Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 742 – 747
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Osteoprotegerin in relation to type 2 diabetes mellitus and the metabolic syndrome in postmenopausal women

Iraj Nabipour[a,*], Mohammadreza Kalantarhormozi[a], Bagher Larijani[b], Majid Assadi[a], Zahra Sanjdideh[a]

[a]*Department of Endocrine and Metabolic Diseases, The Persian Gulf Tropical Medicine Research Center, Bushehr University of Medical Sciences, Bushehr, 7514763448 Iran*
[b]*Tehran Endocrine Research Center, Tehran University of Medical Sciences, Tehran, 1417863181 Iran*

Received 24 August 2009; accepted 18 September 2009

Abstract

Osteoprotegerin (OPG) is an inhibitor of bone resorption. Circulating levels of OPG seem to be elevated in patients with cardiovascular disorders and diabetes. The relationship between OPG and the metabolic syndrome has never been studied in postmenopausal women. In a population-based study, 382 Iranian postmenopausal women were randomly selected. Cardiovascular risk factors, high-sensitivity C-reactive protein, and OPG were measured. The diabetes classification and the metabolic syndrome definition were based on the criteria of the American Diabetes Association and the National Cholesterol Education Program–Adult Treatment Panel III, respectively. The mean serum OPG level was higher in those with type 2 diabetes mellitus than those without diabetes (4.33 ± 1.70 vs 3.84 ± 1.76 pmol/L, $P = .016$). In multiple logistic regression analysis, type 2 diabetes mellitus showed a significant association with serum OPG levels when adjustments were made for age, high-sensitivity C-reactive protein, and cardiovascular risk factors (odds ratio = 2.21; confidence interval, 1.34-3.66; $P = .002$). No significant difference was found between the mean serum OPG levels of those with the metabolic syndrome and those without the metabolic syndrome. Mean OPG levels did not differ significantly between subjects with and without hypertension, dyslipidemia, glucose intolerance, or abdominal obesity according to the National Cholesterol Education Program–Adult Treatment Panel III criteria. In conclusion, circulating OPG levels are significantly associated with diabetes, independent of cardiovascular risk factors in postmenopausal women. However, OPG levels have no correlation with the metabolic syndrome or its components. Further studies are warranted to determine the pathophysiologic origin of elevated OPG in type 2 diabetes mellitus.

1. Introduction

Osteoprotegerin (OPG) is a secreted glycoprotein belonging to the tumor necrosis factor receptor superfamily and acts as a blocking receptor by binding to the receptor activator of nuclear factor–κB ligand and preventing it from binding to the receptor activator of nuclear factor–κB [1]. As a result, bone resorption is inhibited.

Emerging evidence from in vitro studies and mouse genetics indicates that OPG is not merely a protective factor for bone but may, in fact, act as a protective factor for the vascular system [2]. Paradoxically, however, clinical studies suggest that serum OPG levels increase in association with vascular calcification, coronary artery disease, stroke, and future cardiovascular events [3]. Taken together, some data suggest that OPG is induced by atherosclerosis and may be up-regulated as an incomplete compensatory response to vessel insult, possibly limiting vascular calcification [4].

Elevated levels of plasma OPG were reported in newly diagnosed type 2 diabetes mellitus patients, and these levels were associated with endothelium-dependent arterial dilation [5]. In a large observational study in elderly women, plasma concentrations of OPG were higher in diabetic than nondiabetic subjects [6]. Osteoprotegerin had an independent association with asymptomatic coronary artery disease

* Corresponding author. The Persian Gulf Tropical Medicine Research Center, Boostan 19 Alley, Imam Khomeini St, Bushehr, IR Iran. Tel.: +98 771 2541827; fax: +98 771 2541828.
E-mail address: inabipour@gmail.com (I. Nabipour).

0026-0495/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.09.019

in type 2 diabetes mellitus patients [7], and its plasma levels predicted both subclinical atherosclerosis and near-term cardiovascular events in uncomplicated type 2 diabetes mellitus subjects [8]. In some more recent studies in diabetic subjects, a strong association between circulation OPG and microvascular complications [9], urinary albumin excretion [10], and neuropathy [11] was observed. Serum OPG also was associated with carotid intima-media thickness in women with previous gestational diabetes [12].

There are very limited data about the relationship between OPG and the metabolic syndrome [13,14]. In a cohort of patients with peripheral artery disease, serum concentrations of OPG were elevated in patients with obesity and the metabolic syndrome [14]. In contrast, in an aging male population, there was no statistical difference in OPG values between men with or without the metabolic syndrome [13].

However, to date, no data are available on the relationship between OPG and the metabolic syndrome in postmenopausal women. Therefore, the main objective of this population-based study is to investigate this association in a large sample of postmenopausal women.

2. Subjects and methods

2.1. Community sampling and physical examinations

The study design has been described previously [15]. Nevertheless, in brief, a total of 382 postmenopausal women who participated in the extension part of the Iranian Multicentral Osteoporosis Study were evaluated in April 4 to September 22, 2006. The mean age (mean ± SD) of the women was 58.78 ± 7.8 years (range, 50-83 years). They were randomly selected from 13 clusters in Bushehr port (the center of Bushehr province, which has the longest border with the Persian Gulf). All were community dwelling and ambulatory.

Blood pressure was assessed twice at the right arm after a 15-minute rest in the sitting position using a standard mercury sphygmomanometer. A stadiometer was used to measure height and weight. Heavy outer garments and shoes were removed before the participants' height and weight were measured. Body mass index (BMI) was calculated. Waist circumference was defined at the midway level between the costal margins and the iliac crests. Hip circumference was measured at the level of the greater trochanters.

Using the American Diabetes Association criteria, a fasting plasma glucose of 126 mg/dL or greater or use of antidiabetic measures was defined as *diabetes* [16].

The cutoff points of serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) and serum triglycerides (TG) distributions used to assign subjects to different levels of risk were those derived from the National Cholesterol Education Program (NCEP) guidelines in the United States (Adult Treatment Panel [ATP] III, September 2002) [17]. A subject was considered hypertensive if blood pressure was at least 140/90 mm Hg or centrally obese if the waist-to-hip ratio was at least 0.9.

The metabolic syndrome was diagnosed with the criteria indicated by the NCEP-ATP III [17]. According to these criteria, subjects with the metabolic syndrome are those with any combination of 3 or more of the following risk determinants: fasting plasma glucose of at least 110 mg/dL, blood pressure of at least 130/85 mm Hg or antihypertensive treatment, plasma TG of at least 150 mg/dL, HDL-C less than 50 mg/dL, and waist circumference of at least 88 cm in women.

2.2. Laboratory measurements

A fasting blood sample was taken. All samples were promptly centrifuged and separated, and analyses were carried out at the Persian Gulf Health Research Center on the day of blood collection using a Selectra 2 autoanalyzer (Vital Scientific, Spankeren, the Netherlands). Glucose was assayed by the enzymatic (glucose oxidase) colorimetric method using a commercial kit (Pars Azmun, Tehran, Iran). Serum TC and HDL-C were measured using a cholesterol oxidase phenol aminoantipyrine method, and TG was measured using a glycerol-3 phosphate oxidase phenol aminoantipyrine enzymatic method. Serum LDL-C was calculated using the Friedewald formula; LDL-C was not calculated when the TG concentration was greater than 400 mg/dL.

Measurement of C-reactive protein (CRP) by a high-sensitivity (hs) CRP assay, CRP HS enzyme-linked immunosorbent assay (ELISA) (DRG International), was conducted. The minimum detectable concentration of the CRP HS ELISA assay was estimated to be 0.1 mg/L. In addition, the functional sensitivity was determined to be 0.1 mg/L (as determined with interassay coefficient of variation <20%).

Serum OPG levels were measured using an ELISA commercial kit (Biomedica Gruppe, Vienna, Austria). The detection limit of the assay was 0.14 pmol/L. The mean intra- and interassay coefficients of variation of the OPG assay were 4% to 10% and 7% to 8%, respectively.

2.3. Statistical analysis

A 2-tailed *t* test was used to compare the mean values across groups. We found that log transformation of CRP gave a better fit to a Gaussian distribution. The *geometric mean for CRP* was defined as the arithmetic mean of the log-transformed data ±2 SD, raised to the power of 10.

Pearson correlation analysis was used to study the relationships between OPG values and the anthropometric and biochemical variables. Partial correlation analysis was performed to assess the association between OPG measurements and biochemical variables, with adjustment for age and BMI.

Multiple logistic regression analysis was used to ascertain the associations between type 2 diabetes mellitus and

elevated serum OPG levels. Age, high LDL-C, low HDL-C, hypertension, central obesity, elevated CRP (>3 mg/L), and elevated serum OPG levels (values greater than the median) were considered as covariates; and diabetes, as the dependent variable. $P < .05$ was considered statistically significant. Statistical analysis was performed with an IBM computer using the SPSS 9.05 statistical software package (SPSS, Chicago, IL).

3. Results

The characteristics of the study participants according to their metabolic status are shown in Table 1. Of the studied population, 102 subjects (26.7%) had type 2 diabetes mellitus; and 261 women (68.32%) had the metabolic syndrome (NCEP-ATP III criteria). The mean ± SD serum OPG concentration for all 382 postmenopausal women was 3.97 ± 1.75 pmol/L (median, 3.60 pmol/L). The mean serum OPG level was higher in those with type 2 diabetes mellitus than those without diabetes (4.33 ± 1.70 vs 3.84 ± 1.76 pmol/L, $P = .016$).

Bivariate correlation analysis showed a correlation between OPG and age ($r = 0.40$, $P < .0001$), BMI ($r = -0.13$, $P = .007$), and fasting blood glucose ($r = 0.12$, $P = .016$). However, no significant correlations were found between OPG and LDL-C or HDL-C, TG, systolic or diastolic blood pressure, log hs-CRP levels, or waist-to-hip ratio in postmenopausal women (Table 2).

In the total population, a positive correlation between serum OPG level and fasting blood glucose persisted after adjustment for age and BMI ($r = 0.11$, $P = .022$).

In the multiple logistic regression analysis to ascertain the association between type 2 diabetes mellitus and hs-CRP, OPG, and cardiovascular risk factors, higher serum OPG levels were independently associated with diabetes (odds ratio [OR] = 2.21; confidence interval [CI], 1.34-3.66; $P = .002$) (Table 3).

Table 2
Bivariate correlation analysis between cardiovascular risk factors and serum OPG levels in postmenopausal women

	Bivariate correlation	
Parameters	*r*	*P*
Age	0.407	<.0001
Waist circumference	−0.086	.093
BMI	−0.137	.007
Waist-to-hip ratio	0.009	.051
Systolic blood pressure	0.061	.239
Diastolic blood pressure	−0.022	.664
Fasting glucose	0.123	.016
TC	−0.069	.178
LDL-C	−0.057	.264
HDL-C	−0.014	.780
log hs-CRP	0.046	.374

Correlation coefficients and *P* values were calculated using Pearson correlation analysis.

No significant difference was found between the mean serum OPG levels of those with the metabolic syndrome and those without the metabolic syndrome (Table 1).

Mean OPG levels were compared between the 2 groups divided by each component of the metabolic syndrome (Table 4). Mean OPG levels did not differ significantly between subjects with and without hypertension, dyslipidemia, glucose intolerance, or abdominal obesity (Table 4). The metabolic syndrome did not show a significant association with serum OPG levels (OR = 0.92; CI, 0.58-1.45; $P = .739$) after age was adjusted for.

4. Discussion

In this population-based study, there was no association between circulating OPG levels and the metabolic syndrome using the NCEP-ATP III criteria. On the other hand, serum OPG levels were significantly higher in type 2 diabetes mellitus subjects than in healthy postmenopausal women.

Table 1
Characteristics of 382 postmenopausal women, stratified by healthy–type 2 diabetes mellitus status and by healthy–metabolic syndrome status

	Healthy (n = 280)	Type 2 diabetes mellitus (n = 102)	*P*	Healthy (n = 121)	Metabolic syndrome (n = 261)	*P*
Age, y	58.47 ± 8.07	59.67 ± 7.01	.185	57.80 ± 7.35	59.24 ± 7.97	.090
Waist circumference, cm	98.63 ± 10.52	100.48 ± 0.86	.134	94.86 ± 12.09	101.11 ± 9.39	<.0001
BMI, kg/m^2	28.36 ± 5.11	28.01 ± 4.46	.530	27.26 ± 5.76	28.75 ± 4.50	.008
Waist-to-hip ratio	0.92 ± 0.07	0.94 ± 0.05	.010	0.89 ± 0.07	0.93 ± 0.06	<.0001
Systolic blood pressure, mm Hg	125.61 ± 18.25	127.76 ± 2.8	.345	119.93 ± 16.03	128.95 ± 20.46	<.0001
Diastolic blood pressure, mm Hg	78.73 ± 10.57	78.97 ± 10.97	.849	75.55 ± 8.77	80.23 ± 11.12	<.0001
Fasting glucose, mg/dL	93.50 ± 13.56	176.45 ± 69.49	<.0001	95.06 ± 34.31	125.30 ± 56.87	<.0001
TC, mg/dL	235.93 ± 47.84	232.54 ± 48.17	.542	224.60 ± 47.06	239.58 ± 47.90	.005
LDL-C, mg/dL	159.09 ± 43.06	152.59 ± 43.44	.194	152.05 ± 42.66	159.71 ± 43.56	.115
HDL-C, mg/dL	42.04 ± 10.71	38.08 ± 9.07	.001	48.99 ± 10.11	37.39 ± 8.56	<.0001
TG, mg/dL	174.03 ± 93.75	209.48 ± 98.70	.001	117.96 ± 32.28	212.45 ± 100.15	<.0001
log hs-CRP, mg/L	0.251 ± 0.446	0.348 ± 0.470	.066	0.166 ± 0.465	0.329 ± 0.441	.001
OPG, pmol/L	3.85 ± 1.76	4.33 ± 1.70	.016	3.91 ± 1.64	4.01 ± 1.81	.624

Data are given as means ± SD.

Table 3
Odds ratio and 95% CI relating type 2 diabetes mellitus as dependent variable, and OPG and associated risk factors as independent parameters in postmenopausal women

	OR	95% CI	*P*
Low HDL-C	1.77	1.09-2.88	.020
High LDL-C	0.93	0.57-1.52	.791
Hypertension	0.95	0.53-1.69	.861
Age	0.99	0.96-1.02	.823
Waist-to-hip ratio	2.23	1.26-3.95	.005
High OPG	2.21	1.34-3.66	.002
hs-CRP	1.35	0.82-2.22	.231

Low HDL-C (<40 mg/dL), high LDL-C (≥160 mg/dL), hypertension (≥140/90 mm Hg), waist-to-hip ratio (≥0.9), high OPG (≥median), high CRP (≥3 mg/L).

As far as we know, this is the second largest study on the relationship between serum OPG and diabetes in postmenopausal women. Browner et al [6] first reported an association of serum OPG levels with diabetes in a prospective cohort of elderly women. Consistent with our results, circulating OPG levels were found to be increased in patients with type 2 diabetes mellitus with or without complications [5-11].

Both vascular smooth muscle cells and endothelial cells have been shown to produce OPG in vitro, and vascular cells likely significantly contribute to circulating OPG levels measured in serum [18]. Olsen et al [19] reported that the content of OPG was increased in aortic tunica media samples from diabetic individuals. Emerging evidence has demonstrated a link between OPG and vasculopathy, in particular in inhibiting arterial calcification commonly observed in atherosclerosis [1]. Plasma OPG levels increased with the initiation of an atherogenic diet in low-density lipoprotein receptor-deficient mice, yet treatment with OPG resulted in a reduction of vascular calcification [4]. These data support a role for OPG in vasculature as an inhibitor of calcification and a marker, rather than a mediator, of atherosclerosis [4].

Table 4
The differences in OPG values according to the presence or absence of the metabolic syndrome components in postmenopausal women

	N = 382	OPG, pmol/L	*P* value
Waist circumference (cm)			
≥88	333	3.50 ± 1.73	.474
<88	49	4.14 ± 1.89	
TG (mg/dL)			
≥150	208	4.07 ± 1.93	.216
<150	173	3.85 ± 1.52	
HDL-C (mg/dL)			
<50	310	3.53 ± 1.77	.843
≥50	72	3.93 ± 1.69	
Blood pressure (mm Hg)			
≥130/85	187	4.0 ± 1.61	.801
<130/85	189	3.96 ± 1.89	
Fasting glucose (mg/dL)			
≥110	127	4.20 ± 1.64	.080
<110	254	3.86 ± 1.80	

However, it still remains to be fully elucidated that observed increases in OPG in patients with type 2 diabetes mellitus with or without complications and in our observational study are due to a compensatory (incomplete) self-defense mechanism against arterial calcification or not.

In vitro data have demonstrated that insulin down-regulates OPG expression in vascular smooth muscle cells [19]. High doses of insulin accelerated the calcification of human vascular smooth muscle cells, which may be related to insulin-induced down-regulation of the presumed calcification inhibitor OPG [20]. Insulin infusion (acute hyperinsulinemia) decreased plasma OPG, but with diminished effect in individuals with type 2 diabetes mellitus and obesity [21]. Osteoprotegerin levels decreased markedly after insulin therapy in type 1 diabetes mellitus patients [22]. These data support the hypothesis that insulin resistance (lack of insulin action and hyperinsulinemia) in type 2 diabetes mellitus may be part of the mechanism behind the accumulation of OPG.

However, the relationship between OPG and insulin resistance has been poorly studied and subject to controversy. An inverse relationship between OPG and insulin resistance was reported in an aging male population [13] and in healthy premenopausal obese women [23]. In contrast, OPG was positively correlated with insulin resistance in an obese population [24] and in men with type 2 diabetes mellitus [25].

It is now realized that insulin resistance plays a principal role in initiating and perpetuating the pathologic manifestations of the metabolic syndrome [26]. The metabolic syndrome also is associated with atheroscleropathy and vascular calcification [27]. We found that serum OPG levels were not associated with the metabolic syndrome in a large postmenopausal population. Therefore, OPG may not represent the molecular link between vascular calcification and insulin resistance in the metabolic syndrome.

To our knowledge, this study is the first to examine the relationship between circulating OPG levels and the metabolic syndrome in a population-based sample of postmenopausal women. There are only 2 previously published studies [13,14] about the relationship of the metabolic syndrome and OPG levels in the medical literature. Similar to our results, no significant difference in OPG levels was found in subjects with or without the metabolic syndrome in elderly Lebanese men [13].

There is conflicting evidence about whether there is a general relationship between circulating OPG levels and cardiovascular risk factors in type 2 diabetes mellitus subjects [5,8,9,25] and in observational studies [6,13,24,28]. In this study, serum OPG values according to the presence or absence of the metabolic syndrome criteria showed no significant differences. Obesity is viewed as a contributing factor to insulin resistance, and waist circumference may be more closely related to insulin resistance and its consequences than generalized obesity as estimated by BMI [29]. It is very surprising that we found a strong negative correlation between serum OPG and BMI, but there was no correlation

between OPG and abdominal obesity according to the waist-to-hip ratio or waist circumference.

It has been suggested that inflammation-driven hyperglycemia, rather than high glucose levels per se, is involved in the increase of OPG observed in diabetes [18]. We observed that hs-CRP levels were higher in subjects with type 2 diabetes mellitus and metabolic syndrome than in healthy postmenopausal women. However, inconsistent with previous studies [6,7], we did not find any correlation between serum OPG and hs-CRP levels. Therefore, OPG levels could not be linked to an inflammatory state in postmenopausal women.

Our study had some limitations. The primary limitation is that the participants were not uniformly screened for glucose intolerance; some cases of diabetes may have been undiagnosed. In the present study, we used the NCEP-ATP III definition for the metabolic syndrome. Although this definition is most often used, other definitions for the metabolic syndrome do exist. We did not measure free OPG levels and OPG complex forms. Another limitation of our study includes the lack of measurement of insulin resistance from fasting glucose and insulin concentrations using the homeostasis model assessment method. It has been shown that acute hyperglycemia did not increase plasma levels of OPG in healthy subjects, whereas hyperinsulinemia suppressed plasma levels of OPG [30].

In conclusion, this study shows that serum OPG levels are significantly associated with type 2 diabetes mellitus in postmenopausal women, independent of cardiovascular risk factors including hs-CRP. However, OPG levels, at least as we measured them, were not associated with the metabolic syndrome or its components. Thus, it still remains to be fully elucidated that observed increases in OPG in patients with type 2 diabetes mellitus are due to insulin resistance per se.

Therefore, further studies are warranted to determine the pathophysiologic origin of elevated OPG in patients with type 2 diabetes mellitus. By investigating OPG in relation to type 2 diabetes mellitus and the metabolic syndrome, we would gain a new perspective on understanding the pathogenesis of vascular calcification and atherosclerosis in diabetes.

Acknowledgment

This project was supported jointly by a grant from the Ministry of Health; Tehran Endocrine Research Center; Tehran University of Medical Sciences, Tehran IR Iran; and Bushehr Province Research Committee.

References

[1] Reid P, Holen L. Pathophysiological roles of osteoprotegerin (OPG). Eur J Cell Biol 2009;88:1-17.
[2] Schoppet M, Preissner KT, Hofbauer LC. Rank ligand and osteoprotegerin paracrine regulator of bone metabolism and vascular function. Arterioscler Thromb Vasc Biol 2002;22:549-53.
[3] Van Campenhout A, Golledge J. Osteoprotegerin, vascular calcification and atherosclerosis. Atherosclerosis 2009;204:321-9.
[4] Morony S, Tintyt Y, Zhang Z, et al. Osteoprotegerin inhabits vascular calcification without affecting atherosclerosis in ldlr(−/−) mice. Circulation 2008;117:411-20.
[5] Xiang GD, Xu L, Zhao LS, et al. The relationship between plasma osteoprotegerin and endothelium-dependent arterial dilation in type 2 diabetes. Diabetes 2006;55:2126-31.
[6] Browner WS, Lui LY, Cummings SR. Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women. J Clin Endocrinol Metab 2001;86:631-7.
[7] Avignon A, Sultan A, Piot C, et al. Osteoprotegerin is associated with silent coronary artery disease in high-risk but asymptomatic type 2 diabetic patients. Diabetes Care 2005;28:2176-80.
[8] Anand DV, Lahiri A, Lim E, et al. The relationship between plasma osteoprotegerin levels and coronary artery calcification in uncomplicated type 2 diabetic subjects. J Am Coll Cardiol 2006;47:1850-7.
[9] Knudsen ST, Foss CH, Poulsen PL, et al. Increased plasma concentrations of osteoprotegerin in type 2 diabetic patients with microvascular complications. Eur J Endocrinol 2003;149:39-42.
[10] Xiang GD, PU JH, Zhao LS, et al. Association between plasma osteoprotegerin concentrations and urinary albumin excretion in type 2 diabetes. Diabet Med 2009;26:397-403.
[11] Terekeci HM, Senol MG, Top C, et al. Plasma osteoprotegerin concentrations in type 2 diabetic patients and its association with neuropathy. Exp Clin Endocrinol Diabetes 2009;117:119-23.
[12] Akinci B, Demir T, Celtik A, et al. Serum osteoprotegerin is associated with carotid intima media thickness in women with previous gestational diabetes. Diabetes Res Clin Pract 2008;82:172-8.
[13] Gannage-Yared MH, Fares F, Semaant M, et al. Circulation osteoprotegerin is correlated with lipid profile, insulin sensitivity, adiponectin and sex steroids in an ageing male population. Clin Endocrinol 2006;64:652-8.
[14] Golledge J, Leicht AS, Crowther RG, et al. Determinants of endothelial function in a cohort of patients with peripheral artery disease. Cardiology 2008;111:51-6.
[15] Nabipour I, Larijani B, Beigi S, et al. Relationship among insulinlike growth factor I concentrations, bone mineral density, and biochemical markers of bone turnover in postmenopausal women: a population-based study. Menopause 2008;15:934-9.
[16] Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183-97.
[17] Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults: executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.
[18] Secchiero P, Corallini F, Pandolfi A, et al. An increased osteoprotegerin serum release characterizes the early onset of diabetes mellitus and may contribute to endothelial cell dysfunction. Am J Pathol 2006;169:2236-44.
[19] Olesen P, Ledet T, Rasmussen LM. Arterial osteoprotegerin: increased amounts in diabetes and modifiable synthesis from vascular smooth muscle cells by insulin and TNF-alpha. Diabetologia 2005;48:561-8.
[20] Olsen P, Nguyen K, Wogensen L, et al. Calcification of human smooth muscle cells: associations with osteoprotegerin expression and acceleration by high-dose insulin. Am J Physiol Heart Circ Physiol 2007;292:1058-64.
[21] Jorgensen GM, Vind B, Nybo M, et al. Acute hyperinsulinemia decreases plasma osteoprotegerin with diminished effect in type 2 diabetes and obesity. Eur J Endocrinol 2009;161:95-101.
[22] Xiang GD, Sun HL, Zhao LS. Changes of osteoprotegerin before and after insulin therapy in type 1 diabetic patients. Diabetes Res Clin Pract 2007;76:199-206.
[23] Ugur-Altun B, Altun A. Circulation leptin and osteoprotegerin levels affect insulin resistance in healthy premenopausal obese women. Arch Med Res 2007;38:891-6.

[24] Gannage-Yared MH, Yaghi C, Habre B, et al. Osteoprotegerin in relation to body weight, lipid parameters insulin sensitivity, adipocytokines, and C-reactive protein in obese and non-obese young individuals: results from both cross-sectional and interventional study. Eur J Endocrinol 2008;158:353-9.
[25] Yaturu S, Rains J, Jain SK. Relationship of elevated osteoprotegerin with insulin resistance, CRP, and TNF-alpha levels in men with type 2 diabetes. Cytokine 2008;44:168-71.
[26] Lann D, LeRoith D. Insulin resistance as the underlying cause for the metabolic syndrome. Med Clin North Am 2007;91:1063-77.
[27] Hayden MR, Tyagi SC, Kolb L, et al. Vascular ossification-calcification in metabolic syndrome, type 2 diabetes mellitus, chronic kidney disease, and calciphylaxis-calcific uremic arteriolopathy: the emerging role of sodium thiosulfate. Cardiovasc Diabetol 2005;4:4.
[28] Oh ES, Rhee EJ, Oh KW, et al. Circulation osteoprotegerin levels are associated with age, waist-to-hip ratio, serum total cholesterol, and low-density lipoprotein cholesterol levels in healthy Korean women. Metab Clin Exp 2005;54:49-54.
[29] Reaven G. The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. Endocrinol Metab Clin North Am 2004;33:283-303.
[30] Knudsen ST, Jeppesen P, Poulsen PL, et al. Plasma concentrations of osteoprotegerin during normo- and hyperglycaemic clamping. Scand J Clin Lab Invest 2007;67:135-42.