Dehydroepiandrosterone sulfate, osteoprotegerin and its soluble ligand sRANKL and bone metabolism in girls with anorexia nervosa

Siarczan dehydroepiandrosteronu, osteoprotegeryna i jej rozpuszczalny ligand sRANKL a metabolizm kostny u dziewczat z jadłowstrem psychicznym

Zofia Ostrowska\textsuperscript{1}, Katarzyna Ziura\textsuperscript{2}, Joanna Oświęcimska\textsuperscript{2}, Elżbieta Świętochowska\textsuperscript{1}, Kinga Wołkowska-Pokrywa\textsuperscript{1}

\textsuperscript{1} Clinical Biochemistry Division, Department of Biochemistry, Medical University of Silesia, Zabrze, Poland
\textsuperscript{2} Department of Pediatrics, Medical University of Silesia, Zabrze, Poland

Summary

Background: Only scarce data exist concerning the relationship between dehydroepiandrosterone (DHEA) and/or its sulfate form DHEAS and bone status in adolescents with anorexia nervosa (AN).

Aim: We investigated whether a relationship existed between DHEAS and bone metabolism (as assessed based on serum osteocalcin [OC], and collagen type I cross-linked carboxy-terminal telopeptide [CTx]). We also aimed to establish whether the above mentioned relationship might be affected by osteoprotegerin (OPG) and its soluble ligand sRANKL.

Material/Methods: Fifty-six female patients with AN and 21 healthy female subjects aged 13 to 16 years participated in the study. Serum DHEAS, OC, CTx, OPG and sRANKL were measured by ELISA.

Results: Our female patients with AN demonstrated significant suppression of DHEAS and bone markers, an increase in OPG and sRANKL levels, and a reduction of the OPG/sRANKL ratio. DHEAS, CTx and the OPG/sRANKL ratio correlated positively with BMI. A significant positive correlation was also observed between DHEAS and the OPG/sRANKL ratio, OC and the OPG/sRANKL ratio, and CTx and sRANKL. The correlation was negative in the case of DHEAS and CTx, DHEAS and sRANKL, CTx and the OPG/sRANKL ratio, and sRANKL and the OPG/sRANKL ratio.

Discussion/Conclusions: DHEAS suppression in girls with anorexia nervosa was associated with a decrease in the levels of bone markers, an increase in OPG and sRANKL concentrations and a significant decrease in the OPG/sRANKL ratio. DHEAS suppression in girls with anorexia nervosa might have a harmful effect on their bone tissue, probably via a shift in the OPG/RANKL ratio toward a functional excess of sRANKL.

Key words: anorexia nervosa • female adolescents • DHEAS • bone metabolism • OPG • sRANKL

Streszczenie

Wstęp: Nieliczne i niejednoznaczne dane o związku między dehydroepiandrosteronem (DHEA) i/lub jego siarczanem – DHEAS a stanem kośćca u chorych na jadłowstrem psychiczny (anorexia nervosa – AN) skłoniły nas do podjęcia badań w tym zakresie.
Celem pracy było wykazanie, czy u dziewcząt z AN istnieje związek między DHEAS a metabo-
lizmem kostnym (oczynianym na podstawie oznaczeń w surowicy krwi osteokalcyny – OC i C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcucha C-końcowego usieciowanego telopeptydu łańcucha C-
końcowego usieciowanego telopeptydu łańcuch

Material/Metody: U 56 dziewcząt z AN i 21 zdrowych w wieku 13–16 lat oceniono stężenia DHEAS, OC, CTx, OPG i sRANKL w surowicy krwi metodą ELISA.

Wyniki: U dziewcząt z AN wykazano znaczną supresję stężeń DHEAS, markerów kostnych, istotny wzrost stężeń OPG i sRANKL oraz znamienne obniżenie wskaźnika OPG/sRANKL. Stężenia DHEAS, CTx i wartości wskaźnika OPG/sRANKL u tych dziewcząt korelowały dodatnio z BMI. Dodatnią korelacją stwierdzono również między DHEAS a OPG/sRANKL, OC a OPG/sRANKL, CTx a sRANKL. Natomiast w odniesieniu do DHEAS i CTx, DHEAS i sRANKL, CTx i OPG/sRANKL, sRANKL i OPG/sRANKL korelacja była ujemna.

Dyskusja/Wnioski: Supresja stężeń DHEAS u dziewcząt z AN jest związana z obniżeniem stężeń markerów kost-
nych oraz wzrostem stężeń OPG i sRANKL przy zmniejszonym wskaźniku OPG/sRANKL. Zmniejszone u dziewcząt z AN wytwarzanie DHEAS może niekorzystnie wpływać na tkankę kostną, najprawdopodobniej poprzez przesunięcie relacji OPG/sRANKL na korzyść sRANKL.

Słowa kluczowe: jadłowstręt psychiczny • dziewczęta • DHEAS • metabolizm kostny • OPG • sRANKL
the Pediatric Department in Zabrze (Medical University of Silesia in Katowice, Poland), who, following pediatric examination and psychiatric consultation, were diagnosed with AN according to the criteria from the American Psychiatric Association’s Diagnostic and Statistical Manual of Mental Disorders – DSM-IV (1994). All examined girls were at Tanner puberty stages IV and V. The average disease duration was 12.1 months (range 3–36 months). They had normal liver and kidney functions; no severe somatic complications or psychiatric disorders were observed. On recruitment, no patients were taking medications known to affect the nutritional and bone status, including calcium or vitamin D supplements. Exclusion criteria included severe somatic complications or psychiatric disorders were observed. On recruitment, no patients were taking medications known to affect the nutritional and bone status, including calcium or vitamin D supplements. Exclusion criteria included severe somatic complications, i.e., gastrointestinal bleeding, diarrhea, dehydration, peptic ulcer disease, liver and kidney dysfunction, and pharmacotherapy, e.g., anti-anxiety or psychotropic drugs. During hospitalization, patients were placed at bed rest, which is the standard care. The control group comprised 21 healthy regularly menstruating adolescent females between 13 and 17 years of age, with normal body mass and no endocrine or other disorders that could possibly influence adipose and bone tissue metabolism. During the three months preceding the study, the control participants did not receive calcium and/or vitamin D supplements.

The height (stadiometer), body mass (electronic scale), and body mass index (BMI) of each female subject were measured and documented. Between 8 and 9 a.m. (after a 12-hour fast), 8 ml blood samples were collected for the determinations of DHEAS, OC, CTx, OPG and its soluble ligand sRANKL. Centrifuged serum was frozen and stored at −75°C until assay.

Serum DHEAS concentrations were determined using ELISA kits (IBL, Germany); OC with ELISA kits from Diagnostic Systems Laboratories, Inc. (USA). CTx levels were analyzed by the serum CrossLaps ELISA (Nordic Bioscience Diagnostics A/S, Denmark) while OPG and sRANKL were determined by ELISA kits (Biomedica, Austria). The respective sensitivity and intra- and inter-assay errors were: 0.11 µmol/l, 4.8 and 7.5% for DHEAS; 0.05 µmol/l, 5.8 and 7.3% for OC; 0.08 nmol/l, 5.2 and 6.7% for CTx; 0.14 pmol/l, 7 and 7.5% for OPG; 0.04 pmol/l, 5 and 7% for RANKL.

The database was prepared using Excel 2000 (Microsoft Corporation). Statistical analysis was carried out with Statistica 5.5 for Windows (StatSoft Inc., USA). The t-test was used for the significance of the difference between groups (normal distribution of variables). In the case of non-normal distribution, the significance was tested using the Mann-Whitney U test. Relationships between DHEAS, BMI, OC, CTx, OPG, sRANKL and the OPG/sRANKL ratio were estimated with Spearman’s correlation coefficients. The level of significance was set at p≤0.05.

The study was approved by the Bioethics Committee at the Medical University of Silesia in Katowice (KNW/0022/KB1/105/09). Written informed consent was obtained from all examined participants and their parents or legal guardians before participation.

**Results**

The mean values of body mass and BMI were significantly decreased in adolescent females with AN compared to the control. Similarly, mean DHEAS concentrations were lower than those found in control participants with normal body weight. These findings were associated with significant reductions in mean concentrations of OC (bone formation marker) and CTx (bone resorption marker) as well as a significant increase in mean OPG and sRANKL levels and a reduced OPG/sRANKL ratio (Table 1).

### Table 1. Mean values of age, height, body mass, body mass index (BMI), serum levels of dehydroepiandrosterone sulphate (DHEAS), osteocalcin (OC), collagen type I crosslinked carboxyterminal telopeptide (CTx), osteoprotegerin (OPG), soluble receptor activator of nuclear factor-κB ligand (sRANKL) and OPG/sRANKL ratio in girls with anorexia nervosa and the control group

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (n=15)</th>
<th>Anorexia nervosa (n=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>15.50±1.91</td>
<td>14.86±2.91</td>
</tr>
<tr>
<td>Body mass [kg]</td>
<td>52.75±6.69</td>
<td>32.03±2.60*</td>
</tr>
<tr>
<td>Height [m]</td>
<td>1.62±0.85</td>
<td>1.57±0.09</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>21.71±1.53</td>
<td>13.46±0.85*</td>
</tr>
<tr>
<td>DHEAS [µmol/l]</td>
<td>2.95±0.41</td>
<td>2.57±0.38*</td>
</tr>
<tr>
<td>OC [µmol/l]</td>
<td>3.47±0.22</td>
<td>1.61±0.80*</td>
</tr>
<tr>
<td>CTx [nmol/l]</td>
<td>7.76±0.86</td>
<td>7.19±0.72*</td>
</tr>
<tr>
<td>OPG [pmol/l]</td>
<td>3.49±0.69</td>
<td>4.19±0.89*</td>
</tr>
<tr>
<td>sRANKL [pmol/l]</td>
<td>0.280±0.119</td>
<td>0.401±0.148*</td>
</tr>
<tr>
<td>OPG/sRANKL ratio</td>
<td>13.22±4.03</td>
<td>10.49±4.06*</td>
</tr>
</tbody>
</table>

*p≤0.05 versus control group.*
A positive and statistically significant correlation was observed in female adolescents with AN between BMI and both DHEAS and CTx concentrations as well as between BMI and the OPG/sRANKL ratio. A significant negative correlation was found between DHEAS and CTx and DHEAS and sRANKL. DHEAS also correlated positively and significantly with the OPG/sRANKL ratio. CTx concentrations demonstrated a positive and significant correlation with sRANKL. The OPG/sRANKL ratio correlated positively and significantly with OC, and negatively and significantly with CTx and sRANKL concentrations (Table 2).

### Discussion

Anorexia nervosa is associated with low BMD in adults [3,4,16,24,25,26,27,31,33,41,50,53,55,56,58,59,62]. Adults with AN continue to show a lower bone mass and BMD than healthy adults, which suggests that the low BMD in AN is a permanent feature of the disease. However, the mechanisms underlying the low BMD in AN are not fully understood, and further research is needed to elucidate the factors that contribute to the low BMD in AN.

### Table 2. Correlation between values of body mass index (BMI), serum levels of dehydroepiandrosterone sulphate (DHEAS), osteocalcin (OC), collagen type I crosslinked carboxyterminal telopeptide (CTx), osteoprotegerin (OPG), soluble receptor activator of nuclear factor-x8 ligand (sRANKL) and OPG/sRANKL ratio in girls with anorexia nervosa

<table>
<thead>
<tr>
<th>Variables (n=56)</th>
<th>BMI kg/m²</th>
<th>DHEAS µmol/l</th>
<th>OC µmol/l</th>
<th>CTx nmo/l</th>
<th>OPG pmol/l</th>
<th>sRANKL pmol/l</th>
<th>OPG/sRANKL ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI kg/m²</td>
<td>–</td>
<td>0.334*</td>
<td>0.167</td>
<td>0.315*</td>
<td>–1.52</td>
<td>0.101</td>
<td>0.265*</td>
</tr>
<tr>
<td>DHEAS µmol/l</td>
<td>0.334*</td>
<td>–</td>
<td>0.132</td>
<td>–0.387*</td>
<td>0.199</td>
<td>–0.261*</td>
<td>0.245*</td>
</tr>
<tr>
<td>OC µmol/l</td>
<td>0.167</td>
<td>0.132</td>
<td>–</td>
<td>–0.081</td>
<td>0.132</td>
<td>–0.067</td>
<td>0.262*</td>
</tr>
<tr>
<td>CTx nmo/l</td>
<td>0.315*</td>
<td>–0.387*</td>
<td>–0.081</td>
<td>–</td>
<td>–1.50</td>
<td>0.301*</td>
<td>–0.257*</td>
</tr>
<tr>
<td>OPG pmol/l</td>
<td>–0.152</td>
<td>0.199</td>
<td>0.132</td>
<td>–1.50</td>
<td>–</td>
<td>0.046</td>
<td>0.192</td>
</tr>
<tr>
<td>sRANKL pmol/l</td>
<td>0.101</td>
<td>–0.261*</td>
<td>–0.067</td>
<td>0.301*</td>
<td>0.046</td>
<td>–</td>
<td>–0.612*</td>
</tr>
<tr>
<td>OPG/sRANKL ratio</td>
<td>0.265*</td>
<td>0.245*</td>
<td>0.262*</td>
<td>–0.257*</td>
<td>0.192</td>
<td>–0.612*</td>
<td>–</td>
</tr>
</tbody>
</table>

* p≤0.05 – statistically significant values of correlation coefficients.
also correlated negatively with percent fat mass and leptin [39]. Similar to our previous investigations [48,49], our adolescent female patients with AN showed OC and CTx suppression, increase in OPG and sRANKL levels, and OPG/sRANKL ratio reduction. Positive correlations were found between BMI and CTx, BMI and the OPG/sRANKL ratio, CTx and sRANKL, and OC and the OPG/sRANKL ratio, while OC concentrations correlated negatively with CTx and sRANKL. Misra et al. [39] suggest that the decrease in markers of bone resorption may reflect the suppressive effects of higher OPG levels on osteoclastic activity in adolescent female patients. In the Misra et al. study [39], a negative correlation was noted between lumbar spine BMD z-score and OPG, suggesting that higher OPG levels in girls with lower BMD may indeed be a compensatory phenomenon. However, like Misra et al. [39] we did not find a significant association between OPG and a marker of bone resorption. On the other hand, low values of the OPG/sRANKL ratio associated with high concentrations of the above mentioned cytokines as well as some dissociation between the examined cytokines and bone markers suggest that adolescent females with AN might suffer from disturbances in the mechanisms which control the bone remodeling process or in the mechanism that compensates for bone loss. Our results, and especially correlation analysis, confirm the hypothesis that the OPG/sRANKL ratio might prove a more reliable bone turnover and BMD determinant than any of the examined cytokines alone [49]. Munoz-Calvo et al. [42] also demonstrated a significant decrease in the OPG/RANKL ratio in adolescent girls with AN. They also found a significant positive correlation between OPG/RANKL and BMD, but did not observe serum OPG increase or any relationship between OPG and RANKL or OPG and BMD. The authors conclude that the decrease in the OPG/RANKL ratio in girls with AN could only partly explain the increase in bone loss that occurs in these patients.

The differences in bone markers as well as OPG and sRANKL levels in females with AN could be associated with age differences between study subjects (children, teenagers, adult women) and the resulting diversity in terms of ontogenesis, growth, development stage, and disease duration. Differences in OPG levels can be caused by different sensitivity and specificity of investigation kits, and the fact that circulating levels of cytokines under investigation do not always reflect their synthesis by osteoblasts and marrow stromal cells [4,64,65]. OPG mRNA transcripts have also been found in lymphoid cells, kidney, liver and thyroid gland, and many fetal tissues [43]. As in the case of OPG, the clinical applications of the laboratory measurement of sRANKL are limited due to methodological complications, the influences of many physiological factors (e.g. ethnicity, age, cyclic variations, gender, renal and liver function) and hormonal status [4,64,65]. Nonetheless, numerous authors believe that serum OPG and RANKL determinations may be useful in cross-sectional cohort studies [43,64,65].

It has been well established that OPG and/or RANKL expression by osteoblasts and marrow stromal cells in females with AN might, apart from estrogen deficiency, be modulated by changes in the concentrations of other hormones including androgens, somatotropic axis hormones, thyroid hormones, glucocorticoids and hormones which play an essential role in the control of the appetite center, as well as by changes in cytokine production [1,2,5,8,11,23,28,30,32,33,34,35,36,38,39,41]. The effect of some of the above mentioned factors on OPG and/or RANKL production could be opposite to that of estradiol; hence, it is difficult to predict the ultimate outcome of their actions [12,19]. It has recently been suggested that changes in the secretion of adrenal androgens (DHEA in particular), observed in the majority of females with AN, might take part in bone metabolism disturbances [1,8,9,10,51,61]. In vitro studies seem to indicate that this could be mediated by the RANKL/RANK/OPG system [66]. We therefore decided to investigate relationships between DHEAS and bone metabolism in girls with AN and to determine whether such relationships might be affected by OPG and its soluble ligand sRANKL.

Most authors have observed DHEA and/or DHEAS decrease in patients with AN compared to age-matched healthy individuals [5,8,10,17,52,68,69]. We have obtained similar results. It is believed that DHEA and DHEAS suppression seen in these patients might result from elimination of the anabolic effect of IGF-I on DHEA and/or DHEAS secretion. This concept seems to be lent support by the fact that patients with AN have noticeably decreased IGF-I levels which usually correlate positively with DHEA concentrations [2,6,7,8,9,10,11,15,17,22,45,59]. The regulatory effect of pro-resorptive cytokines (i.e., IL-1β, IL-6 and TNF-α) on DHEA production has not been fully elucidated [8,9,10]. Decreased DHEA concentrations might also be indicative of compromised ovarian function and low androstenedione secretion in these patients [8]. Other researchers observed enhanced DHEA and/or DHEAS levels in their patients with AN. However, they believed that the increased concentrations were not linked to the corticotropin-releasing factor/adrenocorticotropic system [21,40]. Still others did not observe any significant changes in DHEA and/or DHEAS in AN [6,23,28,59,60] with the exception of women receiving oral contraceptives [23,28]. The latter had reduced DHEA compared to controls, which the authors considered attributable to decreased albumin levels. [23]. On the other hand, Sirinathsinhji and Mills’ [57] patients with AN had significantly increased DHEA levels while their DHEAS levels were significantly lower compared to healthy participants. These differences in DHEA and/or DHEAS concentrations in patients with AN might be related to gender, age, the stage of the disease and, sometimes, disease duration. The majority of investigations were carried out in young women. Differences in DHEA and DHEAS determinations might also have resulted from different sized, mostly small patient populations. Also, determinations were carried out using different biological material (blood serum or plasma, saliva, diurnal urine collections) and methods of different sensitivity and specificity. Some differences might also be associated with collection times. Although DHEA and DHEAS concentrations do not vary significantly throughout the day, they do show some diurnal variations. These variations tend to be greater in patients with AN than in healthy individuals.

The relationship between DHEA and/or DHEAS and bone metabolism has been investigated by very few authors, and mainly in young females with AN. These studies revealed...
significant suppression of bone formation markers and a significant correlation between increased bone resorption and DHEA and/or DHEAS [8,9,10]. We have obtained similar results; our adolescent females with AN showed significant suppression of DHEAS, which correlated negatively with CTx. All these results indicate that DHEA and/or DHEAS deficiency in patients with AN might be among the factors which ultimately lead to increased bone resorption. Gordon et al. [9] demonstrated that a 1-year course of oral DHEA treatment (50 mg/d) in young women with AN significantly reduced the levels of specific bone resorption markers, i.e., urinary, type I collagen, and cross-linked N-telopeptides (NTx) [9]. Administration of 50, 100, or 200 mg of DHEA daily to young women with AN enrolled in a 3-month trial significantly decreased the NTx levels in both the 50 mg and the 200 mg subgroups. The levels of bone formation markers (BAP and OC) increased simultaneously. A dose-dependent negative correlation was revealed between DHEA or DHEAS and NTx. DHEA and DHEAS concentrations also correlated positively with both E2 and IGF-I [10]. Hence, it can be concluded that the anabolic effect of DHEA on bone formation (BAP and OC) is most likely mediated by IGF-I.

The so far infrequent in vitro and in vivo investigations seem to indicate that the effect of DHEA on bone resorption might be mediated by the RANKL/RANK/OPG system. In vitro studies using isolated osteoblasts show that DHEA at the concentrations 20.01 µM, and especially within 0.1 to 1 µM, increase the ratio of mRNA OPG/mRNA RANKL in osteoblasts [66]. Similar investigations concerning isolated osteoclasts demonstrate that DHEA could decrease the number and area of absorption lacunae only in the presence of osteoblasts, which again, although indirectly, suggests mediation by RANKL/RANK/OPG [66]. Our own studies, involving female adolescents with AN, revealed a significant negative correlation between DHEAS and both CTx and sRANKL, and a positive correlation between DHEAS and the OPG/sRANKL ratio. CTx concentrations also correlated positively and significantly with sRANKL; a correlation between OC and the OPG/sRANKL ratio was also observed. The OPG/sRANKL ratio correlated negatively and significantly with CTx and sRANKL. Thus, it may be suspected that DHEAS suppression might have a harmful effect on bone tissue in girls with AN – probably via a shift in the OPG/RANKL ratio that tilts the balance toward a functional excess of sRANKL. The latter results in an increase in bone resorption and related bone loss.

**CONCLUSIONS**

- There seems to be a relationship between the changes in DHEAS levels observed in girls with anorexia nervosa and changes in the concentrations of bone markers, i.e., osteoprotegerin and its soluble ligand sRANKL.
- A correlation between DHEAS and a decrease in the OPG/sRANKL ratio associated with high osteoprotegerin and sRANKL levels seems to suggest that DHEAS suppression in girls with anorexia nervosa might have a harmful effect on bone tissue – probably via a shift in the OPG/RANKL ratio toward a functional excess of sRANKL.

**REFERENCES**


The authors have no potential conflicts of interest to declare.