Age and Menopausal Status Affect Osteoprotegerin and Osteocalcin Levels in Women Differently, Irrespective of Thyroid Function

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ABSTRACT: Osteoprotegerin (OPG) and osteocalcin (OC) are essential bone proteins. Recent studies have demonstrated that they are not secreted solely by bone cells; they play roles in the vascular function and energy metabolism, and they are influenced by multiple factors. The aim of the current study was to investigate the influence of menopause and age on OPG and OC in women with different thyroid-stimulating hormone (TSH) levels.

MATERIAL AND METHODS: We studied 49 women with elevated TSH, 26 with suppressed TSH, and 67 age-matched euthyroid controls. Of them 64 were menstruating and 78 postmenopausal. Body weight, height, waist circumference (WC), body mass index (BMI), serum TSH, free thyroxin (FT4), OPG, and OC were measured.

RESULTS: Generally, both OPG and OC were higher in the postmenopausal women than in the menstruating subjects (OPG 3.85 ± 1.49 pmol/L vs. 5.84 ± 2.42 pmol/L, P < 0.001; OC 8.84 ± 3.70 ng/dL vs. 12.87 ± 6.45 ng/dL, P < 0.001), and within the two thyroid dysfunction subgroups and the controls (all P < 0.05). OPG correlated with age (postmenopausal rho = 0.57, P < 0.001; premenopausal rho = 0.31, P = 0.015). Among the premenopausal subjects, OPG was higher in those with low TSH than in the controls (P = 0.048). OC correlated negatively with BMI and WC in the postmenopausal group (Spearman rho = −0.25, P = 0.03 and rho = −0.42, P < 0.001 respectively). OC was higher in the postmenopausal subjects with low TSH than in those with elevated TSH (P = 0.024), and correlated positively with FT4 (rho = 0.40, P = 0.002) and negatively with TSH (rho = −0.29, P = 0.013).

CONCLUSIONS: In women, OPG and OC depended differently on age and menopause and, to a lesser extent, on the thyroid function and body composition.

KEYWORDS: osteoprotegerin, osteocalcin, menopause, thyroid

Introduction

Both osteoprotegerin (OPG) and osteocalcin (OC) are produced by the osteoblast (OB) cells, but they are regulated by different mechanisms and enter different metabolic pathways in the body. OPG is a decoy receptor of the receptor activator of nuclear factor kappa ligand (RANKL) and mainly acts to regulate osteoclast differentiation and activation. It is a secretory glycoprotein, belonging to the Tumor necrosis factor (TNF) receptor superfamily. Though initially studied as a molecule regulating bone remodeling, it is now evident that it is produced in various tissues and organs, eg the skin, the liver, the stomach, the intestine, the lungs, and the vascular endothelium. OPG has been implicated in the pathogenesis of atherosclerotic disease and has been thought to be a mediator of arterial calcification. It may be both a marker of the atherosclerotic process in the vascular wall and a protective factor restricting atherogenesis. Some authors view it as a possible marker of cardiovascular disease. OC, on the other
hand, is a secretory glycoprotein, produced almost exclusively by bone OBs, though there are data that it may be secreted by other cell types as well.\textsuperscript{8,9} Several recent papers have suggested an additional function of OC outside the bone tissue: modulating intermediary metabolism.\textsuperscript{10–12} It seems that OC might be a direct regulator of glucose homeostasis, linking the skeleton with the intermediary metabolism, as reviewed by Kassi and Papavassiliou a couple of years ago.\textsuperscript{13} It is also possible that OC and other bone-derived proteins undergoing carboxylation (bone matrix proteins) might be involved in the process of atherosclerotic vascular damage.\textsuperscript{9} Both circulating OPG and OC levels are influenced by age and a variety of other factors. One of them, thyroid function, has a profound impact on bone remodeling and bone cell metabolism, and as such influences the serum concentrations of both molecules. OPG has been found to be increased in both hypothyroidism and hyperthyroidism and to change with the thyroid function.\textsuperscript{14–16} OC, on the other hand, is also influenced by the levels of the thyroid hormone (TH) and the thyroid-stimulating hormone (TSH) and increases with hyperthyroidism, but decreases with depressed TH levels.\textsuperscript{17} Bone metabolism changes with menopause and might, therefore, influence the OPG and OC levels. Botella-Carretero et al recently demonstrated that the menopausal status might be an independent factor determining the OPG levels irrespective of the thyroid function.\textsuperscript{18} The aim of the current study was to investigate the influence of menopause and age on OPG and OC levels in women with different TSH levels who are not taking any TH-affecting medication.

Material
The participants were recruited from the cohort of a nationwide epidemiological screening study carried out by a team at the University Hospital of Endocrinology with the Medical University of Sofia. A total of 2,406 subjects were enrolled in a survey of thyroid disorders, cardiovascular morbidity, and diabetes in six areas in Bulgaria. Of these, 1,348 (56%) were female. All female subjects with elevated or suppressed TSH were assessed for inclusion in the present post-hoc analysis. Women with a history of current thyrotoxicosis or hypothyroidism who were receiving TH replacement or antithyroid medication were excluded. Estrogen or combined estrogen/progestin replacement therapy was an exclusion criterion as well, as were hypercalcemia or hypocalcemia. Forty-nine women (35%) with elevated TSH, and 26 (18%) with suppressed TSH, as found from the thyroid screening, met these criteria. Sixty-seven (67%) age-matched euthyroid women from the same cohort were included as a control group. As a result, a total of 142 female subjects were included in the current analysis. Of them 64 (47%) were menstruating and 78 (53%) were postmenopausal, defined as having passed at least 12 months after the last menstrual period (Table 1). None of the studied women were taking antosteoporotic medication that could influence the OC and/or OPG levels at the time of the study. The bone mineral density of the study subjects was unknown, as was their vitamin D status since these were not included in the screening.

Methods
All participants signed an informed consent that had been approved by the local ethics committee, and filled in a structured questionnaire containing data on current disorders and medication, personal and family medical history, smoking, and menopausal status. Body height, body weight, and waist circumference (WC) were measured as recommended\textsuperscript{19} and the body mass index (BMI) was calculated using the standard formula. The subjects were categorized by WC following the International Diabetes Federation (IDF) criteria: normal WC up to 80 cm, and increased WC >80 cm. Fasting blood sample was collected for TSH, free thyroxin (FT4), OPG and OC measurement.

Ultrasonic TSH was determined by a microparticulate immunoenzyme analysis (MEIA) using an automated

| Table 1. Characteristics of the studied subjects. The data are presented as means ± SD, median and interquartile range, or proportions and 95% confidence interval. The Student t-test was applied for age, BMI, and OC, and Mann–Whitney U-test was used for OPG, WC, TSH, and FT4 to compare the menstruating and the postmenopausal groups. Abdominal obesity prevalence is expressed as the prevalence of subjects with increased WC. |
|---|---|---|
| **MENSTRUATING** | **PM** | **P** |
| **N = 64 (45%, 37–53)** | **N = 78 (55%, 47–63)** |  |
| Age (y) | 39.7 ± 7.8 | 61.0 ± 10.0 | <0.001 |
| BMI (kg/m\(^2\)) | 24.95 ± 4.38 | 27.44 ± 4.92 | 0.002 |
| Waist (cm) | 77 (70.5–88.5) | 89 (81–95) | <0.001 |
| OPG (pmol/L) | 3.61 (2.94–4.38) | 5.3 (4.36–6.11) | <0.001 |
| OC (ng/dL) | 8.84 ± 3.70 | 12.87 ± 6.45 | <0.001 |
| TSH (mU/L) | 2.49 (0.69–4.57) | 4.50 (0.87–8.83) | 0.013 |
| FT4 (pmol/L) | 12.5 (10.6–13.6) | 10.9 (9.2–13.9) | 0.097 |
| Abdominal obesity n (%), 95% CI | 27 (42%, 30–54) | 54 (69%, 58–80) | 0.002 |

**Abbreviation:** PM, postmenopausal.
analyzer, AxSYM (ABBOTT, USA). The analytical sensitivity of the method was 0.01 μIU/mL, and the functional sensitivity was 0.011 μIU/mL. Intra-assay coefficient of variation (CV) was 5.64%. Subjects with values between 0.4 and 5.0 mIU/L were assumed to be euthyroid. TSH below 0.4 mIU/L was considered suppressed and TSH above 5.0 mIU/L was considered elevated. FT4 was measured using a two-step enzyme immunoassay (Access Free T4) with a reference range of 9.0−19.0 pmol/L. FT4 was measured with a presumptive view to identify the subjects with subclinical and overt thyroid dysfunction. The splitting up of the subjects in this way did not yield any useful information, and therefore, the data are not shown. Furthermore, the FT4 levels were used in the correlation analysis and as a factor in the regression analysis.

Serum total OPG was determined by a sandwich ELISA (BioVendor—Laboratorní medicína a s.p, Czech Republic). OPG was expressed in pmol/L. The analytical limit of detection was 0.13 pmol/L. The assay sensitivity was 0.4 pmol/L. The intra-assay CV was 4.5%, the inter-assay CV was 5.64%, and the normal range (mean ± SEM) was 4.1 ± 2.3 pmol/L. OC was determined using a solid phase enzyme amplified sensitivity immunoassay (BioSource hOST-EASIA Kit, Biosource, Belgium). OC was expressed in ng/mL. The method measured intact OC only, and no discrimination of carboxylated and uncarboxylated OC was possible. The assay sensitivity was 0.4 ng/mL. The intra-assay CV was 1.0% and the inter-assay CV was 5.3%. The reference range was 5 to 25 ng/mL.

Statistical Analysis

The numerical data are presented as means and standard deviations or median and interquartile range. The categorical data are presented as proportions and 95% confidence interval. The normality of distribution was assessed by means of a Kolmogorov–Smirnov test. The Student t-test was applied for comparison of normally distributed data. The Mann–Whitney U-test and the Kruskal–Wallis test were used for comparison of non-normally distributed continuous variables and for groups with a low number of cases. Spearman’s rho was used to test the correlations between numerical variables that did not follow the normal distribution. A multivariate regression analysis was also applied. Two-sided P-values below 0.05 were considered statistically significant. 95% confidence intervals were applied in all tests. The data processing was done using SPSS for Windows v. 13 (SPSS Inc., Chicago, IL, USA).

Results

The characteristics of the studied subjects stratified by their menstrual status are presented in Table 1. We found a significantly higher WC, BMI, and TSH in the postmenopausal group. The prevalence of abdominal obesity was significantly higher among the postmenopausal women than in the menstruating ones (Pearson Chi square P < 0.001). The characteristics of the subjects in the two thyroid function groups and the euthyroid control group are presented in Table 2.

### Table 2. Comparison between the characteristics of the menstruating and postmenopausal subjects, grouped by thyroid function. The Student t-test was applied for age, BMI, OPG, FT4, and OC, and Mann–Whitney U-test was used for all analyses in the low TSH group because of the small case number, as well as for WC and FT4. Kruskal–Wallis and median tests were applied to compare the OPG and OC levels among the thyroid function groups.

| Group              | Age (Years) | BMI (kg/m²) | Waist (cm) | OPG (pmol/L) | OC (ng/dL) | TSH (mU/L) | FT4 (pmol/L) |
|--------------------|-------------|-------------|------------|--------------|------------|------------|--------------|
| **Low TSH group**   |             |             |            |              |            |            |              |
| Menstruating, n = 13 | 40.9 ± 6.1  | 23.7 ± 3.8  | 80.3 ± 12.9 | 4.26 ± 1.51‡ | 10.71 ± 4.42 | 0.11 ± 0.09 | 19.9 ± 13.6  |
| PM, n = 13          | 60.5 ± 6.1  | 27.3 ± 5.1  | 85.7 ± 11.8 | 5.76 ± 2.08  | 16.85 ± 7.29* | 0.17 ± 0.11 | 18.0 ± 6.7   |
| P                  | <0.001      | 0.048       | 0.243      | 0.034        | 0.044      | 0.153      | 0.756        |
| **Elevated TSH group** | 43.4 ± 14.2 | 26.3 ± 5.1  | 83.9 ± 13.3 | 4.11 ± 2.43  | 8.37 ± 6.42 | 10.44 ± 1.54 | 10.4 ± 1.8   |
| Menstruating, n = 15 | 61.6 ± 6.7  | 26.7 ± 4.9  | 88.7 ± 10.6 | 5.94 ± 0.94  | 10.76 ± 3.87* | 14.48 ± 12.54 | 9.6 ± 3.0    |
| PM, n = 34          |            |            |            |              |            |            |              |
| P                  | <0.001      | 0.878       | 0.275      | 0.001        | 0.027      | 0.004      | 0.123        |
| **Euthyroid control group** | 37.7 ± 13.4 | 24.9 ± 4.7  | 79.8 ± 12.4 | 3.59 ± 1.94  | 8.38 ± 6.73 | 2.57 ± 0.11 | 12.0 ± 10.6  |
| Menstruating, n = 36 | 60.4 ± 8.3  | 28.4 ± 4.3  | 89.9 ± 13.2 | 5.77 ± 1.64  | 13.64 ± 3.23 | 2.44 ± 1.49 | 12.9 ± 1.8   |
| PM, n = 41          |            |            |            |              |            |            |              |
| P                  | <0.001      | 0.005       | <0.001     | <0.001       | 0.002      | 0.763      | 0.155        |

Notes: ‡P = 0.048; *P = 0.024.

Abbreviation: PM, postmenopausal.
the menstruating group, with a lowest point in the euthyroid subgroup. In the postmenopausal women, OPG correlated strongly and positively with age, Spearman’s rho = 0.57, P < 0.001 (Table 3). In the premenopausal women, the correlation with age was weaker (Spearman’s rho = 0.31, P = 0.015). In neither of the two groups did OPG correlate with WC, BMI, FT4, or TSH, which was further confirmed by the multifactor regression analysis (Table 4). The only significant factor in the latter was the subjects’ age.

**OC**

The OC levels were significantly higher in the postmenopausal group compared to the premenopausal one, irrespective of the thyroid function (Table 2). The OC levels were significantly lower in the postmenopausal women with elevated TSH than in those with suppressed TSH (P = 0.047). The levels in the euthyroid controls fell in between, but did not differ significantly from the two other groups. The differences within the menstruating group were not significant, either. OC correlated negatively with BMI and WC in the postmenopausal group (Spearman rho = −0.23, P = 0.032 and rho = −0.420, P < 0.001), but not with age (Table 3). In the premenopausal group, OC did not correlate with any of the studied parameters, except the BMI (Spearman’s rho = 0.31, P < 0.019).

In the postmenopausal group, the OC levels correlated positively with FT4 (rho = 0.40, P = 0.002) and negatively with TSH (rho = −0.29, P = 0.013). The only significant factors for the OC levels in the multifactor regression analysis were the menopausal status and WC (Table 4).

**Discussion**

We observed higher OPG and OC levels in the postmenopausal women compared to those with preserved menstrual cycle. On the other hand, no parallel was found between OPG and OC with respect to their association with the other studied parameters in our cohort. The thyroid function was associated differently with the levels of the two proteins. OPG levels demonstrated a trend toward lower values in the euthyroid subjects compared to those with elevated or suppressed TSH. Our results are very close to the data reported by Botella-Carretero et al in a small group of thyroxin-treated thyroid cancer subjects. It is noteworthy that the curves of both

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**Table 3. Correlations among the studied parameters.**

| MENSTRUAL STATUS | AGE (Y) | BMI (kg/m²) | WAIST (cm) | TSH (mU/L) | FT4 (pmol/L) |
|------------------|---------|-------------|------------|------------|-------------|
| Menstruating N = 64 | OPG (pmol/L) | r 0.31 0.19 0.16 0.08 −0.24 | P 0.015 0.142 0.220 0.529 0.117 |
|                      | OC (ng/dL) | r −0.02 −0.31 −0.15 −0.23 0.27 | P 0.876 0.019 0.269 0.091 0.083 |
| Postmenopausal N = 78 | OPG (pmol/L) | r 0.57 −0.15 −0.02 0.09 0.15 | P < 0.001 0.195 0.848 0.421 0.283 |
|                      | OC (ng/dL) | r −0.02 −0.23 −0.42 −0.29 0.40 | P 0.898 0.032 <0.001 0.013 0.002 |

**Table 4. Results of the multiple regression analysis with osteocalcin and osteoprotegerin as independent variables.**

|                      | UNSTANDARDIZED COEFFICIENTS | STANDARDIZED COEFFICIENTS | t | SIG. |
|----------------------|-----------------------------|---------------------------|---|------|
| **Osteocalcin**      |                             |                           |   |      |
| (Constant)           | 15.985                      | 3.455                     | 4.626 | <0.001 |
| Age (years)          | −0.031                      | 0.053                     | −0.074 | −0.583 | 0.56  |
| TSH (mU/L)           | −0.065                      | 0.046                     | −0.117 | −1.405 | 0.16  |
| Menstrual status     | 5.384                       | 1.490                     | 0.463 | 3.614 | <0.001 |
| BMI (kg/m²)          | −0.222                      | 0.100                     | −0.188 | −2.233 | 0.027 |
| **Osteoprotegerin**  |                             |                           |   |      |
| (Constant)           | −0.235                      | 1.099                     | −0.214 | 0.83  |
| Age (years)          | 0.112                       | 0.017                     | 0.691 | 6.560 | <0.001 |
| TSH (mU/L)           | −0.009                      | 0.016                     | −0.039 | −0.559 | 0.58  |
| Menstrual status     | −0.348                      | 0.489                     | −0.076 | −0.712 | 0.48  |
| BMI (kg/m²)          | −0.012                      | 0.033                     | −0.027 | −0.376 | 0.71  |
OPG and OC in the two studies are quite similar despite the different settings: a longitudinal follow-up of a single group vs. a comparison of three subpopulations with a different thyroid function. Therefore, our work complements the already existent data. We observed, however, an opposite association with the subjects’ menstrual status and age.

The mechanisms underlying the higher OPG and OC in the postmenopausal hyperthyroid subjects are still not very well understood. One possible explanation is the increase in bone turnover through the effect of TH and reduced TSH or by an altered cytokine production, as demonstrated in other studies.\textsuperscript{17,20} The OPG increase in the hypothyroid state reported by other authors is even less clear. One possible explanation is the endothelial dysfunction observed in hypothyroid subjects, since bone remodeling is decreased in hypothyroidism and should not contribute to high OB-derived molecule level. A study by Martini et al demonstrated an increase in RANKL but not in OPG with the administration of recombinant TSH.\textsuperscript{21} It is also suggested that TSH may exert a bone-sparing effect by the Wnt signaling pathway rather than by the OPG.\textsuperscript{22} Moreover, it is generally unknown what proportion of the circulating OPG is derived from the bone or from other tissues, e.g., the vascular endothelium. OPG has been found to be elevated in subjects with atherosclerotic vascular disease, and to be linked to cardiovascular risk.\textsuperscript{11} Some authors, however, question this relationship.\textsuperscript{23} The increased bone turnover and the state of a mild chronic systemic inflammation with the menopause and aging, on the other hand, may explain the loss of the difference among the three thyroid function groups in the postmenopausal women. We performed a linear regression model in an attempt to assess the effect of multiple possibly interacting factors, especially as the postmenopausal subjects were significantly older than the menstruating ones. The only factor in the model that influenced significantly the OPG levels was age.

In our study, OC showed an almost linear positive relationship with the thyroid function. The increase in OC levels with increasing TH levels was expected, but we observed it only in the postmenopausal women, probably resulting from the more prominent effect of hyperthyroidism on bone remodeling after menopause.\textsuperscript{24,25} The only significant factors associated with the OC levels in the regression analysis, however, were the menopausal status and WC. In the postmenopausal group, OC correlated negatively with WC and BMI. These findings can be interpreted to a certain extent in the light of previously published data.\textsuperscript{26} WC is a measurement of abdominal obesity and is closely linked to insulin resistance.\textsuperscript{27} Our results, therefore, support the hypothesis of an association between OC and insulin sensitivity. Lee et al found a negative association of OC with the markers of insulin resistance in an animal model, thus demonstrating a possible link that closes the loop between bone and energy metabolism.\textsuperscript{28} Feron et al\textsuperscript{29} and Pittas et al\textsuperscript{30} studied the markers of metabolic syndrome in human subjects and reached similar conclusions.

The markedly weaker negative correlation of OC with BMI may mean that OC is related to body composition through the insulin/tissue insulin sensitivity/visceral fat and not through the total fat mass, as suggested by Pepene et al,\textsuperscript{31} who explored the potential role of OC in Polycystic ovary syndrome patients. A negative association of OC with the markers of insulin resistance in postmenopausal women has been reported also by Kanazawa et al.\textsuperscript{32} The same authors observed a negative correlation of OC with intima-media thickness and adiposity in type 2 diabetic subjects. These findings, however, have been brought into question by the data published by Mori et al\textsuperscript{33} and Schwartz et al,\textsuperscript{34} which illustrate how complex and equivocal the subject is.

One possible explanation for the totally different associations of OC in the premenopausal and postmenopausal women observed by us is that the estrogens are a very strong determinant of body composition and bone turnover in women. After the decline of the ovarian estrogen production other mechanisms take over. García-Martín et al demonstrated a positive association of increased follicle stimulating hormone and luteinizing hormone with the markers of bone turnover in postmenopausal women.\textsuperscript{35} The increased peri- and postmenopausal bone turnover is accompanied by a higher release of OC in the systemic circulation, which attenuates the insulin resistance and probably counteracts the trend toward hyperinsulinemia and increasing abdominal fat in aging females.\textsuperscript{36,37} It can be speculated that in the setting of a profound estrogen deficiency, the relative homeostatic interactions between bone and energy metabolism gain more significance.

OPG is also affected by the menopausal transition. Unlike OC, its levels are influenced by age, and this was seen more prominently in the postmenopausal group. Whether this was merely the result of a nonlinearity of the association with an age threshold at which the slope becomes steeper is hard to conclude. Possibly this finding might be related to the increased cardiovascular morbidity in the postmenopausal women. It is still debatable whether estrogen deficiency is the sole cause of the steep increase in cardiovascular diseases in this group. Age might contribute independently by modulating the cellular effects of the sex steroids.\textsuperscript{38} The higher prevalence of abdominal obesity, dyslipidemia, and arterial hypertension form a more prevalent “metabolic syndrome” phenotype in the postmenopausal women.

The current study has several limitations. The thyroid function subgroups are relatively small, especially the group with suppressed TSH. We did not separate the subjects with subclinical and overt thyroid dysfunction because the size of the groups would have dropped further and statistical processing would not have been applicable. The influence of thyroid function might be more demonstrable with larger groups. It would also be interesting to measure the fasting immunoreactive insulin levels and fasting blood glucose. Investigating OPG and OC would also be more informative if bone mineral
REFERENCES

1. Simonet W, Lacey DL, Dunstan CR, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell. 1997;89:309–319.

2. Lacey DL, Timme E, Tan HL, et al. Osteoprotegerin (OPG) ligand is a cytokine that regulates osteoclast differentiation and activation. Cell. 1998;93:165–176.

3. Hofbauer LC, Heufelder AE. The role of osteoprotegerin and receptor activator of NF-κB ligand in the pathogenesis and treatment of metabolic bone diseases. J Clin Endocrinol Metab. 2000;85:2355–2365.

4. Reid P, Holen I. Pathophysiological roles of osteoprotegerin (OPG). Eur J Cell Biol. 2009;88:1–17.

5. Morony S, Tintut Y, Zhang Z, et al. Osteoprotegerin inhibits vascular calcification without affecting atherosclerosis in LDLr−/− mice. Circulation. 2008;117:411–420.

6. Wik A, Mathiesen EB, Johansen SH, et al. Serum oestrogen, sRANKL and carotid plaque formation and growth in a general population: the Tromsø study. J Thromb Haemost. 2008;6:896–905.

7. Gordin D, Soro-Paavonen A, Thomas MC, et al. Osteoprotegerin is an independent predictor of vascular events in Finnish adults with type 1 diabetes. Diabetes Care. 2013;36:1827–1833.

8. Haushcka PY, Lian JB, Cole DE, Gundberg CM. Osteocalcin and matrix protein: vitamin K-dependent protein in bone. Physiol Rev. 1989;69:990–1047.

9. Gissl M, Modder U, Atkinson EJ, Lerman A, Khosla S. Osteocalcin expression by circulating endothelial progenitor cells in patients with coronary atherosclerosis. J Am Coll Cardiol. 2008;52:1314–1325.

10. Vestri HS, Luna-Castro C, Moelarring DR, Gunberg CM, Garvey WT. Osteocalcin is not just for bones: effects on adipocytes and role in human metabolism. Diabetologies. 2008;57: A29 (Abstract).

11. Kanazawa I, Yamaguchi T, Yamamoto M. Serum osteocalcin level is associated with glucose metabolism and atherosclerosis parameters in type 2 diabetes mellitus. J Clin Endocrinol Metab. 2009;94:47–49.

12. Im JA, Yu BP, Jeon JY, Kim SH. Relationship between osteocalcin and glucose metabolism in postmenopausal women. Clin Clin Acta. 2008;396:66–69.

13. Kassi E, Papavassiliou AG. A possible role of osteocalcin in the regulation of insulin secretion: human in vivo evidence? J Endocrinol. 2008;199:151–153.

14. Nagasaku T, Inaba M, Jono S, et al. Increased levels of serum osteoprotegerin in hypothyroid patients and its normalization with restoration of normal thyroid function. Eur J Endocrinol. 2005;152:347–353.

15. Mikosch P, Obermayer-Pietsch B, Jost R, et al. Bone metabolism in patients with differentiated thyroid carcinoma receiving suppressive levothyroxine treatment. Thyroid. 2003;13:347–156.

16. Guan-da X, Hui-ling S, Zhi-song C, Lin-shuang Z. Changes in plasma concentrations of osteoprotegerin before and after levothyroxine replacement therapy in hypothyroid patients. J Clin Endocrinol Metab. 2005;90:5765–5768.

17. El Hadidy EHM, Ghonaim M, El Gawad SSA, El Arfa MA. Impact of severity, duration, and etiology of hyperthyroidism on bone turnover markers and bone mineral density in men. BMC Endocr Disord. 2011;11:15.

18. Botella-Carretero J, Alvarez-Blasco F, San Millan J, Escobar-Morreale H. Thyroid hormone deficiency and postmenopausal status independently increase serum osteoprotegerin concentrations in women. Eur J Endocrinol. 2007;156:539–545.

19. World Health Organization. Waist Circumference and Waist–Hip Ratio: Report of a WHO Expert Consultation, Geneva, 8–11 December 2008; 2011:5.

20. Mysliwiec J, Adamczyk M, Nikulajek A, Gorska M. Interleukin-6 and its considered role in the pathogenesis of thyrotoxicosis-related disturbances of bone turnover in postmenopausal women. Endokrynol Pol. 2011;62:299–302.

21. Martin G, Gennari L, De Paola V, et al. The effects of recombinant TSH on bone turnover markers and serum osteoprotegerin and RANKL levels. Thyroid. 2008;18:455–460.

22. Balizar R, Latif R, Berkwote J, et al. Thyroid-stimulating hormone induces a Wnt-dependent, feed-forward loop for osteoblastogenesis in embryonic stem cell cultures. Proc Natl Acad Sci U S A. 2011;108:16277–16282.

23. Olesen M, Skov V, Megta M, Mumm BH, Rasmussen LM. No influence of OPG and its ligands, RANKL and TRAIL, on proliferation and regulation of the calcification process in primary human vascular smooth muscle cells. Mol Cell Endocrinol. 2002;181:149–156.

24. Kung AVC, Lorenzo T, Tam SCF. Thyrotoxic suppression therapy decreases bone mineral density in postmenopausal women. Clin Endocrinol. 1993;39:535–540.

25. Belaya Z, Melnichenko G, Rozhinskya L. Subclinical hyperthyroidism of variable etiology and its influence on bone in postmenopausal women. Hormones (Athens). 2006;7:62–70.

26. Kindblom JM, Ohlson C, Ljunggren O, et al. Plasma osteocalcin is inversely related to fat mass and plasma glucose in elderly Swedish men. J Bone Miner Res. 2009;24:785–791.

27. Heatley CJ, Wang PW, Chen TY. The relationship between regional abdominal fat distribution and both insulin resistance and subclinical chronic inflammation in non-diabetic adults. Diabetology. 2014;6:49.

28. Lee NK, Sowa H, Hinoi E, et al. Endocrine regulation of energy metabolism by the skeleton. Cell. 2007;130:456–469.

29. Ferron M, Hinoi E, Karatay G, Ducy P. Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. Proc Natl Acad Sci U S A. 2008;105:5266–5270.

30. Pittas A, Harris S, Eliaades M, Stark P, Dawson-Biggs B. Association between serum osteocalcin and markers of metabolic phenotype. J Clin Endocrinol Metab. 2008;93:827–832.

31. Pepene CE. Serum under-carboxylated osteocalcin levels in women with poly cystic ovary syndrome: weight-dependent relationships with endocrine and metabolic traits. J Ovarian Res. 2003;6:4.

32. Kanazawa I, Yamaguchi T, Tada Y, Yamauchi M, Yano S, Sugimoto T. Serum osteocalcin level is positively associated with insulin sensitivity and secretion in patients with type 2 diabetes. Bone. 2011;48:720–725.

33. Mori K, Emoto M, Motoyama K, et al. Undercarboxylated osteocalcin does not correlate with insulin resistance as assessed by euglycemic hyperinsulinemic clamp technique in patients with type 2 diabetes mellitus. Diabetology. 2012;4:53.

34. Schwartz AV, Schafer AL, Grey A, et al. Effects of anti-insuropic therapies on glucose metabolism: results from the FIT, HORIZON-PFT and FREEDOM trials. J Bone Miner Res. 2013;28(6):1348–1354.

35. Garcia-Martín A, Reyes-García R, García-Castro JM, Rosas-Moreno P, Escobar-Jiménez F, Muñoz-Torres M. Role of serum FSH measurement on bone resorption in postmenopausal women. Endocrine. 2012;41:302–308.

36. Akin O, Gol K, Aktruk M, Erkaya S. Evaluation of bone turnover in postmenopausal patients with type 2 diabetes mellitus using biochemical markers and bone mineral density measurements. Gynecol Endocrinol. 2003;17:19–29.

37. Gaspari U. Hyperinsulinemia, a key factor of the metabolic syndrome in postmenopausal women. Maturitas. 2009;62:362–365.

38. Qiao X, McConnell KR, Khalil RA. Sex steroids and vascular responses in hypertension and aging. Gend Med. 2008;5(suppl 3):846–864.