



# Osteoprotegerin — does it play a protective role in the pathogenesis of bone loss in obese perimenopausal women?

Czy osteoprotegeryna jest potencjalnym czynnikiem hamującym ubytek masy kostnej otyłych kobiet w wieku okołomenopauzalnym?

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## Abstract

**Introduction:** Assessment of serum osteoprotegerin (OPG) concentrations in obese patients in comparison to healthy controls and evaluation of a possible correlation between OPG and other markers of bone turnover or calcitropic hormones.

**Material and methods:** 50 obese perimenopausal women without concomitant diseases (BMI  $36.7 \pm 4.1$  kg/m<sup>2</sup>, mean age  $50.4 \pm 4.9$  yrs). The control group consisted of 19 healthy women (BMI  $24.2 \pm 2.1$  kg/m<sup>2</sup>; mean age  $53.8 \pm 5.1$  yrs).

In all patients serum concentration of OPG, C telopeptide of type I collagen containing the crosslinking site (CTX), osteocalcin, parathormone (PTH) and vitamin D (25-OH-D<sub>3</sub>) was assessed. Dual energy x-ray absorptiometry (the DXA method) of the lumbar spine and femoral neck was performed using a Lunar DPXL to measure bone marrow density (BMD).

**Results:** In obese perimenopausal women serum OPG, osteocalcin and 25-OH-D<sub>3</sub> levels were significantly lower, and the serum PTH level was significantly higher in comparison to healthy controls. A significantly positive correlation was found between serum OPG level and age in both obese and control subjects.

**Conclusion:** The serum OPG level in obese perimenopausal women is significantly lower in comparison to healthy controls and does not correlate significantly with biochemical markers of bone turnover, calcitropic hormones and BMD. It probably cannot play a protective role in the pathogenesis of bone loss in obese perimenopausal women.

(Pol J Endocrinol 2007; (58) 1: 7–10)

**Key words:** osteoprotegerin, obesity, bone turnover, calcitropic hormones, BMD

## Streszczenie

**Wstęp:** Celem niniejszej pracy była ocena stężenia osteoprotegeryny (OPG) w surowicy otyłych pacjentek w porównaniu z grupą kontrolną oraz próba wykazania ewentualnych powiązań osteoprotegeryny ze wskaźnikami obrotu kostnego oraz hormonami kalcitropowymi.

**Materiał i metody:** 50 kobiet w wieku okołomenopauzalnym z otyłością prostą bez chorób towarzyszących (BMI  $36,7 \pm 4,1$  kg/m<sup>2</sup>, wiek  $50,4 \pm 4,9$  roku). Grupę kontrolną stanowiło 19 zdrowych kobiet (BMI  $24,2 \pm 2,1$  kg/m<sup>2</sup>; wiek  $53,8 \pm 5,1$  lat).

U wszystkich badanych oznaczono w surowicy stężenia OPG, C-końcowego usieciowanego telopeptydu kolagenu typu I (CTX), osteokalcyny, parathormonu (PTH) oraz 25-OH-D<sub>3</sub>. Badanie gęstości mineralnej kości (BMD, *bone mineral density*) w obrębie odcinka lędźwiowego kręgosłupa oraz szyjki kości udowej wykonano metodą absorpcjometrii podwójnej energii promieniowania rentgenowskiego (DXA, *Dual Energy X-ray Absorptiometry*) przy użyciu aparatu Lunar DPXL.

**Wyniki:** W grupie otyłych chorych obserwowano znamienne niższe stężenie OPG, osteokalcyny i 25-OH-D<sub>3</sub> oraz znamienne wyższe stężenie PTH w porównaniu z grupą kontrolną. Zarówno w grupie badanej, jak i w grupie kontrolnej zaobserwowano dodatnią korelację pomiędzy stężeniem OPG a wiekiem pacjentek.

**Wnioski:** U otyłych kobiet w wieku okołomenopauzalnym stężenie osteoprotegeryny w surowicy krwi jest znamienne niższe w porównaniu z grupą kontrolną i nie koreluje ze wskaźnikami obrotu kostnego, hormonami kalcitropowymi ani z BMD. Osteoprotegeryna prawdopodobnie nie spełnia funkcji ochronnej w patogenezie ubytku masy kostnej u otyłych kobiet w wieku okołomenopauzalnym.

(Endokrymol Pol 2007; (58) 1: 7–10)

**Słowa kluczowe:** osteoprotegeryna, otyłość, obrót kostny, hormony kalcitropowe, BMD



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## Introduction

Obesity in women is associated with a lower risk of osteoporosis, probably because of the beneficial effect of hyperoestrogenemia [1]. When obese women were compared with lean ones of the same age, thinness was found to be an important risk factor for low bone mass and increased bone loss in postmenopausal women [2].

Osteoprotegerin (OPG) was identified as a member of the tumour necrosis factor (TNF) receptor superfamily that acts as an inhibitor of osteoclastogenesis [3]. The biological effects of OPG on bone cells are: inhibition of the terminal stages of osteoclast differentiation [4], suppression of the activation of the mature osteoclast [5] and induction of apoptosis [6]. Additionally OPG antagonises the induction of bone resorption by 1.25 (OH)<sub>2</sub>-D<sub>3</sub> and parathormone (PTH) [7].

The aim of the study was to assess serum OPG concentration in obese patients in comparison to healthy controls. An answer was sought to the question of whether and to what extent serum OPG is a potent protective factor for bone loss in obese perimenopausal women. Secondly we aimed to evaluate the relationship between serum OPG level and both markers of bone turnover and calcitropic hormones.

## Materials and methods

Fifty obese perimenopausal women (BMI  $36.7 \pm 4.1$  kg/m<sup>2</sup>, mean age  $50.4 \pm 4.9$  yrs) were enrolled into the study. All were diagnosed with simple obesity without concomitant diseases. None had undergone ovariectomy. All subjects were informed about the nature of the study and signed forms giving their informed consent. The exclusion criteria included evidence of present or recent (during the preceding 3 months) infectious disease, drug therapy, cigarette smoking and alcohol con-

sumption of more than two drinks a week. The control group consisted of 19 lean healthy women without concomitant diseases (BMI  $24.2 \pm 2.1$  kg/m<sup>2</sup>; mean age  $53.8 \pm 5.1$  yrs).

Body weight and height were measured and BMI values were calculated. Blood samples were withdrawn from each subject between 8.00 and 9.00 am after an overnight fast. After clot formation the samples were centrifuged ( $1000 \times G$ ) at room temperature for 10 min. The serum obtained was drawn into plastic tubes and stored at  $-80^{\circ}\text{C}$  until the time of the assay.

Serum concentrations of OPG, CTX, osteocalcin, PTH and 25-OH-D<sub>3</sub> were assessed for all patients. OPG was estimated using ELISA (BioVentor, Czech Republic). The Electrochemiluminescent ImmunoAssay "ECLIA" (Roche, France) was used for osteocalcin, the C telopeptide of type I collagen containing the crosslinking site (CTX) and PTH assay. 25-(OH)-D<sub>3</sub> was measured by a RIA (The Bio Source, Belgium).

Dual energy x-ray absorptiometry (the DXA method) of the lumbar spine and femoral neck was performed using a Lunar DPXL to measure bone mineral density (BMD). All text and table values are expressed as means  $\pm$  S.D. All statistical analyses were performed using the STATISTICA computer program. The results were analysed using the Kolmogorov-Smirnoff test and Spearman's correlation analysis. P values  $< 0.05$  were considered to be statistically significant.

## Results

In obese perimenopausal women serum OPG, osteocalcin and 25-OH-D<sub>3</sub> levels are significantly lower and PTH serum levels significantly higher in comparison to healthy controls. BMD of the lumbar spine and femoral neck were significantly higher in obese women in comparison to healthy controls (Table I)

**Table I**

*Serum concentration of osteoprotegerin, biochemical markers of bone turnover, calcitropic hormones and BMD in 50 obese subjects in comparison to 19 healthy controls (means  $\pm$  SD)*

**Tabela I**

*Stężenie osteoprotegeryny, biochemicznych wskaźników obrotu kostnego, hormonów kalcitropowych oraz BMD u 50 otyłych chorych w porównaniu z grupą kontrolną (średnia  $\pm$  SD)*

	Subjects	Controls	p
Osteoprotegerin [pmol/l]	$4.39 \pm 1.39$	$5.58 \pm 0.53$	$< 0.005$
PTH [pg/ml]	$50.6 \pm 15.6$	$36.6 \pm 19.8$	$p < 0.01$
25-OH-D <sub>3</sub> [ng/ml]	$28.7 \pm 14.7$	$40.2 \pm 18.7$	$p < 0.001$
CTX [ng/ml]	$0.3 \pm 0.2$	$0.3 \pm 0.1$	ns
Osteocalcin [ng/ml]	$21.2 \pm 8.4$	$28.1 \pm 12.5$	$p < 0.005$
BMD neck [g/cm <sup>2</sup> ]	$1.07 \pm 0.16$	$0.85 \pm 0.11$	$p < 0.001$
BMD L <sub>1</sub> -L <sub>4</sub> [g/cm <sup>2</sup> ]	$1.24 \pm 0.17$	$1.07 \pm 0.13$	$p < 0.05$

The OPG serum level is positively correlated with age in both obese subjects and controls ( $r = 0.28$ ,  $p = 0.048$ ;  $r = 0.5$ ,  $p = 0.03$ ; respectively).

## Discussion

A large number of hormones, cytokines, growth and inflammatory factors are responsible for regulation of bone metabolism. In OPG a further factor has now been recognised that influences osteoclast formation and activity.

RANKL (osteoclast differentiation factor) is produced by an osteoblastic lineage cell and stimulates its receptor RANK, which is located on the osteoclast [8]. The effect of RANKL/RANK interaction is enhanced differentiation, fusion and activation of osteoclasts [7]. Osteoprotegerin (or OCIF, osteoclastogenesis inhibitory factor), acts as a decoy receptor that neutralises RANKL, binding to it and blocking its interaction with RANK, thus inhibiting osteoclast differentiation [3].

There are many factors influencing OPG/RANKL/RANK production, including PTH, calcitriol, glucocorticosteroids, IL-1 and PGE-2. These can either inhibit (PTH, steroids) or stimulate (oestrogen, TGF- $\beta$ ) OPG production [3].

The purpose of the present study was to evaluate whether and to what extent OPG may influence or be responsible for higher bone mass in obese women. We have observed lower serum OPG levels in obese subjects in comparison to healthy controls. Our speculation that higher BMD in the obese may be associated with higher OPG serum levels turned out to be wrong. Moreover, we did not find any significant correlation between BMD and serum OPG levels either in obese women or in controls. We cannot find any reasonable explanation for this phenomenon. Lower serum OPG levels may be caused by the higher serum PTH level (that is observed in the obese) as PTH may decrease OPG production [9]. Andersen and Mosekilde et al. [10, 11] showed that PTH concentration is dependent on the degree of obesity. The finding that serum PTH concentration and the expression of OPG in human bone tissue are inversely correlated was reported in postmenopausal women by Seck et al. [12]. On the other hand, obese patients are characterised by higher oestrogen levels, which may stimulate OPG production.

The finding of lower 25-OH-D<sub>3</sub> in obese patients is not new. The low level of 25-hydroxyvitamin D<sub>3</sub> in obese subjects may be caused by the decreased exposure to sunlight (limited mobility of obese patients) [13], excessive storage of vitamin D in adipose tissue [11] and inhibition of its synthesis in liver by the increased level of 1,25-dihydroxyvitamin D [14].

Both lower osteocalcin and OPG levels may be connected with lower activity of bone metabolism in obese women and less compensating production of OPG in this group of patients.

In the present study we did not find any significant correlation between serum OPG concentration and markers of bone turnover, OPG and calcitropic hormones or between OPG and BMD. A positive correlation was found between OPG and age in both obese subjects and controls.

Other authors, who did not evaluate OPG serum levels in obesity, found a negative correlation between OPG and BMD, a positive correlation with biochemical markers of bone turnover [15], no significant correlation between OPG and markers of bone turnover [16] or a confirmed negative correlation between serum OPG concentrations and BMD in postmenopausal women [17], with no convergence of these findings.

Our findings, when analysed, came as a surprise in view of the established protective effect of OPG on bone. There are many other factors responsible for bone metabolism (excluding cytokines, drugs and hormonal axis) such as diet and physical activity, and so we cannot exclude their influence on our results. We hope that further investigations, including research into the influence of weight reduction therapy on OPG serum levels in obese women, will provide a satisfactory explanation.

## Conclusion

Serum osteoprotegerin concentration in obese perimenopausal women is significantly lower in comparison to healthy controls and does not correlate significantly either with biochemical markers of bone turnover, calcitropic hormones or bone mineral density. It probably cannot play a protective role in the pathogenesis of bone loss in obese perimenopausal women.

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