Osteocalcin as a predictor of the metabolic syndrome in older persons: a population-based study

Mirjam M. Oosterwerff, Natasja M. van Schoor, Paul Lips and Elisabeth M. W. Eekhoff

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Abstract

Background
Recent evidence indicates that the osteoblast-derived protein osteocalcin is able to influence adiposity and glucose homeostasis in mice. Little is known about this relationship in humans.

Objective
To investigate the association of plasma osteocalcin levels with the metabolic syndrome in a community-dwelling cohort of older persons in the Netherlands.

Design and Participants
Data were used from the Longitudinal Aging Study Amsterdam (LASA), an ongoing multidisciplinary cohort study in a representative sample of the older Dutch population (≥ 65 years old). A total of 1284 subjects (629 men and 655 women) between the ages of 65 and 88 years participated in this study.

Measurements
Metabolic syndrome (U.S. National Cholesterol Education Program definition) and its individual components were assessed as well as plasma osteocalcin levels.

Results
Among the participants, the prevalence of the metabolic syndrome was 37.1%. The median osteocalcin level was 2.0 nmol/l. Plasma osteocalcin was inversely associated with the metabolic syndrome. The odds ratio (OR) was 3.68 with 95% confidence interval (CI) 2.53-5.34 for the lowest osteocalcin quartile compared to the highest quartile. The association between osteocalcin and the metabolic syndrome was mainly determined by high triglycerides, low HDL, waist circumference and hypertension.

Conclusion
Low plasma osteocalcin levels are strongly associated with the metabolic syndrome in an older community-dwelling population.
Introduction

Osteocalcin, an osteoblast derived protein, is a biochemical marker of bone formation and plays a role in bone mineralization and calcium homeostasis. Currently, there is growing interest in the additional roles of osteocalcin and evidence is accumulating that it may play a role in the regulation of glucose and fat metabolism, linking bone metabolism with energy homeostasis (1).

From osteocalcin -/- knockout mice studies, it is known that osteocalcin-deficient mice exhibit glucose intolerance, increased fat mass, insulin resistance and decreased energy expenditure (2). Administration of recombinant osteocalcin to wild-type mice caused an increase in blood insulin levels, enhanced glucose tolerance, improved insulin sensitivity and a decrease in development of obesity (3). In human studies, a low plasma osteocalcin has been associated with the parameters adiposity, low insulin secretion and insulin resistance (4-6), as well as the metabolic syndrome (7-11). However, the clinical studies in humans that examined the association between osteocalcin and the metabolic syndrome are limited. Three studies included only men (7, 8, 11) and two other studies were not population-based and included younger populations (9, 10).

Because the metabolic syndrome is a major public burden, with a 5-fold risk of developing type 2 diabetes and 2-3-fold risk of developing cardiovascular disease, (12, 13) the present study was undertaken to investigate whether a low osteocalcin level is associated with the metabolic syndrome and can predict the metabolic syndrome in an older population. To our knowledge, this is the first population-based study in a representative sample of older men and women.

Materials and Methods

Study Sample

Data were collected within the Longitudinal Aging Study Amsterdam (LASA), an ongoing interdisciplinary cohort study on predictors and consequences of changes in autonomy and well-being in the aging population in the Netherlands (for more details see Deeg et al.) (14).

A random sample of men and women aged 55 years and over, stratified by age, sex, urbanization grade and expected 5-year mortality rate was drawn from the population registers of 11 municipalities in three regions of the Netherlands, being a representative sample of the Dutch population. In total, 3107 predominantly Caucasian (>99%) respondents were enrolled in the baseline examination in 1992/1993.

The present study was performed in a subgroup of the LASA population, including persons who participated in the medical interview of the second data collection (1995/1996), which was restricted to subjects who were aged ≥ 65 years on January 1, 1996 (n=1509).
Blood samples were obtained in the same year from 1352 subjects. Metabolic syndrome and osteocalcin levels could be determined for 1284 respondents and these were included in the analysis. Informed consent was obtained from all respondents and the study was approved by the Ethical Review Board of the VU University Medical Center.

**Plasma osteocalcin**

Blood samples were collected in the morning in 1995/1996. Subjects were allowed to have tea and toast, but no dairy products. The blood samples were centrifuged and stored at -20°C. Plasma intact osteocalcin was determined using an immunoradiometric assay in 1997/1998 (Biosource Diagnostics, Fleurus, Belgium). The intra- and inter-assay coefficients of variation were 3% at >1.7 nmol/l and 8% at 3.5 nmol/l, respectively. All analyses were performed at the Endocrine Laboratory of the VU University Medical Center.

**Metabolic syndrome**

Metabolic syndrome was defined as the presence of three or more of the following criteria: triglycerides ≥ 1.7 mmol/l, HDL cholesterol < 1.0 mmol/l for men and < 1.3 mmol/l for women; blood pressure ≥ 160/90 mmHg or antihypertensive medication; waist circumference >102 cm for men and > 88 cm for women; and fructosamine ≥ 0.247 mmol/l or antidiabetic medication in agreement with the definition established by the U.S. National Cholesterol Education Program (NCEP) Adult Treatment Panel III, with an increased cut-off for blood pressure, adjusted for an older population (15). Furthermore, the cut-off of 0.247 mmol/l for fructosamine corresponds to the cut-off of 6.1 mmol/l for fasting plasma glucose in terms of sensitivity and specificity in discriminating subjects with glucose intolerance from subjects with normal glucose tolerance. Fructosamine was used instead of glucose because a fasting state was not required when blood samples were obtained and fructosamine is little affected by eating (16).

**Assessment of components of the metabolic syndrome**

Blood pressure was measured using an Omron 706 automatic device while the subject was sitting. Waist circumference was determined as the average of two measurements calculated to the nearest 0.1 cm midway between the lower rib margin and the iliac crest after normal expiration. Medication use was assessed by recording the medications of the participant directly from the containers. Fructosamine was determined by a colourimetric test, and HDL cholesterol and triglycerides were determined by an enzymatic colourimetric test (Roche Diagnostics, Mannheim, Germany). The interassay coefficients of variation were < 2.8 % for fructosamine and triglycerides and < 6.4% for HDL cholesterol. All laboratory analyses (HDL cholesterol, triglycerides and fructosamine) were performed in EDTA-plasma samples stored at – 80 º C at the department of Clinical Chemistry of the VU
Potential confounders
Age, sex, education, number of chronic diseases, smoking, alcohol use, total physical activity, serum 25-hydroxyvitamin D (25 OHD) and serum parathyroid hormone (PTH) were considered as potential confounders. Data on sex and age were derived from population registries at baseline. Education level was assessed by asking respondents for the highest educational level completed, which was converted into total number of years of education (range 5-18 years). Smoking status was categorized as never, former and current smoker. Alcohol consumption was categorized as none, light, moderate and excessive. Physical activity was assessed with the LASA Physical Activity Questionnaire. The following activities were included: walking outdoors, bicycling, light and heavy household activities, sports activities and a total activity score was calculated as time spent on physical activity in minutes per day. This variable was divided into tertiles for analysis, with the first tertile representing the lowest activity (18). Diabetes and number of chronic diseases were assessed using algorithms in which information obtained from general practitioners, inspection of medicine bottles and self-report was combined. Self reported diabetes has been shown to be in good agreement with the general practitioner’s report (k=0.85) (19).

Statistical analyses
All analyses were performed using SPSS for Windows (version 15.0.1, SPSS, Inc., Chicago, IL). Characteristics of the study sample were presented by metabolic syndrome status and were compared using independent t tests for normally distributed continuous variables and Pearson chi-square tests for dichotomous variables. Continuous variables with a skewed distribution were compared using Mann-Whitney U tests. Associations were checked for linearity. Spearman and Pearson correlation coefficients were calculated to examine multicollinearity (r<0.4).

The analyses were performed with 3 osteocalcin dummy variables, with the highest quartile as reference group, because of the skewed distribution. The analyses were repeated with a log transformed osteocalcin variable. Dichotomous indicators were created for the individual components of the metabolic syndrome. Logistic regression analyses were performed to study the association between the metabolic syndrome and plasma osteocalcin, both unadjusted and adjusted for age, sex, education, smoking, alcohol, chronic diseases, physical activity, serum 25 OHD and PTH. The categorical variables smoking, alcohol and total physical activity were included in the regression analysis as dummies. The analyses were repeated after exclusion of subjects with diabetes, use of lipid-lowering drugs, steroids and bisphosphonates. The associations between osteocalcin and the individual components of the metabolic syndrome were analyzed by including...
each component both separately and together in a logistic regression model. All analyses were tested at the 0.05 level of significance, except for the interaction term, for which 0.10 was tolerated. Effect modification by gender was evaluated and tested by adding the product term of plasma osteocalcin x gender to the univariate model.

**Results**

The baseline characteristics of the subjects are presented in Table 1. Data are presented for subjects according to metabolic syndrome (n= 476) and no metabolic syndrome (n=808). Among all individuals, 629 male and 655 female, the prevalence of metabolic syndrome was 37.1%. The median age was 75.1 (range 64.8-88.4) yr. The median osteocalcin level was 2.01 nmol/litre (interquartile range 1.44-2.63). Subjects with the metabolic syndrome were more often women, had a significantly lower level of education, consumed less alcohol, suffered from more chronic diseases and diabetes and had a higher BMI. Furthermore, they had significantly lower plasma levels of osteocalcin and 25 OHD (p<0.001 and p=0.002). Osteocalcin was divided into quartiles with a range of Q1, 0.05-1.43 nmol/litre, Q2, 1.44-2.00 nmol/litre, Q3, 2.01-2.63 nmol/litre and Q4, 2.64-9.48 nmol/litre. No interaction was found with sex (P>0.10)

The results of the logistic regression analyses are presented in Table 2. After adjustment for confounders, a significant association between plasma osteocalcin levels and the metabolic syndrome was observed. Osteocalcin was represented by dummy variables, with the highest quartile of osteocalcin as reference group. The group with the lowest osteocalcin levels had a significant higher risk of the metabolic syndrome compared to subjects with the highest osteocalcin levels (OR= 2.63, 95% CI 1.89-3.66). After adjustments for relevant confounders (age, sex, education, smoking, alcohol use, chronic diseases, total activity, 25 OHD and PTH), this relationship was even more explicit (OR=3.68, 95% CI 2.53-5.34). In Table 2, in addition, the natural logarithmic (ln) transformed osteocalcin in association with the metabolic syndrome is shown. With increasing osteocalcin levels, the risk for the metabolic syndrome decreases (OR= 0.41, 95% CI 0.31-0.54).

Multivariate analysis results are shown in Table 3. Compared with the highest osteocalcin quartile, lower osteocalcin quartiles were significantly associated with hypertension, high triglycerides, high waist and low HDL levels and not with fructosamine.

After exclusion of diabetic patients (n=116) and exclusion of subjects using lipid-lowering drugs (n=63), steroids (n=58), bisphosphonates (n=16) and vitamin K antagonists (n=80) logistic regression analysis revealed similar results. The OR of the association between the natural logarithmic transformed osteocalcin and the metabolic syndrome was 0.40 (95% CI 0.29-0.55), with 993 subjects included in the analysis.
Table 1. Baseline

<table>
<thead>
<tr>
<th>Individual components</th>
<th>Metabolic syndrome</th>
<th>No metabolic syndrome</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal obesity (%)</td>
<td>81.4</td>
<td>34.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High triglycerides (%)</td>
<td>67.2</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>Hyperglycaemia (%)</td>
<td>42.9</td>
<td>14.8</td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>85.4</td>
<td>49.1</td>
<td></td>
</tr>
<tr>
<td>Low HDL cholesterol (%)</td>
<td>76.4</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Osteocalcin (nmol/l)</td>
<td>1.81[1.33-2.43]</td>
<td>2.13[1.59-2.77]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>25 OHD (nmol/l)</td>
<td>50.8±23.6</td>
<td>55.1±24.2</td>
<td>0.002</td>
</tr>
<tr>
<td>PTH (pmol/l)</td>
<td>3.25[2.51-4.46]</td>
<td>3.11[2.43-4.13]</td>
<td>0.08</td>
</tr>
<tr>
<td>Number of chronic diseases</td>
<td>1.50±1.18</td>
<td>1.10±0.98</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Smoking status (%)
- Never: 38.4
- Former: 46
- Current: 15.5

Alcohol consumption (%)
- None: 32
- Light: 46.9
- Moderate: 15.8
- Excessive: 5.3

Physical activity
- 135[71.8-204.0]
- 135[79.5-203.0]

Diabetes (%)
- 14.9
- 4.2

BMI
- 28.9±3.8
- 25.6±3.9

N=1284. Data are means±SD, %, or median [interquartile range]. P value of χ² tests for dichotomous variables, independent t test for continuous variables and Mann-Whitney U tests for skewed distributed variables.
Discussion

In this population-based study of older individuals, the metabolic syndrome was observed in more than one third of the subjects. Low plasma osteocalcin levels were strongly associated with the metabolic syndrome, independent of several confounders. Waist circumference, high triglycerides, hypertension and low HDL levels were the main contributors to the association between osteocalcin levels and the metabolic syndrome.

Our results are consistent with a recent human study regarding the relationship between osteocalcin and the metabolic syndrome. Tan et al. investigated men aged 20-69 years in China and found an inverse association of plasma osteocalcin with the metabolic syndrome (11). In earlier studies associations were observed between osteocalcin and insulin sensitivity and secretion (4, 5). Kindblom et al. found an inverse association between osteocalcin and fat mass and glucose in men (6). More recently, a few studies have shown inverse correlations between osteocalcin and the metabolic syndrome (7-11). However, in these studies subjects were younger than in our population (9-11), otherwise only men were studied (7, 8, 11). In addition, population-based studies including both men and women were not conducted.

Consistent with the mouse model, in which wild type mice receiving osteocalcin exhibited a dose-dependent decrease in serum triglyceride levels, our study showed higher triglyceride levels in subjects with low osteocalcin levels (2). The waist circumference was strongly negatively associated with osteocalcin, which is in line with animal and human studies. Hypertension was also associated with low plasma osteocalcin, confirming the study of Tan et al (11).

While this study does not prove causality, a causal relationship might lead to therapeutic implications. Synthetic osteocalcin could protect against the metabolic syndrome.

Table 2. Association between plasma osteocalcin and the metabolic syndrome

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>2.63 (1.89-3.66)</td>
<td>3.38 (2.39-4.80)</td>
<td>3.28 (2.29-4.71)</td>
<td>3.68 (2.53-5.34)</td>
</tr>
<tr>
<td>Q2</td>
<td>1.76 (1.26-2.46)</td>
<td>2.18 (1.54-3.08)</td>
<td>2.19 (1.53-3.13)</td>
<td>2.40 (1.66-3.46)</td>
</tr>
<tr>
<td>Q3</td>
<td>1.27 (0.90-1.76)</td>
<td>1.36 (0.96-1.92)</td>
<td>1.37 (0.96-1.95)</td>
<td>1.44 (1.00-2.05)</td>
</tr>
<tr>
<td>Q4</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Ln(osteocalcin)</td>
<td>0.51 (0.40-0.68)</td>
<td>0.43 (0.33-0.56)</td>
<td>0.44 (0.34-0.58)</td>
<td>0.41 (0.31-0.54)</td>
</tr>
</tbody>
</table>

Data are presented with osteocalcin divided in quartiles: Q1 = [0.05-1.43], Q2 = [1.44-2.00], Q3 = [2.01-2.63], Q4 = [2.64-9.48], the highest quartile of osteocalcin being used as reference group. Furthermore, the association between osteocalcin converted to the natural logarithm and metabolic syndrome is presented. Presented as odds ratios and 95% confidence intervals.

Model 1: Univariate. Model 2: as Model 1 and adjusted for sex and age. Model 3: as Model 2 and adjusted for alcohol use, 25 OHD, smoking, education, chronic diseases and physical activity. Model 4: as Model 3 and adjusted for PTH
The ultimate proof for causality would be the effect of recombinant osteocalcin on components of the metabolic syndrome.

Strengths of the present study include the large population-based sample of older individuals, including similar numbers of men and women, in contrast to most published studies. The age-stratified enrolment facilitated the exploration of age interactions. In this study we adjusted for many covariates that might confound the observed association. Osteocalcin has been reported to vary by age, sex, smoking and physical activity (20). In Table 3.

<table>
<thead>
<tr>
<th>Effects on components</th>
<th>Model 2</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>1.80(1.28-2.52)*</td>
<td>2.04(1.42-2.92)*</td>
</tr>
<tr>
<td>Q2</td>
<td>1.52(1.09-2.11)*</td>
<td>1.75(1.24-2.46)*</td>
</tr>
<tr>
<td>Q3</td>
<td>1.23(0.89-1.69)</td>
<td>1.33(0.95-1.84)</td>
</tr>
<tr>
<td>Q4</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>High triglycerides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>2.66(1.87-3.80)*</td>
<td>3.09(2.12-4.53)*</td>
</tr>
<tr>
<td>Q2</td>
<td>1.93(1.35-2.76)*</td>
<td>2.22(1.53-3.23)*</td>
</tr>
<tr>
<td>Q3</td>
<td>1.24(0.86-1.76)</td>
<td>1.32(0.91-1.91)</td>
</tr>
<tr>
<td>Q4</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Hyperglycaemia/fructosamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>1.81(1.26-2.62)*</td>
<td>1.45(0.98-2.14)</td>
</tr>
<tr>
<td>Q2</td>
<td>1.03(0.70-1.51)</td>
<td>0.88(0.59-1.32)</td>
</tr>
<tr>
<td>Q3</td>
<td>1.14(0.79-1.55)</td>
<td>1.06(0.73-1.55)</td>
</tr>
<tr>
<td>Q4</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Low HDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>2.2 (1.57-3.10)*</td>
<td>2.26(1.58-3.25)*</td>
</tr>
<tr>
<td>Q2</td>
<td>1.46(1.03-2.05)*</td>
<td>1.49(1.04-2.13)*</td>
</tr>
<tr>
<td>Q3</td>
<td>1.31(0.94-1.84)</td>
<td>1.39(0.99-1.96)</td>
</tr>
<tr>
<td>Q4</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Waist circumference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>2.82(2.00-4.01)*</td>
<td>3.14(2.17-4.54)*</td>
</tr>
<tr>
<td>Q2</td>
<td>2.30(1.63-3.23)*</td>
<td>2.58(1.81-3.69)*</td>
</tr>
<tr>
<td>Q3</td>
<td>1.38(1.00-1.91)*</td>
<td>1.46(1.04-2.04)*</td>
</tr>
<tr>
<td>Q4</td>
<td>Reference</td>
<td>Reference</td>
</tr>
</tbody>
</table>

Data are presented as odd’s ratios with 95% confidence intervals.
Model 2: adjusted for sex and age
Model 4: model 2 and adjusted for alcohol use, 25 OHD, smoking, education, chronic diseases, physical activity and PTH
The P value <0.05 was considered statistically significant and is depicted in the table as (*).
addition, serum 25 OHD levels have been related to the metabolic syndrome, as well as bone metabolism (21).

The use of early morning blood samples served to minimize diurnal variation of osteocalcin levels. The analyses were repeated after excluding subjects with diabetes and subjects taking lipid lowering drugs, corticosteroids and bisphosphonates. Osteocalcin undergoes a posttranslational modification in which three glutamic acid residues are carboxylated to form γ-carboxyglutamic acid residues. This process is vitamin K dependent and therefore vitamin K antagonists could potentially have an effect on the association between osteocalcin and the metabolic syndrome (22). After excluding subjects using vitamin K antagonists, our results did not change.

Nonetheless, several limitations should be mentioned. We measured plasma intact osteocalcin levels, which comprise both carboxylated and undercarboxylated osteocalcin. From mice studies it is known that undercarboxylated osteocalcin is biologically active (2). In humans it is not yet known whether undercarboxylated osteocalcin is a metabolically active form. Several human studies used total osteocalcin levels in the analyses (7-10). Data on undercarboxylated osteocalcin in humans are still conflicting. Shea at al. did not find an association between undercarboxylated and HOMA-IR in 348 non-diabetic men and women, whereas total osteocalcin was associated with insulin resistance (23). By contrast, Kanazawa et al. observed associations between undercarboxylated osteocalcin and plasma glucose and fat mass in type 2 diabetes in 180 men and 109 women (24). As we did not have the opportunity to measure undercarboxylated osteocalcin, we are unable to draw any conclusion about undercarboxylated osteocalcin. Unfortunately, in the present study, we did not have glucose and HOMA-IR available, but instead fructosamine was measured. We therefore cannot conclude about possible insulin resistance. Although it is assumed that a substantial part of the association can be explained by the effect of osteocalcin on insulin secretion, glucose and insulin resistance, Yeap et al. found an association between osteocalcin and the metabolic syndrome, even after adjustment for glucose. A possible explanation for this phenomenon could be that osteocalcin not only affects the β-cell, but also affects adipose tissue but in a different way (7). Because of the cross-sectional study design, conclusions on a cause/effect relationship cannot be drawn. Finally, our results are specific for a Caucasian population, so the results may not be valid in non-white older populations.

In conclusion, this study shows that low plasma levels of osteocalcin in community-dwelling older persons are associated with a higher risk of the metabolic syndrome, independently of several confounders. In the present study, low plasma osteocalcin was significantly associated with high waist circumference, hypertension, low HDL levels and high triglycerides. In the range between plasma osteocalcin levels 0.05 nmol/l and 2.63 nmol/l, persons are at higher risk of metabolic syndrome, compared to subjects with levels above 2.64 nmol/l. The results of this study do not allow any conclusions on the direction of a
possible causal relationship. A low plasma osteocalcin may be associated with the metabolic syndrome and obesity because obese people simply have a more sedentary lifestyle, which influences bone quality and turnover as well as weight.

More research is needed to fully understand these outcomes, to clarify the direction of causality and to determine whether higher levels of osteocalcin may improve glucose metabolism and weight. Additional studies would be of interest to evaluate the effect of glucose-lowering drugs on the production on osteocalcin, as well as the effect of antiresorptive therapy for osteoporosis on glucose metabolism. Because osteoporosis, as well as the metabolic syndrome is highly prevalent in older persons, this research might be of great importance and merits additional investigation.

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Conflicts of interest

The authors state that they have nothing to declare
Reference List


