Serum Sclerostin Levels In Men With Idiopathic Osteoporosis

B. Lapauw¹, ² S. Vandewalle¹, ² Y. Taes¹, S. Goemaere², H. Zmierczak², J. Collette⁴ & J.M. Kaufman¹, ², ³

¹Department of Endocrinology, ²Unit for Osteoporosis and Metabolic Bone Diseases, ³Laboratory for Hormonology Ghent University Hospital, Ghent, Belgium; ⁴Bone and Cartilage Markers Laboratory, Unilab Lg, CHU Sart Tilman, University of Liège, Liège, Belgium.

Short title: Sclerostin Levels and Idiopathic Osteoporosis.

Key terms: male idiopathic osteoporosis, pQCT, family study, sclerostin.


Corresponding author:
Bruno Lapauw, MD
Department of Endocrinology
Ghent University Hospital
De Pintelaan 185, 6K12 I.E.
B-9000 Ghent, Belgium
Tel: +32-9-332.34.41
Fax: +32-9-332.38.17
E-mail: Bruno.Lapauw@ugent.be

Abstract
Objective: Sclerostin inhibits osteoblast differentiation and bone formation. If aberrant sclerostin action is involved in the less efficient bone acquisition in men with idiopathic low bone mass, this might be reflected in higher serum sclerostin levels.

Methods: In 116 men with idiopathic osteoporosis (<65 yrs), 40 of their sons and healthy controls, areal bone parameters were measured using dual energy X-ray absorptiometry, volumetric and geometric bone parameters using peripheral quantitative computed tomography. Serum analytes were measured using immunoassays, estradiol levels using liquid chromatography-tandem mass spectrometry.

Results: Men with idiopathic low bone mass had lower levels of sclerostin than controls (0.54±0.17 vs. 0.66±0.23 ng/mL; p<0.001). In both groups, sclerostin levels were strongly associated with age; when adjusting for age, no associations with anthropometrics were observed (p>0.14). In multivariate analyses, sclerostin levels displayed a positive association with whole body BMC and aBMD, as well as with trabecular and cortical vBMD at the tibia in the probands. No clear associations were observed in the control group, neither were sclerostin levels associated with BMC at the radius or lumbar spine (all p>0.11). Testosterone, but not estradiol, was inversely related to sclerostin levels in the probands. No difference in sclerostin levels was found in their sons as compared to their controls.

Conclusion: Lower rather than higher serum sclerostin levels in probands with idiopathic low bone mass suggest that aberrant sclerostin secretion is not involved in the pathogenesis of low bone mass in these subjects.
Introduction

Osteoporosis and related fractures are prevalent and lead to increased morbidity and mortality in both sexes (1). In about half of men with osteoporosis, no clear pathogenic cause can be established (2). Our previous findings in a family-based study of men with idiopathic osteoporosis concurred to indicate the existence of a familial defect in the acquisition of bone mass and size, involving both trabecular and cortical bone.

Sclerostin, a glycoprotein secreted by osteocytes partially in response to mechanical loading, is a strong negative regulator of osteoblast differentiation and bone formation through antagonizing effects on the Wnt/β-catenin signalling pathway (3,4). Serum levels of sclerostin increase with aging, are reported to be positively related to overall bone mass, and sex differences have been described (5-8). Furthermore, observational and interventional data suggest that circulating sclerostin levels are modulated by estrogen exposure (6-9). In our family-based study, we reported on lower serum estradiol levels in men with idiopathic low bone mass, suggesting an estrogen-related factor in the pathogenesis of male idiopathic osteoporosis. In addition, these men displayed lower serum P1NP levels reflecting less bone formation (10,11). We therefore hypothesized that lower bone mass in these men might result from constitutionally higher sclerostin levels and/or action, possibly linked to lower estradiol exposition at the tissue level.

Subjects and methods

Subjects
Male study subjects (n=116) were diagnosed with idiopathic low bone mass using following inclusion criteria: age ≤65 yr at presentation, aBMD Z-score ≤-2.0 at lumbar spine or proximal femur. Extensive clinical and laboratory investigations excluded secondary causes of low bone mass. All sons were invited to participate without any DXA-based selection. Subjects with history of hyperthyroidism, metabolic bone disease, delayed puberty, current or past alcohol consumption of ≥5 units/day more than once weekly, malabsorption, hemochromatosis, renal or gonadal dysfunction, malignancy or chronically treated with glucocorticoids, levothyroxin, or (anti)androgens were excluded. For each proband and son, a gender- and age-matched (±2 yr) control was recruited among healthy volunteers without family history of osteoporotic fractures. None had a history of disease or medication use interfering with bone metabolism, were heavy smokers (≥40 pack-years) or drinkers (≥5 units/day more than once weekly). Physical activity was assessed by recording the weekly frequency of recreational and/or professional activities and scored as proposed by Baecke et al. (12). All participants gave written informed consent for participation in this study, approved by the ethics review board of the Ghent University Hospital.

**Anthropometrics, soft tissue composition and areal bone parameters**

Participants’ body weight was measured to the nearest 0.5 kg in light indoor clothing without shoes. Standing height was measured to the nearest 0.1 cm using a wall-mounted Harpenden stadiometer. Areal bone parameters at the lumbar spine, proximal femur (total hip and neck), radius (non-dominant side) and whole body composition were measured using DXA: QDR-2000 (first 68 matched couples; software version 7.20) or QDR-4500A (all subsequent couples; software version 11.2.1; Hologic Inc., MA, USA); all matched couples were measured on the same device and devices were cross-calibrated. Vertebrae with apparent fractures were excluded from analysis; coefficients of variation (CV) were <1% for daily spine and weekly whole body phantom measurements.
In this ongoing study, pQCT measurements were added and extended during the study course and lack for some of the early recruited probands and their sons. Cross-sectional slices (2.0 mm thickness; voxel size 0.4 mm) at the metaphysis (at 4% of bone length from the distal end of the radius) of the non-dominant forearm (all participants) and lower leg (probands only: 66 couples) were taken to determine trabecular volumetric bone mineral density (vBMD) by pQCT (XCT2000, Stratec GmbH, Germany). Cortical bone parameters were determined at the midshaft of the non-dominant forearm (probands: 70 couples; sons: 23 couples) and lower leg (probands: 66 couples) at 33 and 38% of bone length from the distal end of the radius and tibia, respectively. Analyses were performed using the manufacturer’s software (version 5.4). Cross-sectional area (CSA) of the radius/tibia was determined after detecting the outer bone contour at a threshold of 280 mg/cm³. The threshold was set at 180 mg/cm³ for determining trabecular vBMD, and at 710 mg/cm³ for cortical bone. Cortical thickness, endo- and periosteal circumference were estimated using the circular ring model in which the shaft of a long bone is assumed to be an ideal cylinder.

**Laboratory measurements**

Venous blood was obtained between 08:00h and 10:00h after overnight fasting and serum stored at -80°C until analysis. Samples from probands, their sons and respective controls were measured in a single assay run. Commercial immunoassays were used to measure serum leptin (Linco Research Inc., MO, USA), pro-collagen 1 amino-terminal propeptide (P1NP), total testosterone, SHBG (Orion Diagnostica, Finland), C-terminal telopeptides of type 1 collagen (CTX) (Biomedica Medizinprodukte GmbH, Austria), bone-specific alkaline phosphatase (bAP) (Beckman Coulter Inc., CA, USA), osteocalcin (Nordic Bioscience Diagnostics A/S, Denmark) and sclerostin (TECOmedical AG, Switzerland). For sclerostin,
the limit of detection was 0.12 ng/mL and the limit of quantification 0.20 ng/mL; intra-assay CV was <15% for levels between 0.20 and 0.50 ng/mL and <6% for levels > 1.00 ng/mL and inter-assay CV was <6% for levels > 0.50 ng/mL. Serum estradiol was determined by liquid chromatography-tandem mass spectrometry as described previously (13). Free testosterone and estradiol were calculated from testosterone, estradiol, SHBG and albumin concentrations using equations based on the mass action law (14,15). For all measurements, intra- and interassay CV were below 10 and 15%, respectively. Assays were performed at the Laboratory for Hormonology at the Ghent university hospital except for serum sclerostin, which was assayed at the Bone and Cartilage Markers Laboratory, Unilab Lg, CHU Sart Tilman, University of Liège. Kidney function was determined by calculation of estimated glomerular filtration rate (eGFR; mL/min/1.73m²) according to the MDRD-formula.

Statistics

If normally distributed, variables were described as mean ± standard deviation (SD), and as median [1st-3rd quartile] otherwise. If necessary, variables were transformed (natural logarithmic) to meet required model assumptions. Comparisons between groups were performed using independent sample t-tests and ANCOVAs to account for potential confounding by age, body weight or height. Univariate and age-, weight- and height adjusted associations between sclerostin and different patients’ characteristics were explored using multiple linear regression models, with effect estimates reported as standardized regression coefficients (β). The level indicating statistical significance was <0.05 (two-tailed). Statistical analyses were performed using SPSS (version 19.0, SPSS Inc., IL, USA).

Results

General characteristics (Tables 1 and 2)
As previously reported (11), men with idiopathic osteoporosis had lower body weight, height, lean and fat mass than age-matched control subjects. Their bone mass deficit involved trabecular and cortical bone density as well as cortical bone size, and these men presented lower serum levels of estradiol and free estradiol, in part due to higher SHBG levels.

Regarding sclerostin, lower serum levels were observed in men with idiopathic osteoporosis as compared to their controls (Figure 1), whereas no difference was found in their sons as compared to their respective controls. Results were not essentially different when only considering affected sons (data not shown). When adjusting for age and whole body BMC, the observed between-group difference in serum sclerostin levels between probands and their controls was no longer significant ($p=0.13$).

Relation with serum sclerostin levels (Table 3)

In both probands and healthy controls, sclerostin levels were strongly and essentially similarly related to age (Figure 2). In the control group, positive associations between sclerostin levels and body weight, BMI, and total fat mass were no longer significant after adjusting for age; whereas positive associations with leptin levels persisted even after controlling for weight or body fat mass (probands: $\beta=0.341; P=0.062$ and controls: $\beta=0.265; p=0.037$).

When adjusting for age, weight and height, sclerostin levels in the probands were positively related to whole body aBMD and BMC, as well as to trabecular vBMD and BMC and cortical vBMD at the tibia. In controls, sclerostin levels were associated with aBMD at the proximal femur and tended to be positively related to cortical vBMD ($\beta=0.228; p=0.057$). No associations were found with trabecular or cortical bone parameters at the radius, neither were sclerostin levels associated with areal bone parameters at the radius or lumbar spine in either group (all $p>0.06$). Except for endosteal circumference at the tibia ($\beta=-0.23; p=0.032$) in the probands, no significant association between sclerostin levels and bone size parameters were
found. Results were essentially unaltered when adjusting for lean and/or fat mass instead of weight (data not shown).

Sclerostin levels were inversely related to serum levels of P1NP, CTX, bAP and osteocalcin in the control group and with P1NP levels in the probands; findings however which were lost after correcting for participant’s age (data not shown). There was no difference in serum sclerostin levels between those with and without previous fractures (\( p > 0.22 \)) in either group, and no relation with indices of physical activity was observed (data not shown; all \( p > 0.19 \)). Serum sclerostin levels were higher in probands taking vitamin D supplements vs. those who did not (0.57 ± 0.16 vs. 0.48 ± 0.16; \( p = 0.007 \)); and sclerostin levels were inversely related to eGFR in both groups (\( r = -0.26; p = 0.007 \) and \( r = -0.26; p = 0.010 \) resp.). These latter findings lost statistical significance when adjusting for participant’s age (all \( p > 0.18 \)).

In the probands, and in univariate analyses in the control group, negative associations between serum sclerostin and both total (\( r = -0.18 p = 0.047 \)) and free (\( r = -0.20 p = 0.031 \)) testosterone levels were found. No associations with (free) estradiol or SHBG levels were found, but when controlling for testosterone levels, total estradiol levels were found to be positively related to sclerostin levels (\( \beta = 0.20; p = 0.039 \)) in the probands only.

In the sons and their respective controls, sclerostin levels tended to be positively related to total body weight (age-adjusted \( \beta = 0.379; p = 0.098 \) and \( \beta = 0.447; p = 0.028 \) resp.); whereas no significant associations with age, bone parameters or sex steroids were found (data not shown).

**Discussion**
Contrary to the initial working hypothesis, lower rather than higher serum sclerostin levels were observed in men with idiopathic low bone mass as compared to age-matched control subjects. Given that sclerostin is acknowledged as having anti-proliferative and differentiating effects on osteoblasts (3,4), this finding pleads against involvement of aberrant sclerostin secretion in the pathogenesis of low bone mass in these subjects.

This between-group difference in sclerostin levels was no longer significant after adjusting for whole body BMC, and sclerostin levels were positively related to parameters reflecting bone strength in probands and – albeit to a lesser extent – in controls. Although not unequivocally (7,16), earlier studies also reported positive associations between serum sclerostin levels and bone mass in both men and women (5,7,8). These positive associations between bone mass, other parameters of bone strength and serum sclerostin levels, may seem counterintuitive since sclerostin is considered to inhibit bone formation. However, they could be explained by the fact that for the same forces applied, lower bone mass will lead to higher mechanical strain, thereby decreasing sclerostin secretion by osteocytes. Taken together, this suggests that lower serum sclerostin levels in men with idiopathic osteoporosis reflect their lower bone mass and osteocyte number or cell mass.

Corroborating previous findings (5,7-9,16), we also found a strong positive association between serum sclerostin levels and age. Several in vivo studies have shown that mechanical loading decreases, whereas unloading increases local sclerostin production (17-19). Since aging is associated with less physical activity and consequently mechanical loading (20,21), this might at least partially explain the positive association between age and sclerostin levels.

Previous studies reported an inverse association between serum sclerostin and estradiol levels in pre- and postmenopausal women (16), lower sclerostin levels in postmenopausal women under hormonal replacement therapy (7,9) and an interventional study by Mödder et al. (9)
demonstrated that estradiol withdrawal increases serum sclerostin levels in men in whom endogenous sex steroid production was eliminated and then substituted to physiological levels. Contrary to what could be expected from these findings, total estradiol levels were positively related to sclerostin levels when controlling for testosterone levels in our probands.

Further, a consistent negative association was observed between sclerostin and both total and free testosterone levels in our probands, as well as in univariate analyses in the control group. These findings are in line with cross-sectional studies reporting a positive association between serum estradiol and sclerostin levels in older men (7,8) and a negative association between bioavailable testosterone and sclerostin levels in a middle-aged subgroup (40-59 yrs) (7).

These inconsistent findings are difficult to explain but might result from differences in participants’ conditions leading to different sex hormone levels and/or increased bone remodeling.

Strengths of our study are the well-described patient population of men with idiopathic osteoporosis, together with their well-characterized phenotype. Limitations are the limited sample size and the fact that male idiopathic osteoporosis might represent a spectrum of low bone mass due to heterogeneous mechanisms and different causes. Given the age of our probands and controls, we cannot extrapolate on the role of sclerostin during peak bone mass acquisition. However, given that no differences in sclerostin levels were observed between their sons (whether they were affected or not) and age-matched controls, a primary role for sclerostin in the deficient bone acquisition process in men with idiopathic osteoporosis seems unlikely.

In conclusion, this study shows that serum sclerostin levels in men with idiopathic osteoporosis are lower than in healthy control subjects, possibly reflecting differences in overall bone mass. Although further research is needed, aberrant sclerostin secretion seems unlikely to be causally involved in the pathogenesis of idiopathic osteoporosis in men.
**Declaration of interest:** The authors have nothing to disclose.

**Funding:** This work was supported by grants G0662.07 and G0867.11 from 'Fonds voor Wetenschappelijk Onderzoek - Vlaanderen (FWO; Research Foundation - Flanders)' and by an unrestricted grant of Novartis Belgium. YT is a Postdoctoral Fellow and SV is Doctoral Fellow of the FWO.

**Author contributions:** All authors participated in the writing process; SG HZ and JMK designed the study; SG, HZ, JMK, SV, YT and YT recruited subjects; YT and JMK are responsible for all laboratory measurements except for sclerostin which was measured in the lab of JC.

**Acknowledgements:** We are indebted to S. Geboes, K. Toye, K. Mertens, and I. Bocquaert for their excellent technical assistance.

---

Reference List


5. Amrein K, Amrein S, Drexler C, Dimai HP, Dobnig H, Pfeifer K, Tomaszitz A, Pieber TR & Fahrleitner-Pammer A. Sclerostin and Its Association with Physical Activity, Age,
Gender, Body Composition, and Bone Mineral Content in Healthy Adults. *Journal of Clinical Endocrinology & Metabolism* 2012 97 148-154.


18 Spatz JM, Fields EE, Yu EW, Pajevic PD, Bouxsein ML, Sibonga JD, Zwart SR & Smith SM. Serum Sclerostin Increases in Healthy Adult Men during Bed Rest. *Journal of Clinical Endocrinology & Metabolism* 2012 **97** E1736-E1740.


**Figure legends**

Figure 1: Dot plots of serum sclerostin levels in men with idiopathic osteoporosis and age-matched controls. Full line represents mean.

Figure 2: Scatterplots of both group’s age and serum sclerostin levels.
Table 1: General and bone parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Probands (n = 116)</th>
<th>Controls (n = 116)</th>
<th>Sons (n = 40)</th>
<th>Controls (n = 40)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>45.3 ± 12.9</td>
<td>46.0 ± 13.1</td>
<td>28.6 ± 6.0</td>
<td>28.8 ± 6.4</td>
<td>0.68</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.4 ± 9.3</td>
<td>82.3 ± 11.6</td>
<td>73.5 ± 11.3</td>
<td>79.1 ± 10.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.4 ± 6.3</td>
<td>177.1 ± 6.5</td>
<td>180.8 ± 6.7</td>
<td>180.8 ± 6.1</td>
<td>0.002</td>
</tr>
<tr>
<td>Trabecular vBMD at radius 4% (g/cm³)</td>
<td>183 ± 37</td>
<td>226 ± 43</td>
<td>217 ± 32</td>
<td>242 ± 38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cortical vBMD at radius 66% (g/cm²)</td>
<td>1191 ± 50</td>
<td>1207 ± 22</td>
<td>1178 ± 59</td>
<td>1197 ± 46</td>
<td>0.004</td>
</tr>
<tr>
<td>Cortical bone area at radius 66% (cm²)</td>
<td>94 ± 13</td>
<td>103 ± 13</td>
<td>97 ± 10</td>
<td>105 ± 12</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD. * according to an independent Student’s t-test. $ according to Chi Square test. -: not assessed. a pQCT data only available for 23 of the sons.
Table 2: Serum sclerostin and sex steroid levels

<table>
<thead>
<tr>
<th></th>
<th>Proband (n = 116)</th>
<th>Controls (n = 116)</th>
<th>Controls (n = 40)</th>
<th>Controls (n = 40)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sclerostin (ng/mL)</td>
<td>0.54 [0.41 – 0.66]</td>
<td>0.61 [0.51 – 0.78]</td>
<td>0.52 [0.41 – 0.62]</td>
<td>0.54 [0.45 – 0.66]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SHBG (nmol/L)*</td>
<td>29.6 [22.9 - 37.4]</td>
<td>25.8 [20.2 – 37.1]</td>
<td>29.4 [24.3 – 36.9]</td>
<td>24.9 [20.4 - 32.5]</td>
<td>0.027</td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
<td>537 ± 163</td>
<td>554 ± 157</td>
<td>585 ± 179</td>
<td>584 ± 1690</td>
<td>0.42</td>
</tr>
<tr>
<td>Free testosterone (ng/dL)</td>
<td>11.5 ± 3.8</td>
<td>12.9 ± 4.2</td>
<td>12.8 ± 4.3</td>
<td>13.6 ± 3.8</td>
<td>0.009</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>17.4 ± 5.4</td>
<td>21.9 ± 6.2</td>
<td>19.3 ± 5.1</td>
<td>20.2 ± 5.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Free E2 (pg/mL)</td>
<td>0.32 ± 0.12</td>
<td>0.43 ± 0.15</td>
<td>0.37 ± 0.11</td>
<td>0.40 ± 0.11</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD, unless *, presented as median [1st - 3rd quartile] in case of non-Gaussian distribution. * according to an independent Student’s t-test or * Wilcoxon signed rank test where appropriate. Conversion factor for testosterone from ng/dL to nmol/L is 0.0347, for estradiol from pg/mL to pmol/L is 3.671.
Table 3: Unadjusted/age-,weight- and height-adjusted standardized regression coefficients (β) between serum sclerostin levels and general, bone and sex steroid parameters.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Weight</th>
<th>Height</th>
<th>BMI</th>
<th>Lean mass</th>
<th>Fat mass</th>
<th>eGFR</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Probands</strong></td>
<td>-0.389***/0.475***&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.084/0.038&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.148/0.071&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.142/0.003&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-0.030/0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.158/0.080&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.176/0.001</td>
<td>0.280**/0.259*</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>0.485***/0.472***&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.197/0.143&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.168/-0.005&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.292**/0.132&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.027/0.127&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.336**/0.156&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.201**/0.007</td>
<td>0.356**/0.189</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Whole body BMC</th>
<th>Whole body aBMD</th>
<th>Lumbar spine BMC</th>
<th>Lumbar spine aBMD</th>
<th>Total hip BMC</th>
<th>Total hip aBMD</th>
<th>Radius BMC</th>
<th>Radius aBMD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Probands</strong></td>
<td>0.037/0.288**</td>
<td>0.013/0.267**</td>
<td>-0.081/0.165</td>
<td>-0.152/0.088</td>
<td>-0.140/-0.166</td>
<td>-0.150/-0.072</td>
<td>0.048/0.044</td>
<td>-0.007/0.116</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>0.076/0.202</td>
<td>0.066/0.194</td>
<td>0.018/0.114</td>
<td>0.014/0.049</td>
<td>0.068/0.133</td>
<td>0.064/0.247*</td>
<td>0.012/-0.008</td>
<td>0.023/0.047</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Radius trab. BMC</th>
<th>Radius trab. vBMD</th>
<th>Radius cort. BMC</th>
<th>Radius cort. vBMD</th>
<th>Tibia trab. BMC</th>
<th>Tibia trab. vBMD</th>
<th>Tibia cort. BMC</th>
<th>Tibia cort. vBMD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Probands</strong></td>
<td>0.103/0.111</td>
<td>0.012/0.165</td>
<td>0.010/0.070</td>
<td>-0.113/0.078</td>
<td>0.088/0.222*</td>
<td>0.024/0.276**</td>
<td>0.016/0.100</td>
<td>0.049/0.227*</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>0.039/0.038</td>
<td>0.042/0.079</td>
<td>0.065/0.093</td>
<td>-0.079/0.171</td>
<td>0.067/0.136</td>
<td>0.055/0.129</td>
<td>0.066/-0.001</td>
<td>-0.112/0.228</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Estradiol</th>
<th>Free estradiol</th>
<th>Testosterone</th>
<th>Free testosterone</th>
<th>SHBG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Probands</strong></td>
<td>0.071/0.110</td>
<td>-0.036/-0.008</td>
<td>-0.186/-0.184*</td>
<td>-0.311**/-0.202*</td>
<td>0.066/-0.145</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>-0.046/0.106</td>
<td>0.003/0.086</td>
<td>-0.208/-0.018</td>
<td>-0.183/0.010</td>
<td>-0.096/-0.167</td>
</tr>
</tbody>
</table>

* p<0.05; ** p<0.01; *** p<0.001; <sup>a</sup>adjusted for weight and height; <sup>b</sup>adjusted for age and height; <sup>c</sup>adjusted for age and weight; <sup>d</sup>adjusted for age