

# The association of bone turnover markers with pro- and anti-inflammatory adipokines in patients with gestational diabetes

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## Abstract

**Objective.** The aim of the study was to assess differences in circulating osteocalcin (OC) and osteoprotegerin (OPG), as well as in their expression in subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT) and placental tissue obtained from patients with gestational diabetes mellitus (GDM) and normal glucose tolerance (NGT).

**Materials and method.** Serum levels of OC, OPG and soluble nuclear factor- $\kappa$ B ligand (sRANKL) were measured in 49 women with GDM and 30 subjects with NGT between weeks 24–32 of gestation, and three months after childbirth. OC and OPG mRNA expression was measured in 23 patients with GDM and 23 women with NGT at term, using quantitative real-time RT-PCR.

**Results.** The patients with GDM had decreased OC mRNA expression in SAT ( $p=0.015$ ), lower *adiponectin* mRNA expression in VAT ( $p=0.039$ ), and a lower circulating adiponectin level ( $p=0.04$ ). Multiple regression analysis revealed that serum adiponectin was significantly associated with OC mRNA expression in SAT ( $b=0.49$ ,  $p=0.03$ ). Three months postpartum, the OPG/sRANKL ratio was markedly higher in the subjects with prior GDM ( $p=0.03$ ) and correlated positively with HbA1c ( $R=0.33$ ;  $p=0.04$ ), fasting insulin ( $R=0.35$ ;  $p=0.03$ ) and HOMA-IR ( $R=0.34$ ;  $p=0.04$ ).

**Conclusions.** In the patients with GDM decreased OC mRNA expression in SAT might be associated with a reduced stimulatory effect on adiponectin expression in adipose tissue. On the other hand, higher OPG/sRANKL ratio suggests a better protection against bone loss in the subjects with prior GDM.

## Key words

pregnancy, gestational diabetes, osteocalcin, osteoprotegerin, RANKL, adiponectin

## INTRODUCTION

Gestational diabetes mellitus (GDM) affects a population of young women with limited pancreatic beta-cell reserve and an inadequate insulin secretion, unable to meet the increasing tissue insulin demanding of late pregnancy [1]. The pathogenesis of GDM is still far from clear, but it is believed that it results from the insulin-desensitizing effects of placenta-derived hormones, combined with enhanced secretion of proinflammatory adipokines and cytokines from fatty and placental tissue [1]. However, recent studies strongly suggest an entirely new role for bone as an endocrine organ regulating glucose metabolism, indicating that an osteoblast-derived protein osteocalcin (OC) is a hormone increasing insulin production by the pancreas and insulin sensitivity in peripheral tissues, at least in part through enhanced secretion of adiponectin [2, 3]. Experimental models have shown that insulin signaling in osteoblasts promotes decarboxylation and activation of OC, and decreases the expression of osteoprotegerin (OPG) [3] – a decoy receptor of the receptor activator of nuclear factor-

$\kappa$ B ligand (RANKL) and an inhibitor of osteoclastic bone resorption [4]. Recently, Winhofer et al. [5] reported that OC concentrations are elevated in patients with GDM, compared with healthy pregnant women, and suggested that it might be a compensatory mechanism leading to enhanced insulin secretion in order to overcome insulin resistance. Moreover, it has been shown that OC [6] and OPG [7, 8, 9, 10] mRNA and protein are present in subcutaneous and visceral adipose tissue; however, the role of these new adipokines is still an open question. Therefore, in the presented study it is hypothesized that the development of insulin resistance and hyperglycaemia during pregnancy may influence not only OC and OPG serum levels, but also their expression in fatty and placental tissue. To verify this hypothesis, the following were evaluated:

- 1) the differences in circulating OC and OPG and in their expression in subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT) and placental tissue obtained from women with GDM and normal glucose tolerance (NGT);
- 2) possible associations between these two and other pro- and anti-inflammatory adipokines.

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## MATERIALS AND METHOD

**Study population.** The group studied consisted of 49 women with GDM and 30 subjects with NGT, between 24–32 weeks of gestation (Group 1), as well as 23 patients with GDM and 23 women with NGT between 36–40 weeks of gestation (Group 2). GDM was defined according to the criteria of the Polish Diabetological Association, as at least one elevated plasma glucose value during a 75 g oral glucose tolerance test (OGTT), with the following threshold glucose concentrations: fasting  $\geq 100$  mg/dl (5.5 mmol/l), 1 h  $\geq 180$  mg/dl (10.0 mmol/l) and 2 h  $\geq 140$  mg/dl (7.8 mmol/l).

In Group 1, blood samples were collected before the initiation of diet or insulin therapy and three months after childbirth. In Group 2, nine subjects were treated with intensive insulin therapy and 14 with diet alone. All pregnancies were singletons.

Written informed consent was obtained from all participants, and the protocol was approved by the local Ethics Committee at Medical University in Bialystok.

**Analytical methods.** Plasma glucose concentration was measured using the oxidase method (CORMAY, Poland), serum insulin level was assayed by the immunoradiometric method (Biosource Europe SA, Belgium), and glycated haemoglobin (HbA1c) was evaluated by a high performance liquid chromatography technique (BIO-RAD Laboratories, Germany). Serum OC level was measured using commercial immunoassay (Immunodiagnostic System Ltd., UK). Serum OPG and sRANKL concentrations were also estimated by immunoassays (Biomedica, Austria). Serum leptin was measured using a commercial ELISA kit (Millipore Corporation, USA), serum adiponectin was measured using radioimmunoassay (Millipore Corporation, USA) and a HOMA2 (Homeostasis Model Assessment 2) Calculator was used to assess insulin resistance (HOMA-IR), according to the updated HOMA2 model ([www.OCDem.ox.ac.uk](http://www.OCDem.ox.ac.uk)).

**Tissue samples.** Subcutaneous adipose tissue adjacent to the lower abdominal incision, visceral (omental) adipose tissue and placental tissue from the central part of cotyledon, were obtained from 23 women with GDM and 23 subjects with NGT (Group 2) who delivered healthy, singleton infants at term, undergoing elective Caesarean section. Tissue samples were immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until assayed.

**RNA extraction and cDNA synthesis.** Total RNA was isolated and purified using Rneasy Lipid Tissue Midi Kit (Qiagen, Germany) following the manufacturer's protocol. RNA integrity was verified by 1.5% agarose gel electrophoresis/ethidium bromide staining and  $\text{OD}_{260/280}$  absorption ratio  $>1.95$ . One microgram of total RNA was used to prepare cDNA. All RNA samples were treated with DNase (Qiagen Germany) in order to remove a putative contamination with genomic DNA. cDNA synthesis was performed in the MJ Research Thermal Cycler (Model PTC-200, USA), using SuperScript<sup>TM</sup> First-Strand Synthesis System for RT-PCR (Invitrogen, USA) according to the manufacturer's instructions.

**Quantitative real-time RT-PCR (qPCR).** The expression of OC, OPG, OPG receptor, adiponectin, adiponectin receptors ADIPOR1 and ADIPOR2, leptin, interleukin-6

(IL-6), omentin and resistin mRNA were measured by qPCR, using commercially designed primers (Qiagen, Germany, [www1.qiagen.com/GeneGlobe/QTPrimerView](http://www1.qiagen.com/GeneGlobe/QTPrimerView)). 18S rRNA was used as a housekeeping gene (Qiagen, Germany). qPCR was performed in duplicates, in a volume of 20  $\mu\text{l}$ , using the QuantiTect SYBR Green PCR Master Mix (Qiagen, Germany) and carried out in a Chromo4 Real-Time PCR Detector (BIO-RAD, USA). The thermal cycling conditions included an initial activation step at  $95^{\circ}\text{C}$  for 15 min, followed by 40 cycles of 15s at  $94^{\circ}\text{C}$ , 30s at  $55^{\circ}\text{C}$  and 30s at  $72^{\circ}\text{C}$ . At the end of amplification phase, a melting curve analysis of the product formed was carried out in order to confirm the purity of each amplified product. A standard curve using serial dilutions of control cDNA was prepared for each amplicon to enable the assessment of qPCR efficiency.

**Statistical analysis.** The differences in the analyzed genes expression between the groups studied were calculated by REST (Relative Expression Software Tool) 2009, taking into account qPCR efficiency and fold changes of  $C_T$  (threshold cycle) values relative to the control [11]. Other data were analyzed by the STATISTICA 10.0 for Windows Software (StatSoft, Inc, Tulsa, USA). Before analysis, data were tested for normality of distribution using the Shapiro-Wilk test. Differences between the groups were compared by Mann-Whitney U test, and relationships between variables were tested by Spearman's rank correlations. One-way ANOVA with Bonferroni correction was used to compare the changes in the analyzed markers of bone metabolism during and after pregnancy, as well as their expression in various tissues. Multiple regression analysis was applied to establish the variables independently related to OC and OPG serum concentrations and their mRNA expression. P value less than 0.05 was regarded as statistically significant.

## RESULTS

**Clinical characteristics of the groups studied.** The patients with GDM from Group 1 had significantly higher fasting glucose level ( $p=0.005$ ) than the women with NGT. Three months after childbirth, the subjects with previous GDM had lower BMI ( $p=0.03$ ) but higher triglyceride concentration ( $p=0.02$ ) than the women with NGT (Tab. 1). A significant decrease in serum leptin level was also observed postpartum in the subjects with GDM ( $p=0.0009$ ) and NGT ( $p=0.005$ ), compared with the values noted during pregnancy (Tab. 1). The patients with GDM from Group 2 had significantly higher HbA1c ( $p=0.001$ ) and lower serum adiponectin concentration ( $p=0.04$ ), compared with the healthy pregnant women (Table 1).

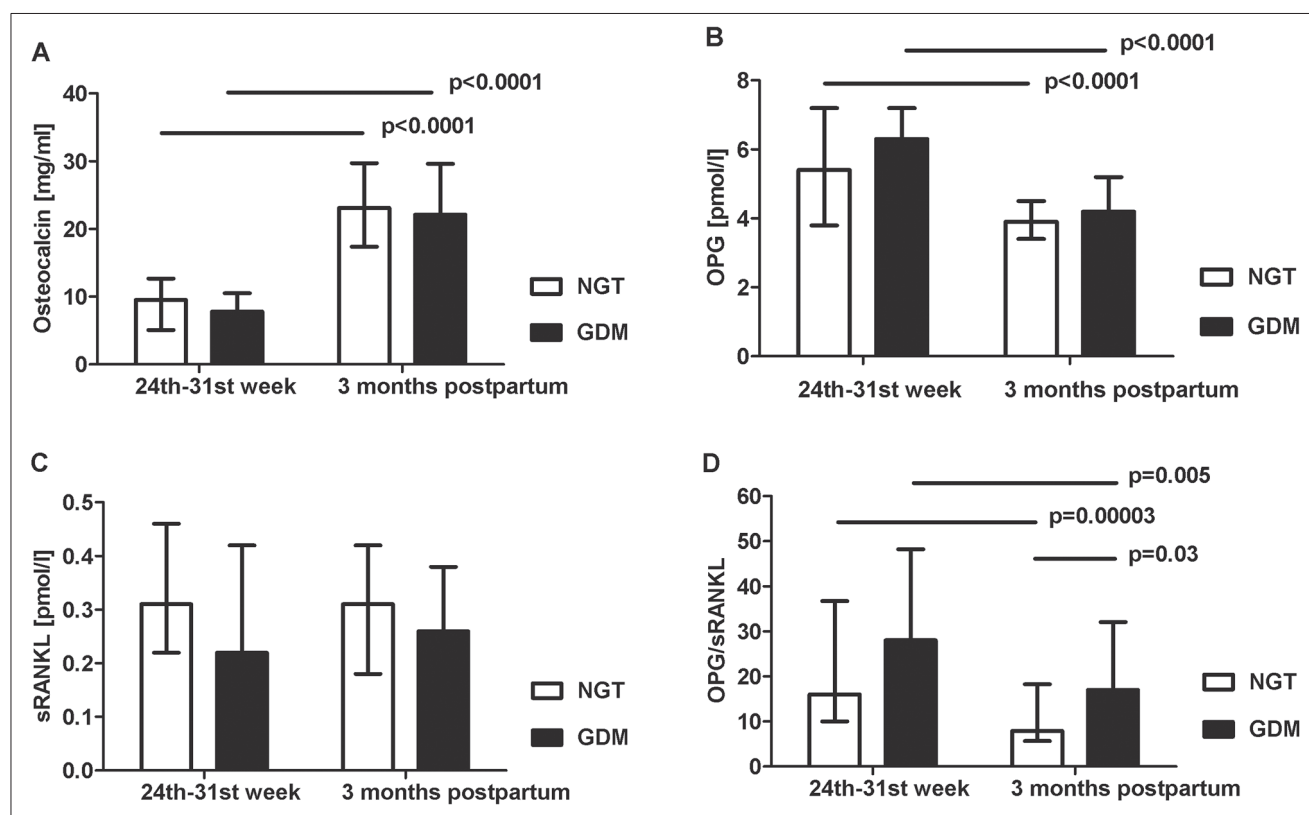
**Serum concentrations of OC, OPG and sRANKL (Group 1).** Serum OC, OPG and sRANKL concentrations did not differ significantly between the patients with and without GDM, both during and after pregnancy; however, the OPG/sRANKL ratio was markedly higher in the subjects with GDM, especially postpartum ( $p=0.03$ ) (Fig.1). Three months after childbirth, OC levels increased ( $p<0.0001$ ), whereas OPG concentrations decreased significantly ( $p<0.0001$ ) in comparison with the values observed during pregnancy. There was also a trend towards higher sRANKL levels after childbirth, but the difference was not significant (Fig. 1).



**Table 1.** Clinical and biochemical characteristics of the groups studied

	Group 1				Group 2	
	24th-32nd week		3 months postpartum		38th-40th week	
	NGT	GDM	NGT	GDM	NGT	GDM
n	30	49	30	49	23	23
Age (years)	29 (27-33)	30.5 (26-33)	30 (27-33)	31 (27-34)	30 (23-34)	32 (25-34)
Parity	2 (1-2)	2 (1-3)	2 (1-2)	2 (1-3)	1 (1-3)	2 (1-3)
Gestational age (week)	28 (25-31)	28 (25-31)			39 (38-40)	38 (37-40)
Prepregnancy BMI (kg/m <sup>2</sup> )	25.6 (22.5-30.5)	23.6 (20.5-27.0)	25.6 (22.5-30.5)	23.6 (20.5-27.0)	26.0 (22.7-29.7)	26.8 (23.8-28.0)
Current BMI (kg/m <sup>2</sup> )	30.1 (27.2-34.4)	28.8 (25.4-30.9)	26.6 (23.5-31.8)	23.4 (21.5-25.7) <sup>c</sup>	32.7 (28.3-34.9)	31.7 (29.3-36.0)
Birth weight (g)			3450 (3200-3750)	3620 (3350-3850)	3350 (2900-4000)	3650 (3000-4000)
Fasting glucose (mmol/l)	4.3 (4.1-4.6)	4.8 (4.3-5.1) <sup>b</sup>	4.5 (4.2-4.8)	4.6 (4.2-4.9)	4.5 (4.2-4.9)	4.8 (4.4-5.0)
Fasting insulin (pmol/l)	99.3 (79.9-131.3)	100 (67.4-127.8)	77.1 (52.8-94.5)	73.6 (61.1-90.3)	92.5 (59.9-126.9)	98.7 (74.6-139.1)
HOMA-IR	2.7 (2.2-3.9)	3.3 (2.0-4.2)	2.3 (1.5-2.7)	2.2 (1.7-2.7)	2.6 (1.6-3.8)	2.9 (2.3-4.3)
HbA1c (%)	4.9 (4.6-5.3)	5.1 (4.8-5.4)	5.2 (4.9-5.4)	5.2 (4.9-5.4)	4.8 (4.5-4.9)	5.1 (4.9-5.6) <sup>a</sup>
Total cholesterol (mmol/l)	6.0 (4.7-7.1)	5.8 (4.9-6.8)	4.6 (4.3-5.0)	5.4 (4.4-6.1)	6.0 (5.1-7.1)	5.7 (5.2-6.0)
HDL-cholesterol (mmol/l)	1.5 (1.4-1.7)	1.5 (1.4-1.7)	1.4 (1.3-1.6)	1.4 (1.3-1.6)	1.35 (1.3-1.5)	1.3 (1.3-1.45)
LDL-cholesterol (mmol/l)	3.8 (2.9-4.6)	3.1 (2.0-4.1)	2.7 (2.4-2.9)	3.0 (2.2-3.5)	3.1 (2.9-3.3)	2.9 (2.5-3.1)
Triglycerides (mmol/l)	2.2 (1.0-2.3)	2.2 (1.6-2.7)	0.7 (0.5-0.9)	1.0 (0.8-1.4) <sup>c</sup>	2.3 (2.0-2.9)	2.7 (2.3-3.2)
Leptin (ng/l)	22.9 (15.6-33.7)	23.0 (14.1-28.5)	14.7 (9.5-28.2)	15.2 (5.9-22.5) <sup>c</sup>	9.1 (4.6-18.5)	7.2 (4.6-9.5)
Adiponectin (ng/ml)	22.2 (15.1-30.7)	18.9 (13.6-31.0)	26.7 (21.3-39.8)	21.9 (16.1-30.7)	35.0 (28.0-45.0)	27.3 (23.6-40.3) <sup>c</sup>

Data are shown as medians (interquartile range), NGT, normal glucose tolerance, GDM, gestational diabetes mellitus, the differences between NGT and GDM groups were significant at <sup>a</sup>p<0.001, <sup>b</sup>p<0.01 and <sup>c</sup>p<0.05 by Mann-Whitney U test.



**Figure 1.** Serum concentrations of osteocalcin (A), osteoprotegerin (OPG) (B), soluble nuclear factor- $\kappa$ B ligand (sRANKL) (C) and OPG/sRANKL ratio (D) in the women with gestational diabetes (GDM) and normal glucose tolerance (NGT)

**Correlations between serum OC, OPG, sRANKL and other variables (Group 1).** Three months postpartum in the women with and without GDM, OC concentration correlated negatively with BMI ( $R=-0.61$ ;  $p=0.04$  and  $R=-0.64$ ;  $p=0.03$ , respectively). In the patients with previous GDM, serum OC was also associated with fasting insulin ( $R=-0.34$ ;  $p=0.04$ ). In the same group, sRANKL level correlated negatively with HOMA-IR ( $R=-0.34$ ;  $p=0.04$ ), whereas the OPG/sRANKL ratio was positively associated with HbA1c ( $R=0.33$ ;  $p=0.04$ ), fasting insulin ( $R=0.35$ ;  $p=0.03$ ) and HOMA-IR ( $R=0.34$ ;  $p=0.04$ ). Multiple regression analysis with OC as the dependent variable and the presence of GDM, age, gestational age, BMI, glucose, insulin, total and HDL-cholesterol and triglyceride concentrations as quantitative variables showed that serum OC level was related to the presence of GDM ( $b=0.57$ ;  $p=0.03$ ), fasting glucose ( $b=-0.83$ ;  $p=0.003$ ) and insulin concentration ( $b=0.92$ ;  $p=0.005$ ;  $R^2=0.42$ ), whereas OPG and sRANKL levels were not associated with any of the variables studied.

**OC and OPG expression in fatty and placental tissue (Group 2).** OC mRNA expression in SAT obtained from the patients with GDM was significantly lower ( $p=0.015$ ) than in the healthy pregnant women, whereas the expression of OPG and OPG receptor mRNA in fatty and placental tissue did not differ markedly between the groups studied (Tab. 2). In the subjects with NGT, OC mRNA expression in SAT was significantly higher than in VAT ( $p=0.004$ ), whereas in the patients with GDM, OC mRNA levels in SAT and VAT were comparable. The highest expression of OC mRNA was observed in the placenta, but with large inter-individual variations. In the women with NGT, OPG mRNA expression in SAT was 6-fold higher than in VAT ( $p=0.004$ ) and 3-fold higher than in placental tissue ( $p=0.006$ ). The same trends were observed in the patients with GDM, but the differences were not significant. No differences in OPG receptor mRNA expression between fatty and placental tissue were observed in both groups studied.

**Other adipokines expression in fatty and placental tissue (Group 2).** The patients with GDM had significantly lower adiponectin mRNA expression in VAT ( $p=0.039$ ) and lower

ADIPOR2 mRNA expression in SAT ( $p=0.006$ ), as well as higher resistin mRNA ( $p=0.013$ ) and ADIPOR1 mRNA expression in placental tissue ( $p=0.03$ ) than the healthy pregnant women. No detectable amounts of adiponectin mRNA were found in placental tissue.

**Correlations between expression of OC, OPG and other adipokines (Group 2).** In the patients with GDM, OC mRNA expression in SAT correlated negatively with resistin mRNA expression ( $R=-0.63$ ,  $p=0.003$ ) and serum leptin level ( $R=-0.54$ ,  $p=0.04$ ), whereas OC mRNA expression in VAT was negatively associated with leptin mRNA expression ( $R=-0.69$ ,  $p=0.006$ ). In the same group, there were positive correlations between OPG mRNA expression and resistin mRNA expression in SAT and VAT ( $R=0.54$ ;  $p=0.01$  and  $R=0.60$ ;  $p=0.006$ , respectively). Additionally, OPG mRNA expression in SAT correlated positively with IL-6 mRNA expression ( $R=0.60$ ,  $p=0.005$ ), whereas OPG mRNA expression in VAT and placental tissue was positively associated with BMI ( $R=0.43$ ,  $p=0.046$  and  $R=0.46$ ,  $p=0.03$ , respectively). In the patients with NGT, OC mRNA expression in SAT correlated positively with serum adiponectin concentration ( $R=0.52$ ,  $p=0.03$ ). In the same group there were negative correlations between OC mRNA expression and resistin mRNA expression in SAT and VAT ( $R=-0.70$ ;  $p=0.001$  and  $R=-0.56$ ;  $p=0.03$ , respectively). Additionally, OPG mRNA expression in SAT correlated positively with resistin mRNA expression ( $R=0.53$ ,  $p=0.02$ ), and negatively with adiponectin mRNA ( $R=-0.62$ ;  $p=0.006$ ) and ADIPOR1 mRNA expression ( $R=-0.72$ ,  $p=0.005$ ). In both groups – with and without GDM – there were also positive correlations between OPG receptor mRNA and omentin mRNA expression in VAT ( $R=0.80$ ;  $p=0.0005$  and  $R=0.68$ ;  $p=0.03$ , respectively).

Multiple regression analysis revealed that OC mRNA expression in SAT was related to serum adiponectin level ( $b=0.52$ ;  $p=0.01$ ;  $R^2=0.32$ ) and conversely, serum adiponectin concentration was significantly associated with OC mRNA expression in SAT ( $b=0.49$ ;  $p=0.03$ ), as well as with gestational age ( $b=0.36$ ;  $p=0.04$ ), glucose ( $b=-0.51$ ;  $p=0.004$ ), insulin ( $b=-0.36$ ;  $p=0.01$ ), total cholesterol ( $b=-0.76$ ;  $p=0.003$ ) and HDL-cholesterol concentration ( $b=0.40$ ;  $p=0.01$ ), together explaining 73% of its variability. Regression analysis also showed that insulin concentration was the only variable influencing OC mRNA expression in VAT ( $b=0.60$ ;  $p=0.003$ ;  $R^2=0.32$ ), whereas OC mRNA expression in placental tissue was not associated with any of the variables analyzed (presence of GDM, age, BMI, glucose, insulin, lipids, adiponectin and leptin concentration). OPG mRNA expression in SAT was significantly predicted by BMI ( $b=-0.52$ ;  $p=0.04$ ), whereas its expression in VAT and placental tissue was related to serum insulin ( $b=0.56$ ;  $p=0.03$ ;  $R^2=0.06$  and  $b=0.56$ ;  $p=0.03$ ;  $R^2=0.61$ , respectively). None of the variables studied was associated with OPG receptor mRNA expression in fatty and placental tissue.

## DISCUSSION

Recent studies have shown that osteoblasts and adipocytes are derived from a common subpopulation of mesenchymal stem cell progenitors, and that osteoblast-derived proteins such as OC and OPG, considered to be bone turnover markers exclusively secreted by bone tissue, are also expressed and

**Table 2.** Fold changes of relative gene expression in subcutaneous adipose tissue, visceral adipose tissue and placental tissue from women with gestational diabetes in comparison with healthy pregnant women

Gene	SAT		VAT		Placenta	
	ExpR	p-value	ExpR	p-value	ExpR	p-value
Osteocalcin	0.592	<b>0.015</b>	0.999	0.998	1.399	0.066
OPG	0.871	0.759	1.241	0.520	1.356	0.256
OPG receptor	0.819	0.323	0.916	0.849	0.834	0.487
Adiponectin	0.782	0.543	0.369	<b>0.039</b>	ND	
ADIPOR1	0.993	0.991	0.700	0.456	2.183	<b>0.030</b>
ADIPOR2	0.326	<b>0.006</b>	0.624	0.340	1.549	0.210
Leptin	1.30	0.57	0.70	0.69	0.59	0.43
Omentin	1.171	0.80	1.054	0.941	0.913	0.724
Resistin	1.594	0.356	0.980	0.955	1.881	<b>0.013</b>
IL-6	1.700	0.293	1.316	0.659	0.708	0.362

Relative gene expression of the respective gene in the healthy pregnant group = 1, ExpR, expression ratio; SAT, subcutaneous adipose tissue, VAT, visceral adipose tissue, OPG, osteoprotegerin, ADIPOR, adiponectin receptor, IL-6, interleukin-6, ND, not detected, differences between groups were calculated by REST 2009 Software



secreted by human adipocytes [6, 7, 8, 9, 10]. However, the crosstalk between these new and other traditional adipokines remains unclear. The presented study revealed that the presence of GDM was associated with a significant decrease in OC mRNA expression in SAT, as well as markedly lower *adiponectin* mRNA expression in VAT and lower circulating adiponectin level. Since multiple regression analysis revealed that OC mRNA expression in SAT significantly influences serum adiponectin concentration, it can be hypothesized that decreased OC expression in the GDM subjects may result in a diminished pool of active OC, and in a reduced stimulatory effect on adiponectin expression and secretion by adipose tissue. The current study also showed that in the patients with both NGT and GDM, OC mRNA expression in SAT was negatively associated with *resistin* mRNA expression. An inverse correlation between circulating OC and resistin – a potential mediator linking inflammation with metabolic disease – had been previously demonstrated in overweight children [12] and elderly patients [13], but their crosstalk in fatty tissue is still unknown. Another adipokine which might have an impact on adipocyte-derived OC, seems to be leptin. Experimental models have shown that enhanced expression of leptin in the hypothalamus increased blood osteocalcin [14], whereas leptin up-regulation of sympathetic signaling in osteoblasts resulted in a decrease in OC bioactivity [15]. The obtained results suggest a negative association between leptin and fat tissue OC expression in the women with GDM; however, further investigations are needed to better understand the precise relationship(s) between these two adipokines.

In contrast to OC, the expression of *OPG* and *OPG receptor* mRNA in fatty and placental tissue did not differ significantly between the patients with and without GDM. The study revealed that in the GDM subjects, *OPG* mRNA expression in SAT was positively associated with *resistin* and *IL-6* mRNA expression, whereas in the women with NGT, *OPG* mRNA expression in SAT correlated positively with *resistin* mRNA, but negatively with *adiponectin* and *ADIPOR1* mRNA expression. In both groups, there were also positive correlations between *OPG receptor* mRNA and *omentin* mRNA expression in VAT.

The results of the presented study seem to be in line with experimental studies which have shown that insulin decreases *OPG* mRNA expression in cultured adipocytes [7], whereas proinflammatory cytokines, such as interleukin-1 $\beta$  and tumor necrosis factor- $\alpha$ , significantly increase the expression of *OPG* in SAT [16]. Moreover, it has been demonstrated that adiponectin suppresses, whereas omentin up-regulates *OPG* expression in osteoblasts [17]; however, the influence of these adipokines on *OPG* expression in adipose tissue remains an open question. It is also noteworthy that in the current study *OPG* mRNA expression in SAT was significantly predicted by BMI, which seems consistent with the finding of higher *OPG* mRNA expression in SAT of obese patients, compared with lean individuals [8].

The presented study did not show significant differences in serum OC concentrations between the patients with and without GDM. In contrast, Winhofer et al. [5] found significantly higher circulating OC in patients with GDM, compared with healthy pregnant women. This discrepancy may result from different diagnostic criteria and different characteristics of the groups studied. However, both studies showed that three months after childbirth, serum

OC concentrations were markedly higher than in the 3rd trimester, which is in line with other reports demonstrating that the OC level decreases in early pregnancy, then increases until delivery, and peaks after childbirth [18]. The reason for a decline in circulating OC in pregnancy is unclear, but it can be hypothesized that pregnancy-induced insulin resistance in bone tissue might result in a decrease in active OC level, with a concomitant inhibition of a direct pro-resorptive effect of insulin and an increase in the expression of *OPG* [3]. Indeed, an elevation of circulating OPG in pregnant women in comparison with the postpartum period was observed in the presented study, as well as in other studies carried out on healthy pregnant subjects [19, 20]. However, it is also possible that this increase might be due to a stimulatory effect of estrogens [21] and an additional secretion of OPG by the placenta.

In the current study, serum OPG and sRANKL concentrations did not differ significantly between the patients with and without GDM, but three months after childbirth the OPG/sRANKL ratio was markedly higher in the GDM subjects, suggesting a better protection against an excessive bone loss, especially in the women with higher HOMA-IR. Other authors also found no significant differences in circulating OPG between patients with and without a history of GDM, both three months [20] and several years after childbirth [22, 23], although elevated OPG levels were observed in women with prior GDM diagnosed as having the metabolic syndrome [24]. Moreover, serum OPG has been proposed as a promising biomarker of atherosclerosis in diabetic patients; however, a causal relationship between increasing OPG and the development of vascular lesions is still an open question [25].

Since available results suggest that *OPG* expression might be related to the amount of fatty tissue [8], the lack of differences in circulating OPG between the subjects with and without GDM, found in this study, may be explained by relatively low BMI values in the group with prior GDM, probably as a result of an effective treatment.

In conclusion, the results obtained suggest that decreased OC mRNA expression in SAT might result in a reduced stimulatory effect on adiponectin expression and unfavourable metabolic profile in the subjects with GDM. However, the contribution of fatty tissue derived OC to the pool of active osteocalcin needs further investigations. On the other hand, the higher OPG/sRANKL ratio, observed in the women with prior GDM, suggests a decreased sensitivity to the pro-resorptive action of insulin and a better protection against an excessive bone loss.

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