Effects of a short-term calcium and vitamin D treatment on serum cytokines, bone markers, insulin and lipid concentrations in healthy post-menopausal women

M.H. Gannagé-Yared[1], M. Azoury[2], I. Mansour[2], R. Baddoura[3], G. Halaby[1] and R. Naaman[2]

[1]Department of Endocrinology, Saint-Joseph University, [2]Laboratoire d'Immunologie Cellulaire, Hôtel-Dieu de France, [3]Department of Rheumatology, Saint-Joseph University, Beirut, Lebanon

ABSTRACT. *In vitro* studies have shown that 1,25 dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] decreases cytokine production by monocytes and lymphocytes. In addition, intravenous or oral pulse calcitriol treatment suppresses interleukin 6 (IL6) and interleukin1β (IL1β) in hemodialysis patients. We studied the effect of a daily 12-week course of 1000 mg calcium and 800 U cholecalciferol on circulating 25 hydroxyvitamin D [25(OH)D], PTH, cytokines, osteoprotegerin (OPG), C-reactive protein (CRP), bone markers, lipid parameters and insulin levels in 47 healthy post-menopausal women. Thirty-nine women completed the study. A significant increase in 25(OH)D and a significant decrease in PTH were observed (p=0.0043 and p<0.0001, respectively). In addition, alkaline phosphatase, osteocalcin and, to a lesser extent, urinary free deoxypiridinoline (DPD) decreased significantly (p<0.0001, p=0.0002 and p=0.026, respectively). No change in circulating IL6, tumor necrosis factor α (TNFα), CRP, OPG, triglycerides, LDL- and HDL-cholesterol, and insulin levels was observed. Correlation studies in the 47 women enrolled in the study revealed inverse significant correlations between OPG on one side and body mass index, LDL-cholesterol, IL6, CRP and insulin levels on the other (p=0.002, p=0.002, p=0.004, p=0.023 and p=0.0001). Also, IL6 was significantly correlated with insulin levels (p=0.0005). In a multivariate model, both insulin and LDL-cholesterol were independently associated with OPG, while only insulin was independently associated with IL6. Our results showed no effect of a short-term calcium-vitamin D treatment on circulating cytokines, CRP, insulin levels and lipid parameters. This could be related to the low circulating cytokine concentrations in healthy subjects or to the short duration of treatment. The interesting association we found between OPG and some cardiovascular risk markers deserves further investigation.

(J. Endocrinol. Invest. 26: 748-753, 2003)

INTRODUCTION

Calcium and vitamin D are essential regulating factors in numerous biological systems. They have bone protective effects (1) and their deficiencies lead to secondary hyperparathyroidism (1, 2) and high bone turnover (3), increasing with time the risk of fractures (1).

In addition, vitamin D is an immunomodulatory hormone with immunosuppressive activity (4, 5). It has been shown that vitamin D inhibits mononuclear and T lymphocyte cell proliferation (4, 6). It is most likely that this suppressive activity is mediated through an effect on cytokine secretion. In fact, *in vitro* studies showed that 1,25 dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] decreases cytokine production, in particular interleukin 1α (IL1α), interleukin 6 (IL6), interleukin 2 (IL2) and tumor necrosis factor α (TNFα) by macrophages and lymphocytes (7-10). The expression of vitamin D receptors on the surface of macrophages and activated T cells (11, 12) suggests a direct effect of vitamin D on these cells. Also, an inverse relation between cytokines and vitamin D has been observed in certain inflammatory diseases such as rheumatoid arthritis (13).

Moreover, vitamin D status may influence insulin secretion; vitamin D deficiency results in decreased

Key-words: Vitamin D, cytokines, osteoprotegerin, bone markers, insulin.

Correspondence: Dr. M.H. Gannagé-Yared, Division of Endocrinology, Hôtel-Dieu de France Hospital, Adib Ishaac Street, Beirut, Lebanon.

E-mail: mhcyared@terra.net.lb.

Accepted April 22, 2003.

insulin response to glucose (14) and inadequate vitamin D status has been implicated as a contributing factor to insulin resistance, obesity, hypertension and dyslipidemia (15, 16).

On the other hand, IL6, the most important endocrine cytokine, is multifunctional and is produced by different cellular types including lymphocytes, monocytes, endothelial cells, osteoblasts and adipocytes (17, 18). IL6 has a profound effect on bone and lipid metabolism. It stimulates osteoclastogenesis (19), is associated with high circulating triglyceride levels (20) and is implicated in insulin resistance (20). IL6 stimulates production in the liver of the acute phase reactant C-reactive protein (CRP) and is, with CRP, a marker of increased myocardial infarction risk and mortality in the elderly (21, 22). Furthermore, high osteoprotegerin (OPG) levels, a newly identified cytokine that regulates osteoclastogenesis (23, 24), were recently associated with diabetes and low bone mineral density (25).

The effect of a calcium-vitamin D treatment on serum cytokines, LDL-cholesterol or fasting insulin levels has not been studied yet in healthy subjects. Furthermore, the mechanism of the vitamin D effect on bone and on insulin resistance has not been elucidated. The present study evaluates the effect of a short-term calcium and vitamin D treatment on cytokines levels (IL6, TNFα), CRP, OPG, bone markers, lipid parameters and insulin levels in healthy post-menopausal women. The potential inhibitory effect of vitamin D on these parameters, more particularly IL6, would possibly explain the vitamin D effect on bone and the relation between vitamin D status and the metabolic syndrome.

MATERIALS AND METHODS

Subjects

Between November 2000 and March 2001, 47 healthy post-menopausal women aged 50-65 yr were recruited in the study. Recruitment was based on voluntary enrolment. Inclusion criteria were the following: a menopausal state confirmed by an FSH level higher than 30 U/l, no systemic infections during the previous month, no systemic disease particularly diabetes and hypertension, no therapy with thiazides, bisphosphonate, calcitonin, calcium, vitamin D_3, vitamin D metabolites, estrogen, antiestrogen or lipid-lowering drugs during the past 6 months. Liver, renal or thyroid dysfunction as well as hypercalcemia, severe dyslipidemia (triglycerides >3.5 mmol/l and cholesterol >7 mmol/l) were excluded by measuring liver enzymes, serum creatinine, calcium, cholesterol, triglycerides and TSH levels. Only women with a 25 hydroxyvitamin D [25(OH)D] level below 25 ng/ml were enrolled in the study. The protocol was approved by the Ethics Committee of our hospital and all participants provided written informed consent.

Procedure

Prior to the study, participants completed a questionnaire and a physical examination. For each subject, body mass index (BMI) was calculated as weight (kg)/height (m^2). Urine and blood were collected at the first visit. Then a daily calcium (1000 mg) and vitamin D (800 IU) treatment was administered for 12 weeks. Morning fasting blood and random urine samples were always collected between 8:00 and 9:30 h from all participants before and after the study was completed. Levels of serum calcium, phosphorus, albumin, creatinine, glucose, cholesterol, triglycerides, HDL-cholesterol, alkaline phosphatase, 25(OH)D, PTH, osteocalcin, IL6, TNFα, OPG and urinary-free DPD were determined. LDL-cholesterol was calculated using the Friedewald equation. Insulin sensitivity was also measured by calculating the Quantitative Insulin Sensitivity Check Index (QUICKI) [QUICKI= 1 / log insulin (mlU/ml) + log glucose (mg/dl)] (26). Out of the 47 women, 8 were excluded from the study due to refusal to continue the treatment.

Laboratory analysis

Serum chemistries were measured by Kodack automate. Serum 25(OH)D was measured by radioimmunoassay after extraction with acetonitrile (Incstar, MN, USA). Intact PTH and osteocalcin were measured by an immunoradiometric assay (International CIS, Gif-sur Yvette, France). Urinary free DPD was measured by a competitive radioimmunoassay (Metra, Mountain view, CA, USA). Serum IL6 and TNFα were determined by ultrasensitive enzyme linked immunosorbent assay (Quantikine IL6 and Quantikine High Sensitivity TNFα, R&D systems, Oxford, UK). The sensitivities of these assays were respectively 0.09 and 0.12 pg/ml. CRP was determined by immunonephelometry on Minineph Nephelometer (The Binding Site, Birmingham, UK). The sensitivity of the assay is 0.08 pg/ml. OPG was measured using a highly sensitive sandwich immunoassay provided by Immunodiagnostik (Bensheim, Germany). The sensitivity of the assay is 0.14 pmol/l. Interassay coefficients of variation were as follows: TNFα less than 20%, IL6 less than 17%, OPG less than 10% and CRP less than 7%. FSH, insulin and TSH were measured by chemiluminescence (Immulite, DPC, Los Angelos, USA). All measurements were performed according to the manufacturer's instructions.

Analysis

Data were analyzed using STATA release. Spearman coefficient of correlation was used to study the correlation between variables. Non-parametric Wilcoxon's test for paired data was used to compare values before and after treatment. Results with p values <0.05 were considered statistically significant.

RESULTS

The mean age and mean BMI of the study population (no.=47) were 57.2±3.9 yr and 28.1±4.7 kg/m^2, respectively. The mean FSH level was 75.8±27.8 IU/l. The effect of the calcium-vitamin D treatment has been studied in the 39 women who completed the study. Correlation studies were performed in the 47 women enrolled in the study.

Effect of calcium-vitamin D treatment on 25(OH)D and PTH levels

Compared with the baseline values, significant increases in serum 25(OH)D levels were found ($p<0.0001$). Significant decreases were also found in serum PTH levels ($p=0.0043$) (Table 1).

Effect of calcium-vitamin D treatment on cytokines, CRP and OPG levels

No effect of the treatment was observed on circulating IL6, TNFα, CRP and OPG levels (Table 1).

Effect of calcium-vitamin D treatment on bone markers

A significant decrease in bone markers was observed; this decrease was observed for the two bone formation markers, alkaline phosphatase and osteocalcin ($p<0.0001$, $p=0.0002$, respectively) and, to a lesser extent, for the bone resorption marker, urinary free DPD ($p=0.026$) (Table 1).

Effect of calcium-vitamin D treatment on metabolic parameters

Triglycerides, LDL-cholesterol, HDL-cholesterol, insulin levels as well as the insulin sensitivity index (QUICKI) were not modified by the treatment (Table 1).

Correlations between serum 25(OH)D_3, PTH and cytokines

No significant correlations were observed between 25(OH)D, PTH levels on one side and circulating IL6, TNFα and OPG levels on the other (Table 2).

Associations between serum OPG, IL6 levels and cardiovascular risk factors

OPG has a significant inverse correlation with BMI, LDL-cholesterol, fasting insulin levels, IL6 and CRP ($r=-0.45$ $p=0.002$, $r=-0.43$, $p=0.002$, $r=-0.55$, $p=0.0001$, $r=-0.41$, $p=0.004$ and $r=-0.33$ $p=0.023$, respectively). In addition, a significant positive correlation between IL6 and insulin levels was found ($r=0.48$, $p=0.0005$) (Table 2).

In a multivariate analysis both LDL-cholesterol and insulin levels were independently associated with OPG levels ($p=0.048$ and $p=0.007$, respectively), while only insulin was associated with IL6 levels ($p=0.002$) (Table 3).

DISCUSSION

We report, in the present study, the absence of effect of a short-term calcium-vitamin D treatment on serum IL6, TNFα, CRP and OPG levels. No effect was also observed on LDL-cholesterol or fasting

Table 1 - *Clinical and biological characteristics of the subject (no.=39) before and after the calcium-vitamin D treatment.*

	Before treatment	After treatment	*p*
Cholesterol (mmol/l) (3.84-6.14)	5.74±0.93	5.67±0.92	0.36
Triglycerides (mmol/l) (0.4-1.8)	1.64±0.7	1.68±0.76	0.58
HDL-cholesterol (mmol/l) (>1.4)	1.31±0.33	1.32±0.29	0.32
LDL-cholesterol (mmol/l) (<3.84)	3.69±0.78	3.59±0.8	0.71
Calcium (mmol/l) (2.1-2.54)	2.36±0.09	2.35±0.08	0.16
Phosphorus (mmol/l) (0.81-1.45)	1.22±0.15	1.25±0.17	0.56
25(OH)vitamin D (ng/ml) (>20)***	10.57±6.62	25.84±6.58	<0.0001
PTH (pg/ml) (11-62)**	48.6±18.6	42.25±16.35	0.0043
Alkaline phosphatase (IU/l) (38-126)***	81.15±20.28	69.46±16.72	<0.0001
Urinary deoxypiridinoline (nmol/mmol creatinine) (<7.4)*	8.42±2.52	7.73±2.02	0.026
Osteocalcin (pg/ml) (8-50.5)***	27.4±9.08	23.95±6.5	0.0002
Insulin (µUI/ml) (6-27)	8.94±3.98	8.69±3.31	0.87
QUICKI (<0.37)	0.35±0.27	0.34±0.27	0.23
IL6 (pg/ml) (<11.5)	2.19±1.16	2.05±1.2	0.43
TNFα (pg/ml) (<4.12)	2.37±0.64	2.49±0.72	0.41
CRP (mg/l) (<3.8)	3.68±3.78	3.76±3.34	0.16
Osteoprotegerin (pmol/l)°	4.27±1.1	4.19±1.13	0.28

Mean and SD are expressed (reference ranges).
*The difference of means is significant at the 0.05 level; **the difference of means is significant at the 0.01 level; ***the difference of means is significant at the 0.001 level.
°No reference range.
25(OH)vitamin D: 25 hydroxyvitamin D; QUICKI: Quantitative Insulin Sensitivity Check Index; IL6: interleukin 6; TNFα: tumor necrosis factor α; CRP: C- reactive protein.

Table 2 - *Linear correlations between IL6, TNFα, CRP, osteoprotegerin and other variables in basal conditions (no.=47).*

	IL6	TNFα	Osteoprotegerin
BMI	0.166	–0.03	–0.45**
25(OH)vitamin D	–0.162	0.136	0.018
PTH	–0.066	–0.036	0.09
LDL-cholesterol	0.194	–0.05	–0.43**
Insulin	0.48**	0.023	–0.55***
QUICKI	–0.38**	0.011	0.52***
IL6		–0.01	–0.406**
TNFα			–0.219

*Correlation is significant at the 0.05 level, **correlation is significant at the 0.01 level, ***correlation is significant at the 0.001 level.
BMI: body mass index; 25(OH)vitamin D: 25 hydroxyvitamin D; QUICKI: Quantitative Insulin Sensitivity Check Index; IL6: interleukin 6; TNFα: tumor necrosis factor α.

serum insulin levels. However, this treatment induced a significant reduction in PTH and biochemical bone markers.

Previous studies have shown that 1,25(OH)D_3 reduces the *in vitro* production of different cytokines (IL6, IL1 and TNFα) by monocytes and lymphocytes (7-10). In addition, a recent report (27) showed that oral pulse or iv calcitriol therapy in hemodialysis patients reduces IL6 and IL1β after a 6-month course treatment, with a greater response in the iv group compared to the oral one. However, the effect of a calcium-vitamin D treatment on circulating cytokines and CRP in healthy subjects has not been investigated yet. Since IL6 stimulates osteoclastogenesis, is correlated with circulating triglycerides and is implicated in insulin resistance, we speculate that a potential *in vivo* effect of a calcium-vitamin D course on serum cytokines and more particularly on serum IL6 could elucidate the effect of vitamin D on bone and metabolism. Our results showed no variation in IL6, TNFα or CRP levels after a 12-week course of treatment. The discordance with the study performed on hemodialysis patients (27) could be due: a) to the administration in that study of calcitriol instead of cholecalciferol; b) to the administration of pharmacological doses by oral pulse or iv route; c) to the high baseline cytokine values in hemodialysis patients as opposed to the low levels observed in healthy subjects; or d) to the longer duration of therapy.

Table 3 - *Multiple linear regression with osteoprotegerin and interleukin 6 as dependent variables in basal conditions (no.=47; R^2=0.4 and R^2= 0.25, respectively).*

Osteoprotegerin	Parameter	SE estimate	Significance
Body mass index	–0.04	0.03	ns
LDL-cholesterol	–0.31	0.15	<0.05
Insulin	–0.11	0.04	<0.01
Interleukin 6	**Parameter**	**SE estimate**	**Significance**
Body mass index	–0.03	0.04	ns
LDL-cholesterol	0.09	0.19	ns
Insulin	0.16	0.05	0.002

The relation between OPG and vitamin D is not clear. OPG is a new cytokine secreted by bone, immune cells and cardiovascular system that belongs to the TNF receptor superfamily (28). OPG inhibits osteoclastogenesis and its deficiency in mice predisposes to osteoporosis (29). However, elevated serum OPG levels were recently observed in osteoporotic postmenopausal women (25). This paradox was interpreted as a compensatory mechanism to counteract low bone density. The OPG system has been found to be regulated by vitamin D; two contradictory studies showed stimulation (30) and inhibition (31) of OPG by 1,25(OH)D_3. In our study, no effect of the calcium-vitamin D treatment was observed on circulating OPG levels. In addition, no correlation between 25(OH)D and OPG levels was found, a finding observed in the Szulc study (32). The issue of OPG and its relation to vitamin D deserves further investigation *in vitro* and in clinical conditions.

The effect of calcium-vitamin D treatment on bone markers is not well defined. Two previous studies assessed the effect of a short course of calcium-vitamin D on bone markers. In the first one, the treatment led to a 50% decrease in urinary N telopeptide and a 20% reduction in serum osteocalcin (33). In the second one (34), a 51% reduction of crosslaps was achieved with no change of free pyridinoline. The effect on urinary free DPD has not been previously studied. The reduction of bone markers we observed was highly significant for the two bone forming markers, alkaline phosphatase and osteocalcin and was moderate for urinary free DPD, suggesting a prompt effect of the treatment on bone remodeling.

The relation between vitamin D or calcium and lipid parameters is not clear. A recent study evaluated the effect of a daily 1000-mg calcium citrate supplementation taken during a 12-month period, on serum lipid concentrations in post-menopausal women (35). A non-significant 6% decline in LDL levels and a significant 7% increase in HDL, without any significant effect on triglycerides were observed. The LDL results are similar to ours. The effect on HDL, even significant, was moderate and was not observed in our study. This could be related to the size of our sample or to the shorter duration of our treatment. Larger studies assessing the

effect of calcium or vitamin D on lipid metabolism or cardiovascular events are needed.

The relation between calcium or vitamin D and insulin resistance is also unclear. Vitamin D deficiency was proven to decrease insulin response to glucose (23). In addition, iv calcitriol improves insulin resistance in uremic patients (36). Other contradictory reports suggest that $1,25(OH)_2D_3$ reduces insulin-induced glucose uptake in rat adipocytes (37). We found no effect of the treatment on fasting plasma insulin or on the QUICKI. Further investigations are needed to evaluate the effect of calcium or vitamin D on insulin resistance in healthy subjects.

Even if it was not the aim of our work, the present study showed interesting correlations between OPG and cardiovascular risk markers. We observed an inverse relationship between OPG and BMI, insulin and LDL-cholesterol levels, and in the multivariate analysis, both insulin and LDL-cholesterol levels were independently associated with OPG levels. This suggests that low OPG levels could predispose to insulin resistance and atherosclerosis. It has been reported that OPG-deficient mice developed premature arterial calcification (29), and that the injection of OPG reversed this phenomenon (38). In addition, Browners et al. (25) recently showed that OPG levels are 30% higher in women with diabetes compared to non-diabetic women. The finding by these Authors of a link between OPG levels and a high prevalence of cardiovascular disease and mortality was surprising. Additional research is probably needed in order to elucidate the relation between OPG, osteoporosis and cardiovascular disease. Finally, we also found that IL6 was correlated with fasting plasma insulin. These results are in agreement with the findings of Bastard and Mohamed-Ali (20, 39), and suggest that IL6 may be involved in insulin resistance.

Our study may have certain limitations. In fact, our sample size is relatively small. However, the difference observed in cytokines, LDL-cholesterol or insulin levels does not seem to have any biological significance. Thus, increasing our sample size would probably not detect statistically significant modifications.

In conclusion, our results demonstrate that a short-term calcium-vitamin D treatment had no effect on IL6, TNFα, CRP, OPG, lipid parameters or insulin serum concentrations. This suggests that the vitamin D treatment effects on bone and insulin resistance are not reflected by a modification of circulating cytokines. In addition, our results showed an interesting inverse relationship between OPG and LDL-cholesterol or insulin levels, suggesting a link between OPG and some cardiovascular risk markers. More research is needed in order to explain those results.

ACKNOWLEDGMENTS

This work was supported by grants from the Conseil de Recherche de l'Université Saint-Joseph.

REFERENCES

1. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev 2001, 22: 477-501.
2. McKenna MJ, Freaney R. Secondary hyperparathyroidism in the elderly: means to defining hypovitaminosis D. Osteoporos Int Suppl 1998, 8: S3-6.
3. Sahota O, Masud T, San P, Hosking DJ. Vitamin D insufficiency increases bone turnover markers and enhances bone loss at the hip in patients with established vertebral osteoporosis. Clin Endocrinol (Oxf) 1999, 51: 217-21.
4. Muller K, Bendtzen K. 1,25-Dihydroxyvitamin D_3 as a natural regulator of human immune functions. J Investig Dermatol Symp Proc 1996, 1: 68-71.
5. Deluca HF, Cantorna MT. Vitamin D: its role and uses in immunology. FASEB J 2001, 15: 2579-85.
6. Muller K, Rieneck K, Hansen MB, Bendtzen K. 1,25-Dihydroxyvitamin D_3-mediated suppression of T lymphocyte functions and failure of T cell-activating cytokines to restore proliferation. Immunol Lett 1992, 34: 37-44.
7. Rigby WFC, Denome S, Fanger MW. Regulation of lymphokine production and human T lymphocyte activation by 1,25-dihydroxyvitamin D_3. Specific inhibition at the level of messenger RNA. J Clin Invest 1987, 79: 1659-64.
8. Muller K, Diamant M, Bendtzen K. Inhibition of production and function of interleukin-6 by 1,25-dihydroxyvitamin D_3. Immunol Lett 1991, 28: 115-20.
9. Muller K, Odein M, Bendtzen K. 1,25-dihydroxyvitamin D_3 selectively reduces interleukin-2 levels and proliferation of human T cell lines *in vitro*. Immunol Lett 1993, 35: 177-82.
10. Willheim M, Thien R, Schrattbauer K, et al. Regulatory effects of 1α,25-dihydroxyvitamin D_3 on the cytokine production of human peripheral blood lymphocytes. J Clin Endocrinol Metab1999, 84: 3739-44.
11. Bhalla AK, Amento EP, Clemens TL, Holick MF, Krane SM. Specific high-affinity receptors for 1,25-dihydroxyvitamin D_3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. J Clin Endocrinol Metab 1985, 57: 1308-10.
12. Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC. 1,25-dihydroxyvitamin D_3 receptors in human leukocytes. Science 1983, 221: 1181-3.
13. Oelzner P, Franke S, Muller A, Hein G, Stein G. Relationship between soluble markers of immune activation and bone turnover in post-menopausal women with rheumatoid arthritis. Rheumatology (Oxford) 1999, 38: 841-7.
14. Nyomba BL, Bouillon R, De Moor P. Influence of vitamin D status on insulin secretion and glucose tolerance in the rabbit. Endocrinology 1984, 115: 191-7.

15. Kumar S, Davies M, Zakaria Y, et al. Improvement in glucose tolerance and β-cell function in a patient with vitamin D deficiency during treatment with vitamin D. Postgrad Med J 1994, 70: 440-3.
16. Boucher BJ. Inadequate vitamin D status: does it contribute to the disorders comprising syndrome X? Br J Nutr 1998, 79: 315-27.
17. Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic roles of interleukin-6 in human diseases. Ann Int Med 1998, 128: 127-37.
18. Papanicolaou DA, Vgontzas AN. Interleukin-6: The endocrine cytokine. J Clin Endocrinol Metab 2000, 85: 1331-3.
19. Rifas L. Bone and cytokines: beyond IL-1, IL-6 and TNF-α. Calcif Tissue Int 1999, 6: 1-7.
20. Bastard JP, Jardel C, Bruckert E, et al. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. J Clin Endocrinol Metab 2000, 85: 3338-42.
21. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and risk of future myocardial infarction among apparently healthy men. Circulation 2000, 101: 1767-72.
22. Harris TB, Ferrucci L, Tracy RP, et al. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. Am J Med 1999, 106: 506-12.
23. Simonet WS, Lacey DL, Dunstan CR, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997, 89: 309-19.
24. Lacey DL, Timms E, Tan HL, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998, 93: 165-76.
25. 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.
26. Katz A, Nambi SS, Mather K, et al. Quantitative Insulin Sensitivity Check Index. A sample method for assessing Insulin sensitivity. J Clin Endocrinol Metab 2000, 85: 2402-10.
27. Turk U, Akbulut M, Yildiz A, et al. Comparative effect of oral pulse and intravenous calcitriol treatment in hemodialysis patients: the effect on serum IL1 and IL6 and bone mineral density. Nephron 2002, 90: 188-94.
28. Schoppet M, Preissner KT, Hofbauer LC. RANK ligand and osteoprotegerin: paracrine regulators of bone metabolism and vascular function. Arterioscler Thromb Vasc Biol 2002, 22: 549-53.
29. Bucay N, Sarosi I, Dunstan CR, et al. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 1998, 12: 1260-8.
30. Hofbauer LC, Dunstan CR, Spelsberg TC, Riggs BL, Khosla S. Osteoprotegerin production by human osteoblast lineage cells is stimulated by vitamin D, bone morphogenetic protein-2 and cytokines. Biochem Biophys Res Commun1998, 275: 776-81.
31. Horwood NJ, Elliot J, Martin TJ, Gillespie MT. Osteotropic agents regulate the expression of osteoclast differentiation factor and osteoprotegerin in osteoblastic stromal cells. Endocrinology 1998, 139: 4743-6.
32. Szulc P, Hofbauer C, Heufelder AE, Roth S, Delmas PD. Osteoprotegerin serum levels in men: correlation with age, estrogen, and testosterone status. J Clin Endocrinol Metab 2001, 86: 3162-5.
33. Prestwood KM, Pannullo AM, Kenny AM, Pilbeam CC, Raisz LG. The effect of a short course of calcium and vitamin D on bone turnover in older women. Osteoporos Int 1996, 6: 314-9.
34. Kamel S, Brazier M, Rogez JC, et al. Different responses of free and peptide-bound cross-links to vitamin D and calcium supplementation in elderly women with vitamin D insufficiency. J Clin Endocrinol Metab 1996, 81: 3717-21.
35. Reid IR, Mason B, Horne A, et al. Effects of a calcium supplementation on serum lipid concentrations in normal older women: a randomized controlled trial. Am J Med 2002, 102: 343-7.
36. Kautzky-Willer A, Pacini G, Barnas U, et al. Intravenous calcitriol normalizes insulin sensitivity in uremic patients. Kidney Int 1995, 47: 200-6.
37. Huang Y, Ishizuka T, Miura A, et al. Effect of 1 alpha, 25-dihydroxyvitamin D_3 and vitamin E on insulin-induced glucose uptake in rat adipocytes. Diabetes Res Clin Pract 2002, 55: 175-83.
38. Price PA, June HH, Buckley JR, Williamson MK. Osteoprotegerin inhibits artery calcification induced by warfarin and by vitamin D. Arterioscler Thromb Vasc Biol 2001, 21: 1610-6.
39. Mohamed-Ali V, Goodrick S, Rawesh A, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosing factor-alpha, *in vivo*. J Clin Endocrinol Metab 1997, 82: 4196-200.