sex hormones and selected bone markers turnover levels in peri- and postmenopausal women.

Material and methods: The study enrolled 84 stable-body mass women (31 perimenopausal and 54 postmenopausal). Anthropometric measurements and serum estrone, testosterone, androstenedione, DHEA-S, osteocalcin, β-CTx, 25-OH-Vitamin D and sclerostin levels were obtained.

Results: There were not any differences between body mass, BMI, body fat and waist circumference between the study groups. The serum androstenedione and DHEA-S levels were similar in both study groups. However, estrone and total testosterone levels were observed to be notably higher in the perimenopausal group, unlike in the postmenopausal group (124.1 pg/mL vs. 98.3 pg/mL, p < 0.01 and 0.3 pg/mL vs. 0.22 pg/mL, p < 0.01, respectively). Higher plasma osteocalcin and β-CTx levels were shown in the postmenopausal rather than in the perimenopausal group (19.8 ng/mL vs. 16.8 ng/mL, p < 0.001 and 0.35 ng/mL vs. 0.29 ng/mL, p < 0.05, respectively). Plasma sclerostin and 25-OH-Vitamin D levels were similar. There was not any correlation between plasma sclerostin levels and the other studied parameters. In the multivariate regression analyses, sclerostin levels were proportional to the androstenedione ones (b = 0.06; p < 0.05) but inversely related to the log10(testosterone) levels (b = -0.18; p < 0.05).

Conclusions: Circulating sclerostin levels are similar in peri- and postmenopausal women and are related to the androstenedione and testosterone levels regardless of the nutritional status.

Key words: sclerostin; bone turnover markers; sex hormones; nutritional status; menopause

INTRODUCTION

Hormonal changes during peri- and postmenopausal age, especially decreasing estradiol levels as the effect of the expired ovarian function, are an established link of the pathogenesis of postmenopausal osteoporosis. The experimental study showed that osteogenesis decreased just 5 days after the removal of the ovary [1]. It has been suggested that estradiol inhibits the apoptosis of osteoblasts [2]. Currently, it is believed that the main signaling pathway regulating bone mass is the Wnt/β-catenin pathway [3, 4] and sex hormones may affect the activity of this pathway [5]. However, the main regulator of this pathway ac-
tivity is sclerostin — Wnt antagonist produced by osteocytes. Sclerostin binds to LRPS and LRPR6 receptors and inhibits the activity of the Wnt/β-catenin pathway [6–9]. Higher sclerostin levels were observed in the post- rather than perimenopausal women and its levels are inversely proportional to the free estradiol index. Thus, it seems that estradiol is the factor regulating sclerostin synthesis [10]. This hypothesis confirms the observation that the administration of estradiol reduces the concentration of circulating sclerostin [11]. However, it is not known whether estradiol affects the synthesis of sclerostin directly or indirectly. Interestingly, the changes in sex hormones during the menstrual cycle did not affect sclerostin levels in regularly menstruating women [12]. It has also been shown that in men testosterone increased circulating sclerostin levels [11]. On the other hand, one study showed that sclerostin levels weakly correlated with bone mass density (BMD), bone turnover and parathormone (PTH) levels in postmenopausal women [13], whereas another study revealed an inverse association between bone mineral density and sclerostin in postmenopausal women. In addition, among women with osteoporosis positive association between sclerostin levels and BMI was observed. There were no correlations between sclerostin levels and circulating vitamin D, PTH, FSH, E2 and thyroid hormones [14]. However, the results assessed the relationship between circulating sclerostin levels and BMI as inconclusive. Some studies showed a positive correlation [14, 15], while others did not observe this association [16]. As a consequence, examining the association between circulating sclerostin levels and the nutritional status, sex hormones and selected bone markers turnover levels in peri- and postmenopausal women was the main objective of the study.

MATERIAL AND METHODS

The cross-sectional study involved 31 perimenopausal and 54 postmenopausal women. The inclusion criteria for perimenopausal women were irregular menstruation, hormonal confirmation of perimenopause and for postmenopausal women the time of their last menstruation, minimum 2 years. The inclusion criteria for both groups included normal thyroid function, stable body mass in the last 3 months and not using a hypocaloric diet in the last 6 months. The exclusion criteria included using any kind of a hormonal therapy, smoking and excessive drinking. Informed consent was obtained from all of the participants and the study protocol was granted the approval of the Ethical Committee of the Medical University of Silesia.

Anthropometric measurements (body mass, height and waist circumference) were carried out, and BMI was calculated in accordance with the standard formula. The participants’ body composition was measured by using the bioimpedance method with the aid of Bodystat 1500 (Douglas, Isle of Man). 10 mL samples of venous blood were taken in the morning between 8.00–9.00 a.m., after an overnight period of fasting (16 h). The blood samples were accumulated following the kit manufacturer’s recommendations. All the serum and plasma samples were stored frozen in -70°C.

Biochemical measurements

Total testosterone, dehydroepiandrosterone sulfate (DHEA-S) were determined by the ECLAIA method using Cobas E411 analyzer (Roche Diagnostics GmbH, Mannheim, Germany) with a lower limit of sensitivity 0.025 ng/mL, 0.003 μmol/L, respectively; the respective intra- and inter-assay coefficients of variations were 4.7% and 8.4% for testosterone, 2.8% and 4.7% for DHEA-S.

Estrone (BioVendor, Czech Republic) and androstendione (DRG Instruments GmbH, Marburg, Germany) were determined by using ELISA with a lower limit of sensitivity 10.0 pg/mL and 0.019 ng/mL, respectively; and the respective intra- and inter-assay coefficients of variations 7.7% and 9.1% for estrone and 9.1% and 12.1% for androstendione.

ELISA kits, all commercially available, were used to measure plasma levels of sclerostin (TECOmedical AG, Sissach, Switzerland; the mean intra- and inter-assay coefficients < 4.0% and the < 4.8%, respectively), 25-OH-Vitamin D (DRG Instruments GmbH for Hybrid XL, Marburg, Germany; the inter-assay precision < 14.2%). Osteocalcin and β-CTx were assessed utilizing ECLIA (Roche Diagnostics GmbH, Mannheim, Germany for Cobas e 411 analyser) set up to sensitivity < 3.3% and < 4.2% respectively.

Statistical analysis

The statistical analysis was carried out utilizing the Statistica 12.0 software (TIBCO Software Inc., Palo Alto, USA). Nominal and ordinal data were expressed as percentages, while interval data were expressed as mean value ± standard deviation in the case of the normal distribution or as median with lower and upper quartile in the case of data with the skewed or non-normal distribution. The distribution of variables was evaluated by means of the Shapiro-Wilk test and quantile-quantile (Q-Q) plot, whereas the homogeneity of variances was assessed by using the Fisher test. To compare the data between the fitness and control group, the t-Student test for independent data (in the case of the normal data distribution or after logarithmic normalization — if appropriate — in the case of the skewed distribution) or the non-parametric U Mann-Whitney test (in non-normal data distribution) were used. The Pearson correlation coefficient was used as a measure of association between the analyzed variables. The multivariate stepwise backward regression analysis was carried
out for plasma sclerostin levels as an independent variable with potentially explanatory variables: postmenopausal status, body mass index BMI (model I), fat percentage (model II), waist (model III) and HOMA-IR values, serum levels of estrone, total testosterone, androstenedione, DHEA-S, 25-OH-Vitamin D, osteocalcin and β-CTx. The Cook-Weisberg test was used to test heteroskedasticity and the Remsey RESET test was used to test the linearity of regression. The variance inflation factor VIF was calculated to check multicollinearity. The goodness of fit of the acquired regression models was assessed with the adjusted determination coefficient R². All the tests were two-tailed. The results were regarded as statistically significant with a p-value of less than 0.05.

**RESULTS**

There were no differences between body mass, BMI, body fat and waist circumference between the study groups. Serum androstenedione and DHEA-S levels were similar in study groups, whereas estrone and total testosterone levels were significant higher in the peri- rather than the postmenopausal group (124.1 pg/mL vs. 98.3 pg/mL, p < 0.01 and 0.3 pg/mL vs. 0.22 pg/mL, p < 0.01, respectively). Higher plasma osteocalcin and β-CTx levels were shown in the postmenopausal rather than the perimenopausal group (19.8 ng/mL vs. 16.8 ng/mL, p < 0.001 and 0.29 ng/mL, p < 0.05, respectively). However, plasma 25-OH-Vitamin D and sclerostin levels were similar. Table 1 presents the characteristics of the study groups.

There was a significant negative correlation between estrone levels and age, body mass and BMI (r = -0.25; p < 0.01, r = -0.24; p < 0.01, r = -0.21; p < 0.01, respectively). The negative correlation between 25-OH-Vitamin D levels and body mass, BMI, fat mass and waist circumference and positive with androstenedione levels was found (r = -0.24; p < 0.01; r = -0.24, p < 0.01; r = -0.25, p < 0.01 and r = -0.25, p < 0.01, r = 0.33; p < 0.001, respectively). Plasma β-CTx levels correlated negatively with estrone levels (r = -0.26; p < 0.01) and plasma osteocalcin correlated positively with DHEA-S and androstenedione levels (r = 0.29; p < 0.001 and r = r = 0.41; p < 0.0001). No correlation between plasma sclerostin levels and the other studied parameters was detected.

Multivariate stepwise backward linear regression models for sclerostin as an independent variable, with explanatory variables: postmenopausal status, BMI values or waist circumference or fat percentage and estrone, total testosterone, androstenedione and DHEAS levels revealed that the the alterations in sclerostin levels are proportional to androstenedione levels and inversely proportional to total testosterone levels. The model with explanatory variables: vitamin D, β-CTx and osteocalcin did not show their effect on the changes in sclerostin levels (Tab. 2).

**DISCUSSION**

So far numerous studies assessed circulating sclerostin levels and the factors affecting them in peri- and postmenopausal women [13, 14, 17, 18]. To the best of our knowledge, the study is most likely to be the first one to assess circulating sclerostin levels and the factors influencing them in peri- and postmenopausal women. In contrast to the studies which showed higher plasma sclerostin levels in postmenopausal rather than perimenopausal women [10, 11] we did not observe any differences between perimenopausal and postmenopausal women.

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### Table 1. Characteristics of study group

<table>
<thead>
<tr>
<th></th>
<th>Perimenopausal N = 31</th>
<th>Postmenopausal N = 54</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>49.0 ± 4.0</td>
<td>52.2 ± 4.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body mass [kg]</td>
<td>76.3 ± 13.6</td>
<td>74.6 ± 11.5</td>
<td>NS</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>27.1 (24.0–32.5)</td>
<td>27.3 (24.3–30.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Body fat [%]</td>
<td>37.8 ± 5.6</td>
<td>37.7 ± 5.8</td>
<td>NS</td>
</tr>
<tr>
<td>Body fat [kg]</td>
<td>26.5 (22.8–37.1)</td>
<td>28.7 (23.3–35.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Waist circumference [cm]</td>
<td>88.7 ± 10.0</td>
<td>89.4 ± 10.2</td>
<td>NS</td>
</tr>
<tr>
<td>Estrone [pg/mL]</td>
<td>124.1 (104.3–153.3)</td>
<td>98.3 (74.3–118.9)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>DHEA-S [mg/mL]</td>
<td>143.7 ± 77.9</td>
<td>141.3 ± 66.2</td>
<td>NS</td>
</tr>
<tr>
<td>Androstenedione [ng/mL]</td>
<td>2.1 ± 0.9</td>
<td>2.4 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Total testosterone [pg/mL]</td>
<td>0.30 (0.23–0.38)</td>
<td>0.22 (0.13–0.29)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>25-OH-Vitamin D [ng/mL]</td>
<td>28.0 (23.1–31.5)</td>
<td>30.0 (22.5–36.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Osteocalcin [ng/mL]</td>
<td>16.84 (11.50–18.60)</td>
<td>19.81 (15.74–23.43)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sclerostin [ng/mL]</td>
<td>0.63 ± 0.2</td>
<td>0.71 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>β-CTx [ng/mL]</td>
<td>0.29 (0.21–0.36)</td>
<td>0.35 (0.27–0.48)</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Mean (SD) or median (lower quartile — upper quartile)
The nutritional status. However, the results of recently pub-

age in healthy subjects regardless of gender. Among other

detected a positive correlation between sclerostin levels and

levels were observed in men rather than in women [23]. An-

be the impact of gender on the fat content. Higher sclerostin

levels are proportional to androstenedione levels and

versed to total testosterone levels. Con-

try to our results, it has been observed that in men testoster-

one replacement increased circulating sclerostin levels [27].

The impact of androstenedione on sclerostin levels may

be explained by the results of the experimental study that

showed that androstenedione could improve the prolifica-

tion and differentiation of osteoblasts in vitro [28].

In accordance with the results of the previously published

study [10, 19] no association between sclerostin and vitamin

D levels was observed. However, contrary to other studies

[17, 22, 28, 29], we did not observe any relationships between

sclerostin and osteocalcin as well as β-CTx levels. It should be

noted that the results of the studies described the association

between sclerostin and β-CTx as inconclusive because both

a positive [28, 29] and a negative [22] correlation were found.

Multivariate stepwise backward linear regression models in

our study revealed that the effect of β-CTx on sclerostin lev-

els is negative and close to significance. Further studies are

necessary to explain the association between sclerostin and

bone turnover markers levels in postmenopausal women.

The main limitation of the present study is the small

sample size and not including women of reproductive age in

the study. Other limitations are also the assessment of body

composition on the basis of the bioimpedance method, not

using the DXA method, which makes it impossible to assess

subcutaneous and visceral fat deposits, and the lack of as-

essment of bone density. However, it should be noted that

our study is the first one to assess the complex association

between sclerostin levels and nutritional status and sex

hormone levels in perimenopausal women.

CONCLUSIONS

Circulating sclerostin levels are similar in peri- and

postmenopausal women and are related to androstenedione

and testosterone levels regardless of the nutritional status. 

Table 2. A multivariate stepwise backward linear regression

<table>
<thead>
<tr>
<th>Sclerostin [ng/mL]</th>
<th>b</th>
<th>SE(b)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione [ng/mL]</td>
<td>0.0595</td>
<td>0.0263</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>(\log_{10}) (total testosterone) [pg/mL]</td>
<td>-0.1786</td>
<td>0.0450</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>(\log_{10}) (β-CTx[ng/mL])</td>
<td>-0.1859</td>
<td>0.0972</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Acknowledgments
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REFERENCES


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