



# Bone metabolism, osteoprotegerin, receptor activator of nuclear factor- $\kappa$ B ligand and selected adipose tissue hormones in girls with anorexia nervosa

Metabolizm kostny, osteoprotegeryna i ligand receptora aktywatora czynnika jądrowego- $\kappa$ B a wybrane hormony tkanki tłuszczowej u dziewcząt z jądłowstrętem psychicznym

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## Abstract

**Introduction:** The aim of this study was to determine whether girls with anorexia nervosa (AN) exhibited any relationships between serum levels of LP, ADIPO, RES, VISE, APE-36, APE-12, and bone markers, OPG and sRANKL.

**Material and methods:** Serum levels of selected adipose tissue hormones, OC, CTx, OPG and sRANKL were assessed using ELISA in 86 study participants suffering from AN and 21 healthy controls, all aged 13 to 18 years.

**Results:** Girls with AN showed a significant reduction in body mass, BMI, serum concentrations of LP, RES, VISE, APE-36, APE-12, OC, CTx and increased ADIPO concentration. These changes were associated with significant increases in OPG and sRANKL and a decrease in the OPG/sRANKL ratio. Significant positive correlations were revealed between BMI and LP, APE-36, CTx, OPG/sRANKL ratio; OC and VISE; OPG and ADIPO; OPG/sRANKL ratio and LP, APE-36, APE-12. Significant negative correlations were revealed between CTx, sRANKL and RES, APE-36; OPG and APE-36, APE-12; OPG/sRANKL ratio and ADIPO. VISE was shown to be an independent predictor of OC. APE-36 and RES turned out to be independent predictors of CTx, and sRANKL, APE-36 and ADIPO were independent predictors of OPG while APE-36, LP and ADIPO were independent predictors of the OPG/sRANKL ratio.

**Conclusions:** Changes in bone markers, OPG, sRANKL and/or the OPG/sRANKL ratio exhibited by girls with AN have been found to be associated with changes in the levels of the selected adipose tissue hormones. Abnormal relationships between bone metabolism and LP, ADIPO, RES, VISE and APE might adversely affect the balance of the OPG/sRANKL system and thus potentially compromise the mechanism which compensates for bone remodelling disturbances. (*Endokrynol Pol* 2014; 65 (1): 33–39)

**Key words:** anorexia nervosa; girls; adipose tissue hormones; bone metabolism; OPG; sRANKL

## Streszczenie

**Wstęp:** Celem pracy było wykazanie, czy u dziewcząt z jądłowstrętem psychicznym istnieje związek między LP, ADIPO, RES, VISE, APE-36 i APE-12 a markerami kostnymi, OPG i sRANKL.

**Materiał i metody:** U 86 dziewcząt z AN i 21 zdrowych w wieku 13–18 lat oceniono stężenia wybranych hormonów tkanki tłuszczowej, OC, CTx, OPG i sRANKL w surowicy metodą ELISA.

**Wyniki:** U dziewcząt z AN wykazano istotne zmniejszenie masy ciała i BMI, obniżenie stężeń LP, RES, VISE, APE-36, APE-12 i markerów kostnych (OC i CTx) oraz wzrost stężenia ADIPO. Zmianom tym towarzyszył istotny wzrost stężeń OPG i sRANKL przy obniżonym wskaźniku OPG/sRANKL. Wykazano znamienne dodatnią korelację między BMI a LP, APE-36, CTx i wskaźnikiem OPG/sRANKL; OC a VISE; OPG a ADIPO; wskaźnikiem OPG/sRANKL a LP, APE-36, APE-12. Istotną, ujemną korelację stwierdzono między CTx, sRANKL a RES i APE-36; OPG a APE-36 i APE-12; wskaźnikiem OPG/sRANKL a ADIPO. Wykazano, że niezależnymi predyktorami są: dla OC — VISE, dla CTx i sRANKL — APE-36 i RES, dla OPG — APE-36 i ADIPO, a dla wskaźnika OPG/sRANKL — APE-36, LP i ADIPO.

**Wnioski:** Obserwowanym u dziewcząt z AN zmianom w stężeniach markerów kostnych, OPG, sRANKL i/lub wskaźniku OPG/sRANKL towarzyszą zmiany w stężeniach badanych hormonów tkanki tłuszczowej. Nieprawidłowości w powiązaniach między metabolizmem kostnym a LP, ADIPO, RES, VISE oraz APE u dziewcząt z AN mogą prowadzić do naruszenia równowagi układu OPG/sRANKL, co może skutkować upośledzeniem mechanizmu kompensującego zaburzenia w przebudowie kośćca. (*Endokrynol Pol* 2014; 65 (1): 33–39)

**Słowa kluczowe:** jądłowstręt psychiczny; dziewczęta; hormony tkanki tłuszczowej; metabolizm kostny; OPG; sRANKL



## Introduction

Anorexia nervosa (AN) is an increasingly common eating disorder, a psychosomatic disease characterised by the pursuit of a 'perfect' silhouette through strictly limiting food intake, excessive exercise, self-provoked vomiting or engagement in other purging behaviours including the use of laxatives or diuretics. The disease leads to significant reduction of adipose tissue mass resulting in drastic slimness or even physical wasting (cachexia) [1, 2]. Patients with AN not only exhibit somatic and psychic complications resulting from dramatic weight loss but also, quite frequently, imbalance of hormones — also adipose tissue hormones [3–22].

It has been widely acknowledged that white adipose tissue, both subcutaneous and visceral, serves as a reservoir of several active peptides, commonly referred to as adipokines, which have both local (autocrine and paracrine) and systemic (endocrine) actions [2]. It has been documented that adipokines are important regulators of appetite and numerous metabolic, endocrine and immune functions of the organism. Several adipokines are mainly produced in subcutaneous white adipose tissue (leptin — LP, adiponectin — ADIPO) while others (resistin — RES, visfatin — VISF) are predominantly secreted in the visceral adipose tissue. The least known of these hormones, apelin (APE), is expressed not only by adipocytes but has also been identified in vascular endothelium and the cells of several other tissues (lung, heart, brain) [2].

Osteoporosis is among the most commonly observed complications of AN. Osteoporosis has a multifactorial aetiology. Disturbances in the production, release and action of osteotropic agents, mainly hormones (including adipose tissue hormones, i.e. LP and maybe also ADIPO, RES, VISF and APE), might lead to bone mineral density (BMD) decrease or, alternatively, absence of the BMD increase expected in adolescence [9, 10, 23] resulting from excessive bone resorption and inadequate formation of new bone. The system of receptor activator of nuclear factor- $\kappa$ B ligand/receptor activator of nuclear factor- $\kappa$ B/osteoprotegerin (RANKL/RANK/OPG) might play an important role in this process [24, 25]. According to a convergence hypothesis, the effect of several local and systemic agents on bone resorption consists of the regulation of OPG and/or RANKL expression [24, 25].

*In vitro* studies indicate that LP stimulates rat bone marrow stromal cells, thus enhancing differentiation to osteoblasts. It also causes an increase in the expression of bone matrix proteins mRNA; the effect depending on the dose and time of administration. Leptin enhances bone mineralisation [26–28] and inhibits osteoclast generation in cultures of murine bone marrow stromal cells [26–28], but does not affect mature osteoclast differen-

tiation [26]. It also increases OPG expression in stromal cells and osteoblasts [29, 30] and/or decreases the expression of RANKL [27, 30, 31]. Leptin acts to enhance *in vitro* differentiation of human bone marrow stem cells to osteoblasts, stimulates *de novo* collagen synthesis and bone matrix mineralisation [32–34] and inhibits adipogenesis [32]. It also inhibits osteoclastogenesis in cultures of human peripheral blood mononuclear cells and spleen cells relative to OPG increase and RANKL decrease [32, 35]. Kanazawa et al. [36] demonstrated that ADIPO and AMP kinase activator stimulated the proliferation, differentiation, and mineralisation of MC3T3-E1 cells, while Luo et al. [37, 38] concluded that ADIPO stimulated human osteoblasts proliferation and differentiation and inhibited OPG expression in human osteoblasts via the MAPK signalling pathway. Thommesen et al. [39] found enhanced RES expression in murine osteoclast and osteoblast precursor cells (RAW 264.7, MC3T3-E1) and in primary human bone marrow stem cells. The expression of resistin mRNA in RAW 264.7, increased during differentiation, seemed to be regulated through PKC- and PKA-dependent mechanisms. Recombinant resistin increased the number of differentiated osteoclasts and stimulated NF- $\kappa$ B promoter activity, indicating a significant role of this adipokine in osteoclastogenesis. Resistin also enhanced the proliferation of MC3T3-E1 cells in a PKA and PKC-dependent manner, but only weakly interfered with genes known to be upregulated during differentiation of MC3T3-E1 into osteoblasts [39].

Other investigations have shown that VISF increases type I collagen production in human osteoblast cultures [40] while APE stimulates proliferation and suppresses apoptosis of mouse osteoblastic cell line MC3T3-E1 [41]. The latter also has a protective effect on cultured rat bone marrow mesenchymal stem cells against apoptosis [42]. All these findings seem to suggest that the above mentioned adipokines might regulate bone remodelling, most probably through the cytokines of the RANKL/RANK/OPG system, mainly produced in osteoblasts and bone marrow stromal cells [24, 25, 27, 30–32, 35, 37–39].

Several investigations involving healthy men and women have demonstrated a negative correlation between osteocalcin (OC), OPG and LP concentrations as well as between RANKL and ADIPO levels. OC concentrations were also positively correlated with ADIPO [43–46]. OPG concentrations were higher in a premenopausal than in a postmenopausal population [47–49]. The OPG/RANKL ratio correlated negatively with LP and positively with ADIPO independent of age, BMI and the time elapsed from menopause [45]. Investigations of relationships between serum LP, ADIPO, RES, VISF, APE and BMD have revealed that

only ADIPO was an independent BMD predictor in postmenopausal women and middle-aged men [47–50]. Other authors found that both LP and ADIPO were determinants of BMD; RES was negatively correlated with BMD, but the correlation did not reach statistical significance ( $p = 0.062$ ) [51].

Little data is available on the relationship between adipokines and bone status in patients with AN, and especially girls [3–10, 11, 23, 52–58]. The literature reports available are mainly concerned with the relation between LP, ADIPO and bone metabolism and/or BMD [8–10, 12, 23, 53, 54, 56–59]. Girls with AN exhibit considerable suppression of bone metabolism markers, i.e. bone-specific alkaline phosphatase, OC, C-terminal cross-linked telopeptide of type I collagen  $\alpha$  chain (CTx), N-terminal cross-linked telopeptide of type-I collagen type I collagen  $\alpha$  chain (NTx) and/or deoxypyridinoline [9, 10, 21–23, 60, 61] correlating with a decrease in BMD and LP levels [9, 10, 23]. Several authors have determined OPG concentrations in girls with AN and found that, similar to young females with AN, the girls had elevated serum levels of this cytokine [4, 8, 21, 22, 61]. Our earlier investigations revealed that serum sRANKL was increased while the OPG/sRANKL ratio was significantly decreased [21, 22, 61]. Similar results were obtained by Munoz-Calvo et al. [57]. However, no complex research studies have been carried out so far in girls with AN concerning the relationships between LP, ADIPO, RES, VISE, APE-36, APE-12 and bone metabolism, OPG and its soluble ligand sRANKL.

Therefore, we decided to thoroughly investigate these relationships. Based on the scarce literature data available, we hypothesised that a BMD decrease or, alternatively, absence of the BMD increase expected in adolescence, might not only be caused by several already well-established factors, but also by the effect of changes in the concentrations of the above mentioned adipokines (or some of them) on the balance of the OPG/sRANKL system.

The aim of this study was: 1) to determine whether girls with AN exhibited any relationships between LP, ADIPO, RES, VISE, APE-36, APE-12 and bone metabolism (as assessed based on serum OC and CTx levels); and 2) to establish whether OPG and its soluble ligand sRANKL might affect such relationships.

## Material and methods

The study involved 86 girls aged 13 to 18 years, hospitalised at the Paediatric Endocrinology Division of the Paediatric Department in Zabrze Medical University of Silesia in Katowice (Poland), who, following paediatric examination and psychiatric consultation, had been diagnosed with anorexia nervosa according to Diagnostic

and Statistical Manual of Mental Disorders (DSM-IV) published by the American Psychiatric Association in 1994. The average disease duration was 12.1 months (range 3–36 months). All examined girls were at Tanner puberty stages IV and V. 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. During hospitalisation, patients were placed at bed rest, which is the standard care. The control group comprised 21 age-matched healthy regularly menstruating adolescent females with no endocrine or other disorders that might possibly influence adipose and bone tissue metabolism. During the three-month period before the study, the control participants did not take calcium or vitamin D supplements.

The height (stadiometer) and body mass (electronic scale) of all participants were measured, and their body mass index (BMI) calculated. Blood samples (8 mL) for determination of LP, ADIPO, RES, VISE, APE-36, APE-12, OC and CTx as well as OPG and sRANKL were collected between 08.00 and 09.00 hours following a 12-hour fast. Centrifuged serum was frozen and stored at  $-75^{\circ}\text{C}$  until assayed for the above mentioned adipose tissue hormones, bone markers, OPG and sRANKL.

Determinations of the concentrations of adipose tissue hormones, OC, CTx, OPG and sRANKL were performed by ELISA using the following kits: LP and ADIPO (Bio-Vendor, LLC, USA), RES (Mediagnost, Germany), VISE, APE-36 and APE-12 (Phoenix Pharmaceuticals Inc., Burlingame, CA, USA), OC (DSL Inc., USA), CTx (Nornic Bioscience Diagnostics A/S, Denmark), OPG and sRANKL (Biomedica, Austria). The respective sensitivity, intra- and inter-assay errors were: 0.5 ng/mL, 7.5 and 9.2% for LP; 0.7 ng/mL, 7 and 8.2% for ADIPO; 0.3 ng/mL, 5 and 6.8% for RES; 0.55 ng/mL, 5 and 14% for VISE; 0.09 ng/mL, 5 and 14% for APE-36; 0.07 ng/mL, 5 and 14% for APE-12; 0.05  $\mu\text{mol/L}$ , 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 sRANKL.

The database was prepared using Excel 2000 (Microsoft Corporation). Statistical analysis was carried out with Statistica 10 for Windows (StatSoft Inc., USA). The t-test was used to determine the significance of intergroup differences (normal distribution of variables). In the case of non-normal distribution, the significance was tested using the Mann-Whitney U test. The relationships between LP, ADIPO, RES, VISE, APE-36, APE-12, OC, CTx, OPG, sRANKL and the OPG/sRANKL ratio were analysed by Spearman's correlation. The level of significance was set at  $p \leq 0.05$ .

Stepwise regression was used to determine whether any, and if so which, of the adipose tissue hormones

under investigation were independent predictors of bone markers (OC and CTx), cytokines of the RANKL/RANK/OPG system and the OPG/sRANKL ratio (model entry was set at  $p = 0.05$ , and model exit at  $p = 0.05$ ).

The study was approved by the Regional Bioethics Committee of the Medical University of Silesia in Katowice (KNW/0022/KB1/105/09).

## Results

Our female adolescents with AN showed a significant reduction in body mass and BMI compared to the control group. A significant decrease in mean serum LP, RES, VISE, APE-36 and APE-12 concentrations, and a significant increase in mean serum ADIPO, were also found. These changes were associated with a significant decrease in mean OC and CTx levels, marked increases in OPG and sRANKL, and a significant decrease in the OPG/sRANKL ratio compared to the controls (Table I).

Girls with AN exhibited significant positive correlations between BMI and LP, APE-36, CTx and the OPG/sRANKL ratio (Table II). CTx and sRANKL were negatively and significantly correlated with RES and APE-36; OPG was negatively and significantly correlated with APE-36 and APE-12, while the OPG/sRANKL ratio correlated negatively and significantly with ADIPO. Positive and significant correlations were observed between OC and VISE; OPG and ADIPO as well as between the OPG/sRANKL and LP, APE-36 and APE-12 (Table III).

VISE was shown to be an independent predictor of OC ( $R^2 = 0.071$ ,  $p = 0.020$ ). APE-36 and RES turned out to be independent predictors of CTx and sRANKL ( $R^2 = 0.062$ ,  $p = 0.015$  and  $R^2 = 0.082$ ,  $p = 0.041$ , respectively). APE-36 and ADIPO were independent predictors of OPG ( $R^2 = 0.173$ ,  $p = 0.008$ ), while APE-36, LP and ADIPO were independent predictors of the OPG/sRANKL ratio ( $R^2 = 0.264$ ,  $p = 0.005$ ).

## Discussion

Girls with AN suffer from a considerable decrease of bone mass [8–10, 12, 23, 54, 60, 62, 63] and bone metabolism disturbances [3–11, 21–23, 53, 54, 57, 61, 62]. The majority of authors emphasise the suppression of bone formation and bone resorption markers in these patients which appears correlated with BMI and/or BMD [4, 5, 12, 21, 22, 52, 53, 61, 63].

Our results are consistent with these findings. However, only few of the above mentioned authors determined OPG concentrations in girls with AN [4, 8, 21, 22, 57, 61]. It was found that, similar to young females with AN [64], girls with AN exhibited a considerable increase of serum OPG. Misra et al. [4] suggested that decreased levels of bone resorption markers in girls

**Table I.** Mean values of age, height, body mass, body mass index (BMI), mean serum levels of chosen adipose tissue hormones, osteocalcin (OC), collagen type I crosslinked carboxyterminal telopeptide (CTx), osteoprotegerin (OPG), soluble receptor activator of nuclear factor- $\kappa$ B ligand (sRANKL) and OPG/sRANKL ratio values in girls with anorexia nervosa and the control group

**Tabela I.** Średni wiek, masa ciała, wskaźnik masy ciała (BMI), średnie stężenia wybranych hormonów tkanki tłuszczowej, osteokalcyny (OC), karboksyterminalnego usieciowanego telopeptydu łańcucha  $\alpha$ 1 kolagenu typu I (CTx), osteoprotegeryny (OPG), rozpuszczalnego ligandu receptora aktywatora czynnika jądrowego- $\kappa$ B (sRANKL) i wartości wskaźnika OPG/sRANKL u dziewcząt z jadłowstrętem psychicznym i w grupie kontrolnej

Variables	Groups	
	Anorexia nervosa (n = 86)	Control group (n = 21)
Age (years)	15.45 ± 1.32	15.35 ± 1.72
Height [m]	1.62 ± 0.04	1.63 ± 0.05
Body mass [kg]	<b>39.50 ± 5.24*</b>	52.33 ± 5.56
BMI [kg/m <sup>2</sup> ]	<b>14.79 ± 1.62*</b>	19.71 ± 1.26
Leptin (LP) [ng/mL]	<b>4.96 ± 1.01*</b>	14.62 ± 1.13
Adiponectin (ADIPO) [μg/mL]	<b>43.67 ± 5.21*</b>	24.35 ± 2.52
Resistin (RES) [ng/mL]	<b>2.86 ± 0.45*</b>	4.20 ± 0.39
Visfatin (VISE) [ng/mL]	<b>1.00 ± 0.26*</b>	2.49 ± 0.38
Apelin-36 (APE-36) [pg/mL]	<b>98.11 ± 8.51*</b>	129.15 ± 14.32
Apelin-12 (APE-12) [pg/mL]	<b>96.81 ± 8.90*</b>	130.02 ± 16.61
OC [μmol/L]	<b>0.91 ± 20.80*</b>	3.12 ± 1.5
CTx [nmol/L]	<b>7.22 ± 0.92*</b>	7.99 ± 0.91
OPG [pmol/L]	<b>5.21 ± 0.78*</b>	3.41 ± 0.69
sRANKL [pmol/L]	<b>0.45 ± 0.14*</b>	0.28 ± 0.12
OPG/sRANKL ratio	<b>9.72 ± 0.83*</b>	13.98 ± 1.08

\* $p \leq 0.05$  v. control group

with AN might indicate a suppressive effect of elevated serum OPG on osteoclast activity and differentiation. However, it should be emphasised that Misra et al. [4] did not investigate the levels of RANKL and the values of the OPG/sRANKL ratio. Munoz-Calvo et al. [57] and Ostrowska et al. [21, 22, 61] determined serum OPG and sRANKL in female adolescents with AN and revealed significant suppression of the OPG/sRANKL ratio in patients with normal [57] or significantly increased OPG levels [21, 22, 61] and significantly increased sRANKL [21, 22, 57, 61].

Low values of the OPG/sRANKL ratio with a parallel increase in serum OPG and sRANKL demonstrated in our previous studies [21, 22, 61] and confirmed in the present investigations, along with some degree of desynchronisation of the relationships between the

**Table II.** Correlation between body mass index (BMI), chosen adipose tissue hormones, osteocalcin (OC), collagen type I crosslinked carboxyterminal telopeptide (CTx), osteoprotegerin (OPG), soluble receptor activator of nuclear factor- $\kappa$ B ligand (sRANKL) and OPG/sRANKL ratio in girls with anorexia nervosa ( $n = 86$ )

**Tabela II.** Korelacja między wskaźnikiem masy ciała (BMI), wybranymi hormonami tkanki tłuszczowej, osteokalcyną (OC), karboksyterminalnym usieciowanym telopeptydem łańcucha  $\alpha$ 1 kolagenu typu I (CTx), osteoprotegeryną (OPG), rozpuszczalnym ligandem receptora aktywatora czynnika jądrowego- $\kappa$ B (sRANKL) i wartościami wskaźnika OPG/sRANKL u dziewcząt z jadłowstrętem psychicznym ( $n = 86$ )

Variables	Values of correlation coefficients — R
BMI [kg/m <sup>2</sup> ]	
Leptin (LP) [ng/mL]	<b>0.271*</b>
Adiponectin (ADIPO) [ $\mu$ g/mL]	NS
Resistin (RES) [ng/mL]	NS
Visfatin (VISF) [ng/mL]	NS
Apelin-36 (APE-36) [pg/mL]	<b>0.214*</b>
Apelin-12 (APE-12) [pg/mL]	NS
OC [ $\mu$ mol/L]	NS
CTx [nmol/L]	<b>0.315*</b>
OPG [pmol/L]	NS
sRANKL [pmol/L]	NS
OPG/sRANKL ratio	<b>0.268*</b>

\* $p \leq 0.05$  — statistically significant values of correlation coefficients

above mentioned cytokines and bone markers [21, 22], seem to suggest that girls with AN might exhibit a compromise in the mechanism which controls bone remodelling and/or mechanism compensating for enhanced bone turnover. Munoz-Calvo et al. [57] also reported a significant decrease in the OPG/RANKL ratio correlated with an increase in serum RANKL as well as a positive and significant correlation between the OPG/RANKL ratio and BMD. However, they did not observe a significant OPG increase in serum of girls with AN, or any relationships between OPG, RANKL and BMD. According to Munoz-Calvo et al. [57], the decrease in the OPG/RANKL ratio in girls with AN only partly explains increased bone loss observed in these patients. It has been well established that  $17\beta$ -oestradiol is the major regulator of bone metabolism and BMD in both women and girls suffering from AN. However, it is also known that cytokines under investigation, in particular OPG, are modified not only by  $17\beta$ -oestradiol, but also by other hormones, cytokines and mesenchymal transcription factors [24, 25] whose effect on cytokines concentrations is opposite to that of  $17\beta$ -oestradiol. Therefore, the ultimate effect of all these actions is difficult to predict.

*In vitro* and *in vivo* studies have indicated that, apart from several well-known hormones, bone metabolism may also be modified by some adipose tissue hormones [8, 26–28, 30–34, 40, 42–50, 56–58, 65–68]. It has also been

**Table III.** Correlation<sup>a</sup> between chosen adipose tissue hormones, osteocalcin (OC), collagen type I crosslinked carboxyterminal telopeptide (CTx), osteoprotegerin (OPG), soluble receptor activator of nuclear factor- $\kappa$ B ligand (sRANKL) and OPG/sRANKL ratio in girls with anorexia nervosa ( $n = 86$ )

**Tabela III.** Korelacja<sup>a</sup> między wybranymi hormonami tkanki tłuszczowej, osteokalcyną (OC), karboksyterminalnym usieciowanym telopeptydem łańcucha  $\alpha$ 1 kolagenu typu I (CTx), osteoprotegeryną (OPG), rozpuszczalnym ligandem receptora aktywatora czynnika jądrowego- $\kappa$ B (sRANKL) i wartościami wskaźnika OPG/sRANKL u dziewcząt z jadłowstrętem psychicznym ( $n = 86$ )

Variables	Bone markers		Cytokines of RANKL/RANK/OPG system		OPG/sRANKL ratio
	OC [ $\mu$ mol/L]	CTx [nmol/L]	OPG [pmol/L]	sRANKL [pmol/L]	
Leptin (LP) [ng/mL]	NS	NS	NS	NS	<b>0.252*</b>
Adiponectin (ADIPO) [ $\mu$ g/mL]	NS	NS	<b>0.234*</b>	NS	<b>-0.245*</b>
Resistin (RES) [ng/mL]	NS	<b>-0.215*</b>	NS	<b>-0.236*</b>	NS
Visfatin (VISF) [ng/mL]	<b>0.266*</b>	NS	NS	NS	NS
Apelin-36 (APE-36) [pg/mL]	NS	<b>-0.212*</b>	<b>-0.269*</b>	<b>-0.240*</b>	<b>0.443*</b>
Apelin-12 (APE-12) [pg/mL]	NS	NS	<b>-0.361*</b>	NS	<b>0.225*</b>
Stepwise regression model <sup>b</sup>	VISF	APE-36 RES	APE-36 ADIPO	APE-36 RES	APE-36 LP ADIPO
R <sup>2</sup>	0.071	0.062	0.173	0.082	0.264
p	0.020	0.015	0.008	0.041	0.005

<sup>a</sup>Displayed value represents the model variance explained by these parameters; <sup>b</sup>Parameters entering the stepwise regression model; NS — non significant values of correlation coefficients ( $p > 0.05$ ); \* $p \leq 0.05$  — statistically significant values of correlation coefficients

suggested that the latter might regulate bone remodeling both directly and indirectly, i.e. via affecting OPG and/or RANKL expression [24, 25, 31, 38, 45, 56].

The findings concerning serum LP, ADIPO, RES and VISF concentrations in patients with AN, and especially in adolescent females suffering from the disease, are not unanimous. Our previous [12, 13–19] and present studies in girls with AN revealed suppression of LP, RES and VISF levels while ADIPO concentrations were increased. The results of other authors' investigations are similar to ours [5–8, 53–56, 60, 66, 69–73]. Only a few of these studies have also assessed the relationships between the above mentioned adipose tissue hormones (or some of them) and BMD and/or bone metabolism in patients with AN [3–8, 53–55, 56, 66]. The same regards the relationships between LP and/or ADIPO, RES, VISF and bone status with a focus on possible interaction of cytokines of the RANKL/RANK/OPG system [4, 8, 55, 56, 66]. Several findings [4, 8, 56, 66] indicate a relationship between LP and/or ADIPO and RES and bone status. *In vitro* studies [4, 31, 38, 45] seem to suggest that the above mentioned adipokines, and especially LP and ADIPO, modify bone metabolism, most probably via their effect on RANKL and/or OPG expression.

Our investigations of girls with AN demonstrated that serum LP, RES, VISF, APE-36 and APE-12 suppression and ADIPO elevation were associated with a decrease in bone markers concentrations, low values of the OPG/sRANKL ratio with a parallel increase in serum OPG and sRANKL. CTx and sRANKL correlated negatively and significantly with RES and APE-36; OPG correlated negatively and significantly with APE-36 and APE-12, while the OPG/sRANKL ratio was negatively and significantly correlated with ADIPO. However, significant positive correlations were revealed between OC and VISF; OPG and ADIPO; the OPG/sRANKL ratio and LP, APE-36 and APE-12. Our findings confirm *in vivo* results of other authors [3–11, 23, 52, 53–59, 66], who hypothesised a relationship between bone status and some of the above mentioned adipose tissue hormones (i.e. LP, RES and ADIPO) in adolescent females with AN. We would also like to emphasise the role of VISF and APE in the regulation of bone remodelling in these patients. *In vitro* investigations into the effect of LP [26, 27, 31–35, 74], ADIPO [36–38, 74], RES [39], VISF [40] and APE [41, 42] on bone tissue cells are consistent with the above hypothesis. Stepwise regression analysis carried out in our patients revealed that VISF was an independent predictor of OC. APE-36 and RES turned out to be independent predictors of CTx and sRANKL; APE-36 and ADIPO were independent predictors of OPG, while APE-36, LP and ADIPO were independent predictors of the OPG/sRANKL ratio. Thus, it can be hypothesised that decreased LP, RES, VISF and APE

production and increased ADIPO secretion observed in girls with AN might have an adverse effect on bone tissue, probably via a shift in the OPG/sRANKL ratio toward a functional excess of sRANKL.

## Conclusions

Changes in bone markers, OPG, sRANKL and/or the OPG/sRANKL ratio exhibited by girls with anorexia nervosa have been found to be associated with changes in the levels of the selected adipose tissue hormones.

Abnormal relationships between bone metabolism and leptin, adiponectin, resistin, visfatin and apelin observed in girls suffering from anorexia nervosa might adversely affect the balance of the OPG/sRANKL system and potentially compromise the mechanism which compensates for bone remodelling disturbances.

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