0013-7227/03/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 88(3):1024–1028

doi: 10.1210/jc.2002-020775

Increased Osteoprotegerin Serum Levels in Men with Coronary Artery Disease

MICHAEL SCHOPPET, ALEXANDER M. SATTLER, JUERGEN R. SCHAEFER, MATTHIAS HERZUM, BERNHARD MAISCH, AND LORENZ C. HOFBAUER

Departments of Internal Medicine and Cardiology (M.S., A.M.S., J.R.S., M.H., B.M.) and Gastroenterology, Endocrinology, and Metabolism (L.C.H.), Philipps University, Baldingerstrasse, D-35033 Marburg, Germany

Osteoprotegerin (OPG) regulates osteoclast and immune functions and appears to represent a protective factor for the vascular system. However, the role of OPG in human atherosclerosis has not been evaluated. In this study, we assessed OPG serum levels in 522 age-matched men who, on the basis of coronary angiography, had either absence of coronary artery disease (CAD) or presence of single-vessel disease, double-vessel disease, or severe triple-vessel disease. OPG serum levels were positively correlated with age ($r = 0.28$; $P < 0.001$) and were higher in men with diabetes mellitus ($P < 0.01$). OPG serum levels in men without CAD were 5.4 ± 2.0 pmol/liter, compared with 6.1 ± 2.1 pmol/liter in single-vessel disease ($P < 0.005$), 5.9 ± 2.4 in double-vessel disease ($P < 0.05$), and 6.3 ± 2.3 pmol/liter in triple-vessel disease ($P < 0.001$). Moreover, OPG serum levels were positively correlated with the severity of CAD as determined by a CAD scoring system ($r = 0.17$; $P < 0.01$). In conclusion, our data underline that OPG serum levels are associated with the severity of CAD and are increased in elderly men and patients with diabetes mellitus. We conclude that increased OPG serum levels may reflect advanced cardiovascular disease in men. (*J Clin Endocrinol Metab* 88: 1024–1028, 2003)

OSTEOPROTEGERIN (OPG), A MEMBER of the TNF receptor family, was found to inhibit receptor activator of nuclear factor-κB ligand (RANKL)-mediated osteoclastic bone resorption *in vitro* and *in vivo* (1–3). RANKL acts on its specific receptor, receptor activator of nuclear factor-κB, which is expressed on osteoclasts and dendritic cells (4). In addition to bone metabolism, RANKL and OPG are essential for modulation of dendritic cell functions, regulation of lymph node organogenesis, and lymphocyte development (5–7). *In vitro*, OPG appears to influence B cell development and functions (8) and may exert antiapoptotic effects by binding TNF-related apoptosis-inducing ligand, an inducer of apoptosis in susceptible cells (9).

OPG is secreted by a variety of tissues, including the cardiovascular system, where it is expressed in the heart and the vascular wall in rodents (1). OPG-deficient mice exhibit severe osteoporosis and vascular calcification of the aorta and renal arteries (10), a phenotype that can be prevented by delivery of the OPG transgene from midgestation (11). In another animal model of arterial calcification induced by warfarin or vitamin D intoxication, sc administration of OPG was able to prevent vascular lesions (12). *In vitro*, OPG prolongs endothelial cell survival by preventing apoptosis (13). However, the role of OPG in atherosclerosis has not yet been studied in humans.

We hypothesized that alterations of the OPG cytokine system may promote vascular disease in humans. In this study, we measured serum levels of OPG in 522 men who underwent coronary angiography and their correlation to the severity of coronary artery disease (CAD) and the presence of cardiovascular risk factors.

Abbreviations: CAD, Coronary artery disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor-κB ligand.

Subjects and Methods

Subjects

The study population consisted of 522 Caucasian men undergoing diagnostic coronary angiography for suspected CAD. The study was approved by the Institutional Review Board, and written informed consent was obtained from all patients. CAD was defined as narrowing of more than 50% of at least one major coronary artery, and coronary angiographies were interpreted by at least two experienced cardiologists. On the basis of these coronary angiographies, the number of affected coronary arteries was determined. In addition, the severity of CAD was determined using a modified Gensini score as previously reported (14).

These men were a subset from a well defined cohort of patients that was initiated to optimize strategies for the prevention of CAD (15). Data available for each patient included coronary angiography findings and detailed cardiovascular risk profiles. Patients with malignancies, osteoporosis, and renal disease (creatinine levels > 2.0 mg/dl) and patients receiving systemic glucocorticoids or immunosuppressants were excluded from the study.

Measurements

Serum samples were collected before coronary angiography of fasting patients and subsequently stored at −20 C until analysis. OPG serum concentrations were analyzed blinded to any clinical information using an ELISA system from Immundiagnostik (Bensheim, Germany; Ref. 16). In brief, a monoclonal IgG antibody was used as capture antibody, and a biotin-labeled polyclonal antihuman OPG antibody was used as detection antibody. Triglyceride, total cholesterol, and high-density lipoprotein (HDL) serum levels were determined using standard enzymatic methods (Roche Diagnostics, Mannheim, Germany), and low-density lipoprotein (LDL) levels were calculated according to the Friedewald equation. Lipoprotein (a), Apo AI, and Apo B serum levels were determined using standard nephelometric methods (Dade-Behring, Marburg, Germany). Homocysteine serum levels were measured by an ELISA system from Bio-Rad Laboratories, Inc. (Munich, Germany). Arterial hypertension was defined as systolic blood pressure repeatedly measured greater than 140 mm Hg, diastolic blood pressure greater than 90 mm Hg, or current use of antihypertensive drugs. Patients were considered as diabetic if fasting glucose was repeatedly

above 120 mg/dl or if they were receiving antidiabetic oral drugs or insulin injections.

Statistical analysis

Statistical analysis was performed using the SPSS software for Windows, version 9.0.1 (SPSS, Inc., Chicago, IL). Summary statistics for continuous variables were recorded as the mean ± SD, and categorical data were summarized as frequencies and percentages. Multiple group comparisons were performed using Kruskal-Wallis test followed by Mann-Whitney *U* test if significant differences were detected. Correlations between continuous variables were calculated according to Spearman-Rho. Multivariate logistic regression analysis was performed with the presence of CAD as the dependent variable and OPG serum levels, age, body mass index, HDL, LDL, triglyceride serum levels, and arterial hypertension as independent variables. All numeric data are presented as the mean ± SD, and a P value of less than 0.05 was considered statistically significant.

Results

OPG serum levels in men, with CAD patients compared with men without CAD

On the basis of coronary angiographies, the 522 men were categorized as subjects without CAD (n = 124), patients with single-vessel disease (n = 113), patients with double-vessel disease (n = 107), or patients with severe triple-vessel disease (n = 178). The characteristics of these four groups are given in Table 1. Because OPG serum levels were found to be positively correlated with age (r = 0.28; $P < 0.001$), the four groups were age-adjusted (Table 1). As expected, the percentage of patients with diabetes mellitus was higher in patients with advanced CAD, consistent with the established role of diabetes mellitus in the pathogenesis of CAD, whereas all groups had comparable creatinine serum levels. OPG serum levels in men without CAD were 5.4 ± 2.0 pmol/liter, and significantly higher in patients with single-vessel disease (6.1 ± 2.1 pmol/liter; $P < 0.005$), double-vessel disease (5.9 ± 2.4 pmol/liter; $P < 0.05$), or triple-vessel disease (6.3 ± 2.3 pmol/liter; $P < 0.001$; Fig. 1). When all CAD groups were combined, OPG serum levels were also significantly higher in men with CAD (6.1 ± 2.3 pmol/liter) compared with men without CAD (5.4 ± 2.0 pmol/liter; $P < 0.001$). Moreover, OPG serum levels were higher in men with diabetes mellitus (6.6 ± 2.4 pmol/liter) than in men without diabetes mellitus (5.8 ± 2.2 pmol/liter; $P < 0.01$). To rule out a confounding effect of diabetes mellitus on OPG serum levels, we reanalyzed the data after excluding patients with diabetes mellitus. In men without diabetes mellitus, OPG serum levels were 5.4 ± 2.0 pmol/liter in men without CAD, and higher in patients with single-vessel disease (6.0 ± 2.1 pmol/liter; $P < 0.05$), double-vessel disease (5.7 ± 2.1 pmol/liter; $P = 0.196$), or triple-vessel disease (6.2 ± 2.3 pmol/liter; $P = 0.001$). When all CAD groups were combined, OPG serum levels were also significantly higher in nondiabetic men with CAD (6.0 ± 2.2 pmol/liter) compared with nondiabetic men without CAD (5.4 ± 2.0 pmol/liter; $P < 0.005$).

Using a CAD scoring system that assesses the severity of CAD rather than the number of affected coronary arteries, OPG serum levels were found to be positively correlated with the severity of CAD (r = 0.17; $P < 0.01$; Fig. 2).

OPG serum levels and cardiovascular risk factors

Next, we assessed the association of OPG serum levels with established cardiovascular risk factors, including various lipids and lipoproteins, arterial hypertension, and homocysteine serum concentrations. Of note, OPG serum levels were positively correlated with homocysteine serum levels (r = 0.19; $P < 0.001$) and negatively correlated with triglyceride serum concentrations (r = −0.14; $P < 0.001$), but were not correlated with the body mass index or any other parameter of lipid metabolism such as total cholesterol, HDL or LDL cholesterol, Apo AI and Apo B, or lipoprotein (a). How-

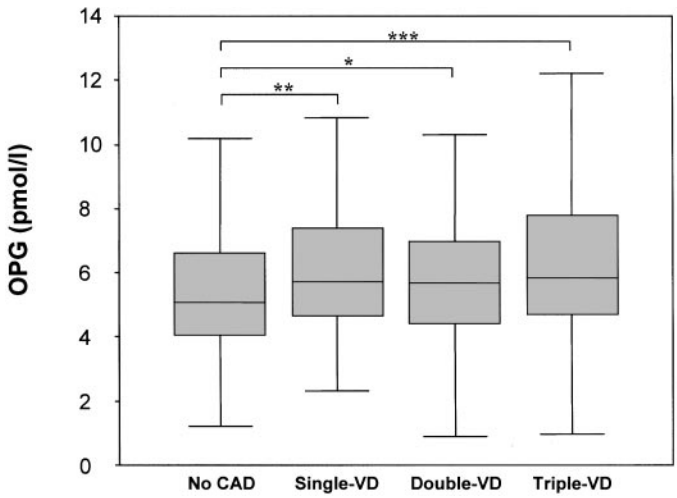

FIG. 1. OPG serum levels in men without CAD (No CAD) compared with patients with single-, double-, or triple-vessel disease (VD). *Boxes* represent the 95% confidence intervals, with the mean superimposed as a *horizontal line*. *Error bars* indicate ranges of OPG serum levels. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$.

TABLE 1. Characteristics of men with or without CAD

Number of affected coronary arteries	0	1	2	3
No. of men	124	113	107	178
Age (yr)	59 ± 9	60 ± 9	60 ± 9	60 ± 9
OPG concentrations (pmol/liter)	5.4 ± 2.0 (1.2–14.4)	6.1 ± 2.1 (2.3–13.2)	5.9 ± 2.4 (0.9–14.5)	6.3 ± 2.3 (1.0–16.7)
Arterial hypertension (%)	37	28	41	42
Diabetes mellitus (%)	3	16	19	16
Creatinine (mg/dl)	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.2
BMI (kg/m^2)	29.1 ± 5.2	29.2 ± 5.1	29.6 ± 5.0	29.1 ± 4.5

The number of affected coronary arteries was determined by coronary angiography. All numbers represent the mean ± SD. In addition, the ranges of OPG concentrations are given. BMI, Body mass index.

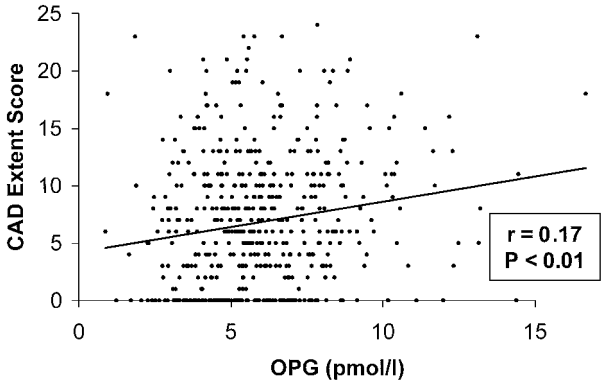

FIG. 2. Correlation of OPG serum levels with the severity of CAD as determined by a CAD scoring system in 522 age-adjusted men.

TABLE 2. Association of CAD with OPG serum levels and established risk factors of CAD in 522 men

Variable	Odds ratio	95% CI	*P*
OPG (pmol/liter)	1.27	1.07–1.50	<0.01
Age (yr)	1.00	0.96–1.03	0.83
BMI (kg/m^2)	1.01	0.95–1.08	0.72
HDL (mg/dl)	0.97	0.95–0.99	<0.05
LDL (mg/dl)	0.99	0.99–1.00	0.12
Triglycerides (mg/dl)	1.00	0.99–1.00	0.33
Hypertension	0.65	0.29–1.47	0.30

BMI, Body mass index; CI, confidence interval.

ever, triglyceride serum levels were negatively ($r = -0.14$; $P < 0.001$) correlated with age, and homocysteine serum concentrations were positively ($r = 0.15$; $P < 0.001$) correlated with age. Furthermore, OPG serum levels tended to be higher in hypertensive patients (6.1 ± 2.4 pmol/liter) compared with normotensive patients (5.8 ± 2.1 pmol/liter; $P = 0.32$) which was due to the fact that men with arterial hypertension were older than men with normal blood pressure (62 ± 8 *vs.* 58 ± 9 yr; $P < 0.001$). In our cohort of men with creatinine serum levels less than 2.0 mg/dl, OPG serum levels were not correlated with creatinine serum levels ($r = -0.14$; $P = 0.757$).

As analyzed by multivariate logistic regression analysis (Table 2), OPG serum levels were independently and positively associated with the presence of CAD ($P < 0.01$), whereas HDL serum levels were negatively associated with the presence of CAD ($P < 0.05$). The variable diabetes mellitus was excluded from multivariate analysis because the percentage of patients with diabetes was considerably lower in men without CAD (3%) compared with men with CAD (16–19%).

Discussion

OPG is produced by cells of the cardiovascular system, including coronary artery smooth muscle cells and endothelial cells (17, 18), and OPG represents a protective factor for the vascular system (19), suggesting that alterations of OPG serum levels may be associated with CAD. In our cohort of men who underwent coronary angiography for suspected CAD, we found OPG serum levels to be significantly higher in patients with advanced CAD. Because CAD has a higher prevalence in men and because OPG serum levels are dependent on the gender (higher levels in women) and subject to the estrogen status (20), we evaluated OPG serum levels in men. Increased OPG serum levels have recently been reported in postmenopausal women with diabetes mellitus compared with postmenopausal women without diabetes mellitus and were found to be associated with increased cardiovascular mortality (21). Consistent with this, OPG serum levels were higher in men with diabetes mellitus compared with those without diabetes mellitus. Because diabetes mellitus is one of the major established risk factors for CAD, the number of diabetic patients was higher in both CAD groups compared with patients without CAD. However, reanalysis of OPG serum levels for participants without diabetes mellitus revealed a similar positive association of OPG serum levels with the severity of CAD, thus excluding a potential confounding effect of diabetes mellitus. We elected to assess OPG serum levels in age-matched groups, because OPG serum levels increase with aging (16), which was confirmed in our study. Moreover, we controlled for increased OPG serum concentrations in patients with renal failure (22), and therefore excluded patients with creatinine serum concentrations above 2.0 mg/dl in our study.

The finding that the presumed protective factor OPG is elevated in disease has also been described for osteoporosis and was interpreted as a counter-regulatory mechanism to protect against bone loss (20). In the vascular system, increased OPG production may indicate endothelial damage, intimal hyperplasia, smooth muscle cell hypertrophy, or advanced plaque calcification (19). Although the precise mechanisms remain unclear, arterial hypertension and diabetes mellitus, both of which become more important during aging, may enhance production or release of OPG protein from the vascular system. Thus, elevated serum levels of the putative protective factor OPG may represent an insufficient compensatory mechanism to prevent further vascular damage. Alternatively, inflammatory mechanisms and mediators, *e.g.* proinflammatory cytokines, may promote vascular disease and increase OPG serum levels alike.

In mice, targeted deletion of the OPG gene resulted in severe calcification of the aorta and renal arteries (10). A more recent paper detected RANKL and OPG immunoreactivity in the normal vascular wall and in early atherosclerotic lesions in humans, whereas OPG was expressed in advanced calcified lesions and RANKL adjacent to calcium deposits (23). Vascular calcification, with its reduced compliance and altered mechanical properties, is a predisposing factor of plaque rupture (24, 25) and a predictor of cardiovascular mortality (26). Clinically, progression of atherosclerotic calcification is associated with bone loss in postmenopausal women (27), and osteoporosis and arterial calcification frequently coincide (28), indicating an imbalance of calcium allocation with a shift from bone to the vascular wall, both of which may be modulated by RANKL and OPG.

Because OPG is ubiquitously produced by a variety of tissues and not restricted to the cardiovascular system, it is possible that other sources may have contributed to the

OPG serum pool and that considerable OPG concentration gradients between the normal vascular wall and sites of atherosclerotic lesions may exist. Another limitation of our study is measurement of total OPG, because the detection system used cannot discriminate between free OPG and OPG complexed to its ligand, RANKL. Therefore, increased OPG serum levels measured by this (16) and other commercial assays (20, 21) may be due to an increase of free OPG, an increase of RANKL-OPG complexes, or both. Development of assays that detect specifically the free fractions of OPG and its ligand, RANKL, will substantially improve assessment of the RANKL-OPG system. Of note, OPG serum levels represent a steady-state between OPG production by various tissues and clearance or degradation. Internalization of OPG via syndecan-1 and subsequent lysosomal degradation has recently been described for myeloma cells (29). However, no such mechanism has been reported for normal bone or vascular cells. Clearly, the clinical significance of these findings needs to be evaluated in more detail, because the differences of OPG serum levels in patients with or without CAD, although statistically significant, were small and do not allow individual CAD risk assessment.

The therapeutic potential of exogenous administration of OPG to patients with CAD remains unclear. Although OPG administration had protective vascular effects in various animal models (10–12), the ensuing serum levels were considerably higher than those detected in our cohort. It will therefore be important to assess the mechanism(s) of how exogenously administered OPG targets to the vascular wall. Nonetheless, our findings provide important insights into the concurrent mechanism of osteoporosis and vascular disease as discussed elsewhere (28). In conclusion, our data show that OPG serum levels increase with the severity of CAD in age-matched men and are higher in men with diabetes mellitus. These findings indicate that alterations of the OPG system may contribute to the pathogenesis of human vascular disease, although further studies are required to determine the diagnostic and therapeutic implications in more detail.

Acknowledgments

We thank S. Fischer, U. Otte, and S. Motzny for excellent technical assistance and Dr. M. Christ for helpful suggestions.

Received May 20, 2002. Accepted November 20, 2002.

Address all correspondence and requests for reprints to: Lorenz C. Hofbauer, M.D., Division of Gastroenterology, Endocrinology, and Metabolism, Department of Medicine, Philipps University, Baldingerstrasse, D-35033 Marburg, Germany. E-mail: hofbauer@post.med.uni-marburg.de.

This work was supported by grants from the Deutsche Forschungsgemeinschaft (Ho 1875/2-1) and from the Deutsche Krebshilfe (10-1697-Ho1) to L.C.H. and the Kempkes-Stiftung to J.R.S.

M.S. and A.M.S. contributed equally to this work.

References

1. **Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang M-S, Lüthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan H-L, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Amgen EST Program, Boyle WJ** 1997 Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 89:309–319
2. **Hsu H, Lacey DL, Dunstan CR, Solovyev I, Colombero A, Timms E, Tan H-L, Elliott G, Kelley MJ, Sarosi I, Wang L, Xia X-Z, Elliott R, Chiu L, Black T, Scully S, Capparelli C, Morony S, Shimamoto G, Bass MB, Boyle WJ** 1999 Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. Proc Natl Acad Sci USA 96:3540–3545
3. **Lacey DL, Timms E, Tan HL, Kelly MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ** 1998 Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93:3597–3602
4. **Miyamoto T, Ohneda O, Arai F, Iwamoto K, Okada S, Takagi K, Anderson DM, Suda T** 2001 Bifurcation of osteoclasts and dendritic cells from common progenitors. Blood 98:2544–2554
5. **Anderson DM, Marakovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L** 1997 A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 390:175–179
6. **Kong YY, Boyle WJ, Penninger JM** 2000 Osteoprotegerin ligand: a regulator of immune responses and bone physiology. Immunol Today 21:495–502
7. **Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ, Penninger JM** 1999 OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 397:315–323
8. **Yun TJ, Tallquist MD, Aicher A, Rafferty KL, Marshall AJ, Moon JJ, Ewings ME, Mohaupt M, Herring SW, Clark EA** 2001 Osteoprotegerin, a crucial regulator of bone metabolism, also regulates B cell development and function. J Immunol 166:1482–1491
9. **Emery JG, Mc Donnell P, Brigham Burke M, Deen KC, Lyn S, Silverman C, Dul E, Appelbaum ER, Eichman C, DiPrinzio R, Dodds RA, James IE, Rosenberg M, Lee JC, Young PR** 1998 Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. J Biol Chem 273:14363–14367
10. **Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS** 1998 Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 12:1260–1268
11. **Min H, Morony S, Sarosi I, Dunstan CR, Capparelli C, Scully S, Van G, Kaufman S, Kostenuik PJ, Lacey DL, Boyle WJ, Simonet WS** 2000 Osteoprotegerin reverses osteoporosis by inhibiting endosteal osteoclasts and prevents vascular calcification by blocking a process resembling osteoclastogenesis. J Exp Med 192:463–474
12. **Price PA, June HH, Buckley JR, Williamson MK** 2001 Osteoprotegerin inhibits artery calcification induced by warfarin and by vitamin D. Arterioscler Thromb Vasc Biol 21:1610–1616
13. **Malyankar UM, Scatena M, Suchland KL, Yun TJ, Clark EA, Giachelli CM** 2000 Osteoprotegerin is an $\alpha_v\beta_3$-induced, NF-κB-dependent survival factor for endothelial cells. J Biol Chem 275:20959–20962
14. **Reardon MF, Nestel PJ, Craig IH, Harper RW** 1985 Lipoprotein predictors of the severity of coronary artery disease in men and women. Circulation 71: 881–888
15. **Schaefer JR, Simon B, Soufi M, Sattler A, Noll B, Herzum M, Maisch B** 2000 Strategies to optimize CAD prevention in modern cardiology. Herz 25:113–116
16. **Szulc P, Hofbauer LC, Heufelder AE, Roth S, Delmas PD** 2001 Osteoprotegerin serum levels in men: correlation with age, estrogen, and testosterone status. J Clin Endocrinol Metab 86:3162–3165
17. **Hofbauer LC, Shui C, Riggs BL, Dunstan CR, Spelsberg TC, O'Brien T, Khosla S** 2001 Effects of immunosuppressants on receptor activator of NF-κB ligand and osteoprotegerin production by human osteoblastic and coronary artery smooth muscle cells. Biochem Biophys Res Commun 280: 334–339
18. **Collin-Osdoby P, Rothe L, Anderson F, Nelson M, Maloney W, Osdoby P** 2001 Receptor activator of NF-κB and osteoprotegerin expression by human microvascular endothelial cells, regulation by inflammatory cytokines, and role in human osteoclastogenesis. J Biol Chem 276:20659–20672
19. **Schoppet M, Preissner KT, Hofbauer LC** 2002 RANK ligand and osteoprotegerin. Paracrine regulators of bone metabolism and vascular function. Arterioscler Thromb Vasc Biol 22:549–553
20. **Yano K, Tsuda E, Washida N, Kobayashi F, Goto M, Harada A, Ikeda K, Higashio K, Yamada Y** 1999 Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: increased serum concentrations in postmenopausal women with osteoporosis. J Bone Miner Res 14: 518–527
21. **Browner WS, Lui LY, Cummings SR** 2001 Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women. J Clin Endocrinol Metab 86:631–637
22. **Kazama JJ, Shigematsu T, Yano K, Tsuda E, Miura M, Iwasaki Y, Kawaguchi Y, Gejyo F, Kurokawa K, Fukagawa M** 2002 Increased circulating levels of

osteoclastogenesis-inhibitory factor (osteoprotegerin) in patients with chronic renal failure. Am J Kidney Dis 39:525–532

23. **Dhore CR, Cleutjens JP, Lutgens E, Cleutjens KB, Geusens PP, Kitslaar PJ, Tordoir JH, Spronk HM, Vermeer C, Daemen MJ** 2001 Differential expression of bone matrix regulatory proteins in human atherosclerotic plaques. Arterioscler Thromb Vasc Biol 21:1998–2003
24. **Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD, Lusis AJ** 1995 Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. Circulation 91:2488–2496
25. **Wexler L, Brundage B, Crouse J, Detrano R, Fuster V, Maddahi J, Rumberger J, Stanford W, White R, Taubert K** 1996 Coronary artery calcification: pathophysiology, epidemiology, imaging methods, and clinical implications. A statement for health professionals from the American Heart Association. Writing Group. Coronary artery calcification: pathophysiology, epidemiology, imaging methods, and clinical implications. Circulation 94:1175–1192
26. **Lehto S, Niskanen L, Suhonen M, Ronnemaa T, Laakso M** 1996 A neglected harbinger of cardiovascular complications in non-insulin-dependent diabetes mellitus. Arterioscler Thromb Vasc Biol 16:978–983
27. **Hak AE, Pols HA, van Hemert AM, Hofman A, Witteman JC** 2000 Progression of aortic calcification is associated with metacarpal bone loss during menopause: a population-based longitudinal study. Arterioscler Thromb Vasc Biol 20:1926–1931
28. **Hofbauer LC, Schoppet M** 2001 Osteoprotegerin: a link between osteoporosis and arterial calcification? Lancet 358:257–259
29. **Standal T, Seidel C, Hjertner O, Plesner T, Sanderson RD, Waage A, Borset M, Sundan A** 2002 Osteoprotegerin is bound, internalized, and degraded by multiple myeloma cells. Blood 100:3002–3007