Endokrynologia Polska
DOI: 10.5603/EP.2014.0061
Tom/Volume 65; Numer/Number 6/2014
ISSN 0423–104X

Selected adipose tissue hormones, bone metabolism, osteoprotegerin and receptor activator of nuclear factor-κB ligand in postmenopausal obese women

Wybrane hormony tkanki tłuszczowej, metabolizm kostny a osteoprotegeryna i ligand receptora aktywatora czynnika jądrowego-κB u otyłych kobiet po menopauzie

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Abstract

Introduction: It has been suggested that changes in the production of adipose tissue hormones in obese postmenopausal women might affect their bone status. The aim of this study was to determine whether obese postmenopausal women exhibited any relationship between serum levels of LP, ADIPO, RES, VISF, APE and bone metabolism markers (OC and CTx), OPG, sRANKL, the OPG/sRANKL ratio as well as BMD.

Material and methods: 80 postmenopausal women (60 obese and 20 healthy) underwent BMD measurement using dual-energy X-ray absorptiometry (DXA) at lumbar spine L_2–L_4. Serum levels of selected adipose tissue hormones, OC, CTx, OPG and its soluble ligand, sRANKL, were assessed by ELISA.

Results: Obese postmenopausal women demonstrated a significant increase in body mass, BMI and WHR associated with significant increases in LP and RES levels, a decrease in ADIPO concentration, suppression of OC, CTx, OPG and sRANKL and an increase in the OPG/sRANKL ratio and BMD. BMI correlated positively with BMD, LP, RES, OPG and the OPG/sRANKL ratio, whereas in the case of ADIPO, OC, CTx, sRANKL the relationship was negative. WHR was positively correlated with the OPG/sRANKL ratio, and negatively with ADIPO and APE. A positive correlation was found between BMD and LP, APE and the OPG/sRANKL ratio, while the correlation between BMD and ADIPO, CTx, sRANKL was negative. Significant positive correlations were also revealed between OC, CTx and ADIPO; OPG and ADIPO; sRANKL and ADIPO, RES; the OPG/sRANKL ratio and LP. OC correlated negatively with LP, RES, VISF, APE; CTx with LP, VISF, APE; OPG with LP; sRANKL with LP and APE; the OPG/sRANKL ratio with VISF. ADIPO was an independent predictor of OC, OPG and sRANKL, while LP turned out to be an independent predictor of CTx, OPG, sRANKL and the OPG/sRANKL ratio.

Conclusions: Obesity in postmenopausal women can lead to changes in BMD, circulating levels of bone markers, OPG, sRANKL and/or the OPG/sRANKL ratio; these changes are associated with alterations in the concentrations of adipose tissue hormones under investigation. The relationships between bone status indicators and adipose tissue hormones, especially LP and ADIPO, seem to suggest that changes in these hormones observed in obese postmenopausal women might have a protective effect on bone tissue, most probably via a shift in the OPG/sRANKL ratio towards a functional excess of OPG. **(Endokrynol Pol 2014; 65 (6): 438–448)**

Key words: *menopause; obesity; adipose tissue hormones; bone metabolism; OPG, sRANKL*

Streszczenie

Wstęp: Istnieją sugestie, że zmiany w produkcji hormonów tkanki tłuszczowej u otyłych kobiet po menopauzie mogą wpływać na stan kośćca. Celem pracy było wykazanie, czy u otyłych kobiet po menopauzie istnieje związek między stężeniami w surowicy LP, ADIPO, RES, VISF, APE a markerami metabolizmu kostnego (OC i CTx), OPG, sRANKL, wskaźnikiem OPG/sRANKL oraz BMD.

Materiał i metody: U 80 kobiet po menopauzie (60 otyłych i 20 zdrowych) wykonano badanie BMD metodą dwuwiązkowej absorpcjometrii rentgenowskiej (DXA) obejmujące część lędźwiową kręgosłupa L_2–L_4 oraz oznaczono metodą ELISA stężenia wybranych hormonów tkanki tłuszczowej, OC, CTx, OPG i jej rozpuszczalnego ligandu sRANKL.

Wyniki: Istotnemu wzrostowi masy ciała oraz wskaźników BMI i WHR u otyłych kobiet po menopauzie towarzyszyło znamienne zwiększenie stężeń LP i RES, obniżenie stężenia ADIPO, supresja stężeń OC, CTx, OPG i sRANKL oraz wzrost wskaźnika OPG/sRANKL i BMD. Wykazano, istotną dodatnią korelację między BMI a BMD, LP, RES, OPG i wskaźnikiem OPG/sRANKL oraz ujemną z ADIPO, OC, CTx i sRANKL. Wartości wskaźnika WHR korelowały dodatnio ze wskaźnikiem OPG/sRANKL a ujemnie ze stężeniami ADIPO i

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APE. Wykazano ponadto dodatnią korelację między BMD a stężeniami LP, APE i wskaźnikiem OPG/sRANKL oraz ujemną ze stężeniami ADIPO, CTx, sRANKL. Stwierdzono także dodatnią korelację między stężeniami: OC, CTx a ADIPO; OPG a ADIPO; sRANKL a ADIPO i RES; wskaźnikiem OPG/sRANKL a LP. Ujemną korelację wykazano natomiast między OC a LP, RES , VISF i APE; CTx a LP, VISF i APE; OPG a LP; sRANKL a LP i APE; wskaźnikiem OPG/sRANKL a VISF. Stwierdzono, że niezależnym predyktorem dla OC, OPG i sRANKL jest ADIPO, a dla CTx, OPG, sRANKL i wskaźnika OPG/sRANKL — LP.

Wnioski: Otyłość u kobiet po menopauzie wywołuje zmiany w BMD, stężeniach markerów kostnych, OPG, sRANKL i/lub wskaźniku OPG/sRANKL, którym towarzyszą zmiany w stężeniach badanych hormonów tkanki tłuszczowej. Wykazane zależności między wykładnikami stanu kośćca a badanymi hormonami tkanki tłuszczowej, zwłaszcza LP i ADIPO, sugerują, że obserwowane u otyłych kobiet po menopauzie zmiany w stężeniach tych hormonów mogą wpływać ochronnie na tkankę kostną, najprawdopodobniej poprzez przesunięcie relacji OPG do sRANKL na korzyść funkcjonalnej przewagi OPG. **(Endokrynol Pol 2014; 65 (6): 438–448)**

Słowa kluczowe: *menopauza; otyłość; hormony tkanki tłuszczowej; metabolizm kostny; OPG; sRANKL*

Introduction

White adipose tissue, both subcutaneous and visceral, is a reservoir of a number of hormones which have both local (autocrine/paracrine) and systemic (endocrine) actions [1–3]. These hormones are important regulators of appetite and numerous metabolic, endocrine and immune functions of the organism [3–8]. Some of them are mainly produced in subcutaneous adipose tissue (leptin — LP, adiponectin — ADIPO) [2, 3, 9]; others (resistin — RES, visfatin — VISF) are predominantly secreted in the visceral adipose tissue [2, 3]. 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 or brain) [10].

The most recent *in vitro* studies indicate that adipose tissue hormones might affect bone status. LP stimulates osteoblast differentiation of human bone marrow stromal cells *in vitro*, enhances *de novo* collagen synthesis and bone matrix mineralisation [11–13]. It also inhibits adipogenesis [11], suppresses osteoclastogenesis in cultures of human peripheral blood mononuclear cells and spleen cells, and stimulates and inhibits the expression of osteoprotegerin (OPG) and receptor activator of nuclear factor-κB ligand (RANKL), respectively [11, 14]. ADIPO stimulates proliferation, differentiation, and mineralisation of osteoblastic MC3T3-E1 cells [15, 16]. It also stimulates RANKL and inhibits OPG expression in human osteoblasts [16, 17]. RES is expressed in murine preosteoclasts and preosteoblasts (RAW 264.7, MC3T3-E1), in primary human bone marrow stem cells and in mature osteoblasts [18]. Recombinant RES increases the number of differentiated osteoclasts and stimulates NF-κB promoter activity, which indicates a significant role of this adipocytokine in osteoclastogenesis. RES also enhances the proliferation of preosteoblastic cells, but only weakly interferes with genes commonly regarded as regulators of preosteoblast differentiation into osteoblasts [18]. VISF has been show to stimulate human osteoblasts proliferation and increase type I collagen production, depending on the dose and time of administration [19]. VISF also enhances mineralisation of bone matrix osteoblasts. APE stimulated proliferation and suppressed apoptosis of mouse osteoblastic cell line MC3T3-E1 [20]. It also has a protective effect on cultured rat bone marrow mesenchymal stem cells [21]. Data from *in vitro* studies seems to suggest that adipose tissue hormones might regulate bone remodelling, not only directly, but also via the cytokines of the RANKL/ /RANK/OPG system [22, 23]. It has been documented that human adipocytes exhibit OPG and/or RANKL expression *in vitro* [24–26] and regulate OPG and RANKL expression by human osteoblasts [26]. Fat cells downregulate RANKL production by osteoblasts but, to a much larger extent, upregulate OPG secretion. Kühn et al. [26] found that the OPG/RANKL ratio in primary human pre-osteoblasts increased nine-fold (mRNA and protein) when stimulated with adipocyte-secreted factors. Moreover, osteoblasts which were prestimulated with adipocyte-secreted factors inhibited osteoclasts formation.

The association between the adipose tissue hormones and bone tissue has been confirmed by *in vivo* studies, which, however, do not always yield unambiguous results, not only in healthy persons but also in persons with eating disorders (including anorexia nervosa — AN — or obesity).

Investigations carried out in apparently healthy women and/or men generally revealed a negative correlation between osteocalcin (OC), OPG and LP as well as between RANKL and ADIPO [27]. However, OC and/ /or bone alkaline phosphatase (B-ALP), collagen type I crosslinked aminoterminal telopeptide (NTx) and/or collagen type I crosslinked carboxyterminal telopeptide (CTx) were positively correlated with ADIPO both in healthy women [28, 29] and men [30, 31]. Additionally, OPG concentrations were higher in post- compared to pre-menopausal women [27]. The OPG/RANKL ratio was positively correlated with ADIPO and inversely associated with LP independent of the effect of age, BMI and menopausal status [27]. In studies involving pre- and postmenopausal healthy women, it was demonstrated that serum LP levels were negatively correlated with bone mineral density (BMD), whereas ADIPO did not seem to exert any effect on bone mass [32]. In other studies, serum ADIPO negatively associated with BMD,

and more significantly in postmenopausal women [28]. In contrast, other studies have indicated that ADIPO exerts an activity to increase bone mass by supporting osteoclastogenesis and activating osteoblastogenesis [33, 34]. When studying the relationships between LP, ADIPO, RES, VISF, APE and BMD, the majority of researchers concluded that ADIPO was the only independent BMD predictor in postmenopausal women [29, 35] and elderly men [31]. According to some authors, both LP and ADIPO turned out to be determinants of BMD in older women and men [36]. Oh et al. [37] found that from among researched adipocytokines (LP, ADIPO, RES), only serum RES level showed a significant negative correlation with lumbar spine BMD in middle-aged men, although the variance was small.

Our studies of females with AN demonstrated that serum LP, RES, VISF and APEL suppression and ADIPO elevation were associated with a decrease in bone markers levels, low values of the OPG/sRANKL ratio with a parallel increase in serum OPG and sRANKL [38]. Of the researched adipocytokines, VISF was shown to be an independent predictor of OC; APE and RES turned out to be independent predictors of CTx, and sRANKL; APE and ADIPO were independent predictors of OPG; and APE, LP and ADIPO were independent predictors of the OPG/ /sRANKL ratio. Abnormal concentrations of the above mentioned adipose tissue hormones observed in females with AN might affect the balance of the OPG/sRANKL system and potentially compromise the mechanism which compensates for bone remodelling disturbances [38].

The results in obese women evidenced significant increases in serum LP, RES and/or APE concentrations and a serum ADIPO decrease compared to age-matched non-obese women [39–47]. Other research has not observed significant differences between serum VISF concentrations in obese and non-obese individuals [48]. Still others demonstrated a decrease [49] or increase [50–52] of serum VISF levels in obese subjects compared to a control group. On the other hand, obese women exhibited a significant reduction in the circulating levels of bone markers, cytokines of the RANKL/RANK/OPG system (especially RANKL) as well as an increase in the OPG/RANKL ratio compared to age-matched lean women [44, 45, 53, 54]. In general, these changes were more pronounced in post- compared to premenopausal obese women [44, 45, 53, 54].

Only a few researchers have studied the effect of obesity on the relationship between adipose tissue hormones and bone status indicators in postmenopausal women. Gannage-Yared et al. [55] did not find any correlation between LP, ADIPO and OPG levels when they compared obese and non-obese young individuals. Our previous studies on obese postmenopausal women revealed a significant positive correlation between LP and BMD, OPG as well as the OPG/sRANKL ratio, and a negative correlation between LP and ICTP, CTx, sRANKL [44, 45]. Saleem et al. [56], who investigated the relationships between LP, ADIPO concentrations and bone markers in postmenopausal women diagnosed with metabolic syndrome (MetS), only found a negative correlation between OC and LP, and a positive correlation between OC and ADIPO. Wu et al. [57] showed that an increase in trunk fat in older women and men with MetS correlated significantly with ADIPO reduction, while an increase in leg fat was correlated with increased circulating levels of this adipocytokine. Although *in vitro* studies tend to indicate relationships between RES, VISF or APE levels and bone tissue [18–21], no literature reports have been found regarding the effect of obesity on the relationship between the above mentioned adipocytokines and bone status indicators in postmenopausal women.

The aim of this study was to determine whether obese postmenopausal women exhibited any relationship between serum levels of LP, ADIPO, RES, VISF, APE and bone metabolism markers (OC and CTx), OPG, sRANKL, the OPG/sRANKL ratio as well as BMD.

Material and methods

The study comprised 60 obese postmenopausal women hospitalised at the Endocrinology Division of the Department of Pathophysiology and Endocrinology in Zabrze Medical University of Silesia in Katowice (Poland). After the exclusion of hormonal causes, the study participants were diagnosed with simple obesity. Insulin resistance, abnormal lipid profile or glucose tolerance test were excluded based on the results of the preliminary biochemical analyses. Other exclusion criteria included liver and kidney failure, coronary heart disease, arterial hypertension, diabetes, systemic connective tissue disorders, neoplastic and autoimmune disease, thyroid hormone or steroid therapy (oestrogen/ /progestin therapy) and nonsteroidal anti-inflammatory agents. Women receiving antiresorptive or immunosuppressive therapy were also excluded. All study subjects had adult-onset obesity. The control group consisted of 20 postmenopausal women with normal body weight.

All women underwent BMD measurement using dual-energy X-ray absorptiometry (DXA) at lumbar spine (L_2–L_4) on a Lunar DPX, USA. All measurements were performed by one operator. Densitometry values were presented as BMD [g/cm^2], T-score (the number of standard deviations [SD] from young adults) and Z-score (the number of SD from age-matched subjects). The coefficient of variation (CV%) was 1.6%.

Blood samples were collected between 8am and 9am following a 12-hour fast. Centrifuged serum was frozen and stored at –75°C until analysis. Determina-

tions of the concentrations of adipose tissue hormones, OC, CTx, OPG and sRANKL were performed by ELISA method using the following kits: LP and ADIPO (Bio-Vendor, LLC, USA), RES (Mediagnost, Germany), VISF, APE (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 VISF; 0.09 ng/mL, 5 and 14% for APE; 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 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 body mass index (BMI), waist-to-hip circumference ratio (WHR), BMD, LP, ADIPO, RES, VISF, APE, 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 analysis was used to determine whether and which of the adipose tissue hormones under investigation were independent predictors of bone markers, OPG, sRANKL 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 (NN-013-24/I/03/04).

Results

Our obese postmenopausal women showed a significant increase in mean body mass, BMI and WHR values compared to the control group. A significant increase in mean serum LP and RES concentrations and a significant decrease in mean serum ADIPO were also found. These changes were associated with a significant decrease in mean serum OC and CTx levels, marked decreases in OPG and sRANKL, and a significant increase in the OPG/sRANKL ratio compared to the control group. The mean concentrations of VISF and APE in obese postmenopausal women were only slightly increased compared to the control. Lumbar spine bone mineral density was significantly higher in obese postmenopausal women compared to the control, whether expressed as BMD [g/cm^2], T-score or Z-score (Table I).

In obese postmenopausal women, BMI values correlated significantly and positively with serum concentrations of LP, RES and OPG, the OPG/sRANKL ratio and BMD (presented as [g/m^2]). A significant and negative correlation was found between BMI values and serum concentrations of ADIPO, OC, CTx, sRANKL. Obese

Table I. *Mean values of age, height, body mass, body mass index (BMI), waist to hip circumference ratio (WHR), bone mineral density ($BMD_{L2–L4}$, presented as [g/cm^2] and T-score or Z-score), mean serum levels of selected adipose tissue hormones, osteocalcin (OC), collagen type I crosslinked carboxyterminal telopeptide (CTx), osteoprotegerin (OPG), soluble receptor activator of nuclear factor-κB ligand (sRANKL) and OPG/sRANKL ratio values in postmenopausal obese women and in the control group*

Tabela I. *Średni wiek, wzrost, masa ciała, wskaźnik masy ciała (BMI), wskaźnik talia–biodra (WHR), gęstość mineralna kości ($BMD_{L2–L4}$, wyrażoną w [g/cm^2] oraz jako T-score i Z-score), średnie stężenia wybranych hormonów tkanki tłuszczowej, osteokalcyny (OC), karboksyterminalnego usieciowanego telopeptydu łańcucha α1 kolagenu typu I (CTx), osteoprotegeryny (OPG), rozpuszczalnego ligandu receptora aktywatora czynnika jądrowego-κB (sRANKL) i wartości wskaźnika OPG/sRANKL u otyłych kobiet po menopauzie i w grupie kontrolnej*

Variables	Groups	
	Obese women (n = 60)	Control group (n = 20)
Age (years)	57.36 ± 3.69	57.25 ± 3.86
Height [m]	1.60 ± 0.08	1.57 ± 0.03
Body mass [kg]	88.68 ± 2.51*	74.98 ± 4.97
BMI [kg/m^2]	35.89 ± 1.62*	24.36 ± 0.46
WHR	0.89 ± 0.07*	0.79 ± 0.03
BMDL2-L4 [g/cm^2]	1.172 ± 0.033*	1.117 ± 0.016
T-score	–0.001 ± 0.21*	–1.08 ± 0.17
Z-score	0.38 ± 0.23*	–0.68 ± 0.18
Last menstrual period (years)	51.73 ± 2.14	50.00 ± 4.24
Time since physiological menopause (years)	6.45 ± 2.57	7.25 ± 2.22
Duration of obesity (years)	16.51 ± 4.83	–
Leptin (LP) [ng/mL]	70.36 ± 28.99*	11.05 ± 4.03
Adiponectin (ADIPO) [μg/mL]	8.80 ± 3.18*	10.62 ± 3.61
Resistin (RES) [ng/mL]	3.36 ± 1.05*	2.76 ± 1.01
Visfatin (VISF) [ng/mL]	53.62 ± 3.22	48.54 ± 3.32
Apelin (APE) [pg/mL]	141.72 ± 20.90	136.48 ± 19.32
OC [μmol/L]	5.21 ± 0.57*	6.14 ± 0.70
CTx [nmol/L]	3.02 ± 0.23*	5.74 ± 0.62
OPG [pmol/L]	4.47 ± 0.31*	5.45 ± 0.38
sRANKL [pmol/L]	0.87 ± 0.07*	1.06 ± 0.08
OPG/sRANKL ratio	24.74 ± 4.43*	12.46 ± 1.88

*$p \leq 0.05$ *vs.* control group; T-score — the number of standard deviations [SD] from young adults; Z-score — the number of SD from age-matched subjects

postmenopausal women exhibited a significant and positive correlation between WHR values and OPG/sRANKL ratio. WHR values correlated also, significantly and negatively, with serum levels of ADIPO and APE. In obese postmenopausal women, a significant and positive correlation was found between BMD and serum levels of LP, APE as well as the OPG/sRANKL ratio. The correlation between BMD and serum levels of ADIPO, CTx as well as sRANKL was significant and negative (Table II).

In obese postmenopausal women, positive and significant correlations were revealed between serum concentrations of OC, CTx, OPG, sRANKL and ADIPO as well as between sRANKL and RES. The OPG/sRANKL ratio correlated significantly and positively with serum LP levels. However, negative and significant correlations in these women were revealed between serum concentrations of OC and LP, RES, VISF, APE; CTx and LP, VISF, APE; OPG and LP; sRANKL and LP, APE. The OPG/sRANKL ratio correlated significantly and negatively with VISF (Table II).

Stepwise regression analysis carried out in postmenopausal obese women revealed that ADIPO was an independent predictor of OC ($R^2 = 0.342$, $p = 0.013$). LP turned out to be an independent predictor of CTx ($R^2 = 0.890$, $p < 0.001$). ADIPO and LP were independent predictors of OPG ($R^2 = 0.422$, $p < 0.001$) and sRANKL ($R^2 = 0.300$, $p = 0.024$), while LP was an independent predictor of the OPG/sRANKL ratio ($R^2 = 0.426$, $p = 0.005$) (Table III).

Table II. *Correlation between values of body mass index (BMI), waist to hip circumference ratio (WHR), bone mineral density ($BMD_{L2–L4}$, presented as [g/cm²]) and selected adipose tissue hormones, 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 postmenopausal obese women (n = 60)*

Tabela II. *Korelacja między wartościami wskaźnika masy ciała (BMI), wskaźnika talia–biodra (WHR), gęstością mineralną kości ($BMD_{L2–L4}$, wyrażoną w [g/cm²]), wybranymi hormonami tkanki tłuszczowej, osteokalcyną (OC), karboksyterminalnym usieciowanym telopeptydem łańcucha α1 kolagenu typu I (CTx), osteoprotegeryną (OPG), rozpuszczalnym ligandem receptora aktywatora czynnika jądrowego-κB (sRANKL) i wartościami wskaźnika OPG/sRANKL u otyłych kobiet po menopauzie (n = 60)*

Variables		Values of correlation coefficients — R
BMI [kg/m²]	BMDL2-L4 [g/cm²]	0.259*
	Leptin (LP) [ng/mL]	0.479*
	Adiponectin (ADIPO) [μg/mL]	–0.305*
	Resistin (RES) [ng/mL]	0.454*
	Visfatin (VISF) [ng/mL]	NS
	Apelin (APE) [pg/mL]	NS
	OC [μmol/L]	–0.514*
	CTx [nmol/L]	–0.507*
	OPG [pmol/L]	0.466*
	sRANKL [pmol/L]	–0.377*
	OPG/sRANKL ratio	0.356*
WHR	BMD [g/cm²]	NS
	Leptin (LP) [ng/mL]	NS
	Adiponectin (ADIPO) [μg/mL]	–0.430*
	Resistin (RES) [ng/mL]	NS
	Visfatin (VISF) [ng/mL]	NS
	Apelin (APE) [pg/mL]	–0.428*
	OC [μmol/L]	NS
	CTx [nmol/L]	NS
	OPG [pmol/L]	NS
	sRANKL [pmol/L]	–0.348*
	OPG/sRANKL ratio	0.448*
BMD [g/cm²]	Leptin (LP) [ng/mL]	0.271*
	Adiponectin (ADIPO) [μg/mL]	–0.695*
	Resistin (RES) [ng/mL]	NS
	Visfatin (VISF) [ng/mL]	NS
	Apelin (APE) [pg/mL]	0.254*
	OC [μmol/L]	NS
	CTx [nmol/L]	–0.625*
	OPG [pmol/L]	NS
	sRANKL [pmol/L]	–0.417*
	OPG/sRANKL ratio	0.521*

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

Discussion

Recent investigations seem to indicate that obesity is not a risk factor for osteoporosis [45, 58–63]. It has been demonstrated that postmenopausal women who are ≥ 30% overweight exhibit higher BMD measured at lumbar spine (L_2–L_4), proximal femur and the radius compared to slim postmenopausal women [58, 60, 61, 64]. Numerous authors have emphasised a significant positive correlation between BMI and BMD measured at lumbar spine and proximal epiphysis of the femur [58, 60, 61]. Compared to non-obese postmenopausal controls, our obese postmenopausal study participants (no insulin resistance, normal lipid profile and normal glycaemic response curve) showed an increase in L_2–L_4 BMD, which was positively correlated with BMI; the results are consistent with those of other authors [45, 54, 58–61]. BMD increase was associated with a decrease in bone turnover markers, OPG and sRANKL levels (the decrease was greater in the case of CTx than OC and in the case of sRANKL than OPG) and an increase in the OPG/sRANKL ratio. Our previous investigations revealed that mean circadian concentrations of CTx, sRANKL and the values of OPG/sRANKL ratio were

Table III. *Correlation[a] between selected adipose tissue hormones, osteocalcin (OC), collagen type I crosslinked carboxyterminal telopeptide (CTx), osteoprotegerin (OPG), soluble receptor activator of nuclear factor-kB ligand (sRANKL) and OPG/sRANKL ratio in obese women (n = 60)*

Tabela III. *Korelacja[a] między wybranymi hormonami tkanki tłuszczowej, osteokalcyną (OC), karboksyterminalnym usieciowanym telopeptydem łańcucha α 1 kolagenu typu I (CTx), osteoprotegeryną (OPG), rozpuszczalnym ligandem receptora aktywatora czynnika jądrowego-κB (sRANKL) i wartościami wskaźnika OPG/sRANKL u otyłych kobiet po menopauzie (n = 60)*

Variables	Bone markers		Cytokines of RANKL/RANK/OPG system		OPG/sRANKL ratio
	OC [μmol/L]	CTx [nmol/L]	OPG [pmol/L]	sRANKL [pmol/L]	
Leptin (LP) [ng/mL]	–0.738*	–0.762*	–0.257*	–0.293*	0.667*
Adiponectin (ADIPO) [μg/mL]	0.928*	0.548*	0.280*	0.289*	NS
Resistin (RES) [ng/mL]	–0.333*	NS	NS	0.325*	NS
Visfatin (VISF) [ng/mL]	–0.738*	–0,690*	NS	NS	–0.381*
Apelin (APE) [pg/mL]	–0.690*	–0.833*	NS	–0.301*	NS
Stepwise regression model[b]	ADIPO	LP	ADIPO LP	ADIPO LP	LP
R2	0.342	0.890	0.422	0.300	0.426
p	0.013	< 0.001	< 0.001	0.024	0.005

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

significantly correlated with BMD in postmenopausal obese women [45, 54].

In our present study, the above relationships were confirmed. Obese postmenopausal women exhibited significant and negative correlations between BMD and CTx as well as sRANKL, while the correlation between BMD and the OPG/sRANKL ratio was positive. Moreover, BMI correlated significantly and negatively with bone markers concentrations and sRANKL, while the correlation between BMI and OPG as well as the OPG/sRANKL ratio was positive. Although no correlations were found between BMD and WHR, the latter correlated significantly and negatively with sRANKL and positively with the OPG/sRANKL ratio. The obtained results show that, as the degree of obesity increases, bone formation and resorption are both suppressed. The balance between these two processes is also disturbed. The cytokines of the RANKL/RANK/OPG system, and especially RANKL, might play an important role in the mechanism of these disturbances. Based on the results of the correlation analysis, it might be assumed that obesity-, adipose tissue distribution- and age-related changes in RANKL and the OPG/RANKL ratio might modify bone metabolism as evidenced by BMD changes seen in obese postmenopausal women.

The observed changes in bone status indicators might be associated with impaired production of some osteotropic factors (mainly hormones) frequently seen in obese postmenopausal women [53, 54, 65–67]. It is assumed that factors which are essential for the maintenance of normal bone mineral density in obese individuals include oestrogens, androgens (and, in particular, adrenal androgens), melatonin, changes in calcium and vitamin D_3 metabolism as well as changes in the relationships between hormones of the somatotropic axis [44, 45, 53, 54, 58, 62, 67]. Recent studies, and especially the results obtained in *in vitro* models, have indicated that some adipocyte-secreted factors, including adipose tissue hormones, such as LP [11–14], ADIPO [15–17], RES [18], and maybe also VISF [19] and APE [20, 21], might play a role in the mechanism which prevents obese women from bone mass loss after menopause. A direct and/or OPG- and/or RANKL-mediated action of the above mentioned adipocytokines might affect bone remodelling in obese postmenopausal women [17, 22, 23, 27, 68]. As particular adipocytokines may exert a similar or opposite effect on bone tissue cells and OPG and/or RANKL expression in human osteoablasts *in vitro* [11–22, 24, 26], it is quite difficult to predict the net effect of their actions on the balance of the OPG/RANKL system and/or bone remodelling and bone mass *in vivo*. The most recent *in vitro* studies [24, 26] seem to indicate a final stimulatory effect. An et al. [24] found OPG and RANKL expression in 3T3L1 murine adipocytes. They also observed that OPG mRNA expression increased with the differentiation of 3T3L1 adipocytes, while RANKL expression was only slightly altered. OPG mRNA was expressed at higher levels in white adipose tissue than in brown adipose tissue and

was more abundant in the subcutaneous portion [24]. Kühn et al. [26] reported that adipocytes regulated RANKL and OPG expression in human osteoblasts. While RANKL expression was only slightly affected, the expression of OPG, a cytokine which blocks RANKL/ /RANK interaction in osteoclasts, was significantly altered. Osteoclasts formation from precursor cells significantly decreases during osteoblast preincubation with adipocyte-secreted factors. Hence the assumption that adipocytes not only stimulate preosteoblast proliferation, but also affect osteoblast/osteoclast interaction, thus reducing the number of osteoclasts which play an important role in the resorption process. These *in vitro* findings could account for higher bone mass in obese individuals, especially women, and attribute this effect to some adipocyte-secreted factors, including adipose tissue hormones, on bone formation [26].

The majority of literature reports focus on the effect of menopause on the relationships between selected adipose tissue hormones (especially LP and/or ADIPO) and various bone status indicators in non-obese women with or without osteoporosis. Only a few researchers have investigated the influence of obesity and menopause on the above mentioned relationships.

Numerous researchers have emphasised a beneficial effect of LP on bone tissue in normal weight pre- and postmenopausal women [45, 59, 69–71]. The effect depends on BMI and body fat percentage. A majority of research reports indicate that lean women exhibit a BMI- and body fat percentage-dependent positive correlation between LP and BMD, and a negative correlation between LP and bone markers, and especially bone resorption markers [59, 69–71]. Nevertheless, some authors have only observed a slight positive correlation between LP and BMD [72–75] and a significant negative correlation between LP and bone markers [73–75] in both pre- and postmenopausal women. Other investigators concluded that LP was associated with BMD but only in postmenopausal women [76, 77]; still others found a significant positive correlation between LP and BMD in both pre- and postmenopausal women [69]. Tenta et al. [27] demonstrated that LP levels were negatively and significantly correlated with OC, OPG and the OPG/RANKL ratio among healthy women. They believe that the negative correlation between LP and the OPG/RANKL ratio might represent a component of a mechanism protecting these women against postmenopausal bone loss. Blain et al. [70] concluded that LP was an independent BMD predictor of whole body and femoral neck BMD in postmenopausal women. Women suffering from postmenopausal osteoporosis exhibited weaker correlations between BMD, bone markers and LP compared to their healthy counterparts [78, 79]. Tenta et al.'s [27] investigations of healthy non-obese women demonstrated that ADIPO levels were positively and significantly correlated with the OPG/ /RANKL ratio independent of the effect of age, body mass index and menopausal status. Several investigations carried out in apparently healthy women have indicated a negative and significant correlation between BMD and ADIPO concentrations [28, 31, 39, 80, 81]. On the contrary, other studies indicated that ADIPO exerts an activity to increase bone mass by supporting osteoclastogenesis and activating osteoblastogenesis [33, 34]. Zhang et al. [29] and Wu et al. [35] have shown that from among researched adipocytokines (LP, ADIPO, RES, VISF and APE), only ADIPO, but not fat mass, LP, RES, VISF or APE, was an independent predictor of BMD in postmenopausal women. Zhang et al. [29] also suggested that ADIPO may exert a negative effect on bone mass by promoting excessive bone resorption associated with bone loss in post-menopausal women. Some other investigators did not reveal a significant association between circulating ADIPO levels and BMD in apparently healthy women of child bearing age [32]. Sodi et al. [82] indicated that there is no significant difference in the circulating concentration of fasting early morning total- or high molecular weight (HMW)-ADIPO in postmenopausal women with or without osteoporosis. However, the negative correlation between HMW/total-ADIPO ratio and OPG showed in these women indicate that ADIPO could influence bone metabolism by altering osteoblast production of OPG, thereby affecting osteoclasts mediated bone resorption [82]. Other researchers observed an OPG increase in postmenopausal women with osteoporosis and LP, ADIPO as well as RANKL upregulation in postmenopausal women without osteoporosis [83].

As mentioned above, only a few researchers have studied the effect of obesity and menopause on the relationship between selected adipose tissue hormones and bone status indicators in women. Several studies have only assessed the association between obesity and the levels of some selected adipose tissue hormones in women of different ages. In general, the obtained results evidenced significant increases in LP and/or RES levels and serum ADIPO decrease in obese women. These changes were typically associated with obesity markers including BMI, WHR and/or body fat mass [39–46] indicating a relationship between these adipocytokines and obesity. We obtained similar results in our studies on obese postmenopausal women. LP and RES concentrations were significantly and positively correlated with BMI, whereas ADIPO levels correlated negatively with both BMI and WHR. Nevertheless, several researchers did not find any relationship between RES levels and obesity markers (BMI, WHR, body fat mass) in obese female teenagers or moderately obese women [42, 84].

The findings of VISF determinations have been also questionable. Similar to our results, Berndt et al. [48] also did not observe significant differences between serum VISF concentrations in obese and lean individuals. In addition, they did not find differences in VISF mRNA expression between visceral and subcutaneous adipose tissue. Other researchers demonstrated a decrease [49] or increase [50–52] of VISF concentrations in obese subjects. According to some authors [43, 85–90], the association between LP, ADIPO, RES and VISF and obesity is lent support by changes in adipocytokine concentrations following weight reduction (diet or surgery) in individuals with moderate or severe obesity. Significant weight reduction in women with moderate or severe obesity, apparently resulting from dietary treatment, has been found to be associated with decreased LP and RES and increased ADIPO circulating concentrations [85–87]. Weight loss after bariatric surgery in women and/or men with morbid obesity was associated with decreased LP, RES, VISF and increased ADIPO levels [43, 88–90]. Boucher et al. [47] postulated a relationship between obesity and APE upregulation. But it seems that obesity itself is not solely responsible for APE increase; circulating levels of this adipocytokine are not significantly correlated with BMI [10, 91]. We did not reveal significant differences between serum APE in obese and non-obese postmenopausal women. Neither did we find a relationship between APE and BMI, although APE concentrations correlated significantly and positively with WHR.

Our obese postmenopausal participants had a positive correlation between LP and BMD as well as the OPG/sRANKL ratio while the relationship between LP and OC, CTx, OPG and sRANKL levels was negative. Moreover, LP turned out to be an independent predictor of CTx, OPG, sRANKL and the OPG/sRANKL ratio in these women. Contrary to our present results, the correlation between LP and OPG, which we had previously observed in obese postmenopausal women [44,45], did not reach the level of statistical significance. Gannage-Yared et al. [55], did not find any correlation between LP and OPG levels, when they compared obese and non-obese young individuals. Women diagnosed with postmenopausal osteoporosis showed a weak correlation between LP and BMD and bone markers compared to the control group [78, 79], whereas obese postmenopausal women exhibited a stronger correlation between LP and BMD, bone markers (PICP, B-ALP or OC, CTx or NTx) than their non-obese postmenopausal counterparts [44, 45, 69, 70]. This was most probably associated with decreased capacity of LP transport in obese individuals providing a mechanism for LP resistance in the central nervous system (CNS) [92]. The levels of LP in the serum and cerebrospinal fluid (CSF) are well-correlated in normal weight individuals, whereas obese people exhibit high LP concentrations in the serum as opposed to only slight LP increases in the CSF [92]. It appears that LP mainly triggers bone tissue resorption and not formation *in vivo* [71]. It is generally believed that, only early in life, LP might stimulate bone growth and size through direct angiogenic and chondro-osteogenic effects. Later, in the mature skeleton, the hormone may decrease bone metabolism by stimulating the OPG/RANKL pathway. LP may also exert negative effects on bone tissue through a hypothalamic pathway mediated by the sympathetic nervous system. These peripheral and central pathways of LP action could counterbalance each other. The peripheral and positive effects predominate when LP central resistance occurs with obesity onset [14]. These findings are consistent with our previous [44, 45] and present results obtained for healthy obese postmenopausal women (with no insulin resistance, normal lipid profile and normal glucose tolerance test).

Our findings seem to suggest that LP increase observed in obese postmenopausal women might have a beneficial effect on bone metabolism.

Considerably less data is available regarding the effects of obesity and menopause on the relationship between bone status indicators and ADIPO levels in women. The majority of research reports have focused on ADIPO concentrations and the association of this adipocytokine with body mass determinants, bone markers, OPG and/or BMD in obese, and, particularly, moderately obese women. Some authors [55] did not find any correlation between ADIPO and OPG levels when they compared obese and lean young individuals. In general, the obtained results indicate that obese women exhibited a BMI- and body fat percentage-dependent decrease in circulating ADIPO levels [9, 40–42]. A significant negative correlation between ADIPO concentrations and OPG has also been emphasised [40,82]. Postmenopausal women with MetS exhibited a high positive correlation between ADIPO levels and serum bone formation marker — OC [56]. Wu et al. [57] reported that trunk fat mass increase in older women with MetS was significantly associated with lower ADIPO concentrations while leg fat mass was significantly associated with higher ADIPO levels [57]. Our findings seem to suggest that ADIPO deficiency observed in obese postmenopausal women (with no insulin resistance, normal lipid profile and normal glucose tolerance test) might have a beneficial effect on bone metabolism. A negative relation between serum ADIPO levels and BMD as well as a positive relationship with serum bone markers, OPG and sRANKL were confirmed among obese postmenopausal women. Moreover, ADIPO turned out to be an independent predictor of OC, OPG and sRANKL in these patients.

Though *in vitro* studies indicate significant relationships between bone tissue metabolism and RES, VISF or APE levels [18–21], literature reports do not provide evidence confirming the occurrence of such relationships in lean and obese persons. The few available *in vivo* investigations suggest the absence of a relationship between serum levels of the above mentioned adipocytokines and BMD in non-obese women and men of different age [29, 31, 36, 37]. However, our results in obese postmenopausal women seem to indicate not only a significant association between BMD and serum APE (positive), but also a significant correlation between serum levels of OC and RES, VISF, APE (positive); serum levels of CTx and VISF, RES (negative); serum levels of sRANKL and RES (positive), APE (negative); the OPG/sRANKL ratio and serum VISF (negative). No significant correlations were found between serum OPG and RES, VISF or APE levels.

Hence, we conclude that changes in the concentrations of the above mentioned adipose tissue hormones (a trend towards VISF and APE increase, plus a significant increase in RES concentrations) might have an effect (most probably beneficial) on bone metabolism and bone mass.

Disparities in correlation analysis regarding adipose tissue hormones and bone status determinants between women non-obese, obese (with no insulin resistance, normal lipid profile and normal glucose tolerance test), and suffering from MetS, might be associated with age differences, time from menopause and obesity-related complications [45, 59]. It should also be noted that osteoblasts and bone marrow stromal cells are not the only producers of OPG and RANKL [22, 23] as well as adipose tissue hormones (LP, ADIPO, RES, VISF, APE) are not only secreted by adipocytes [1, 2, 4]. Hence, the differences in the levels of these substances in the blood. The differences in the results of *in vitro* and *in vivo* studies might arise from the fact that the ultimate effect of adipose tissue hormones on *in vivo* bone status determinants might be modified by several well-known factors including hormones and cytokines [22, 23, 45, 65, 66].

Based on the above cited results, and our own findings, it is possible that obesity itself as well as obesity-related alterations in adipose tissue hormones might modify bone resorption in postmenopausal women not only directly but also via changes in the RANKL/RANKL/OPG system. Hence, it might be assumed that these alterations are included in a complex mechanism that acts to protect obese women against the development of postmenopausal osteoporosis.

The results of the present investigations indicate that the net effect of the investigated adipose tissue hormones on OPG and sRANKL concentrations in obese postmenopausal women most probably consists of a shift in the OPG/sRANKL ratio towards a functional excess of OPG. This is evidenced by the inhibition of the resorption process as reflected by a decrease in CTx, which functions as a resorption marker. Despite the disparities between the results of *in vitro* and *in vivo* studies, it might be assumed that adipose tissue hormones might exert a positive effect on the OPG/sRANKL ratio via reducing the pool of active osteoclasts, thus diminishing the bone resorption process. This effect seems independent of whether adipose tissue hormones increase OPG expression, inhibit RANKL production or affect the expression of both cytokines.

Conclusions

Obesity in postmenopausal women can lead to changes in BMD, circulating levels of bone markers, OPG, sRANKL and/or the OPG/sRANKL ratio; these changes are associated with alterations in the concentrations of adipose tissue hormones under investigation.

The relationships between bone status indicators and adipose tissue hormones, especially LP and ADIPO, seem to suggest that postmenopausal changes in these hormones observed in obese women might have a protective effect on bone tissue, most probably via a shift in the OPG/sRANKL ratio towards a functional excess of OPG.

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