Original article

Relationship between serum levels of osteocalcin and atherosclerotic disease in type 2 diabetes


Aims. – To analyze the relationship between serum levels of osteocalcin and parameters of atherosclerosis in patients with type 2 diabetes mellitus (T2DM).

Methods. – This cross-sectional study of 78 patients with T2DM evaluated intima–media thickness, and the prevalence of coronary heart disease, atherosclerotic plaques and aortic calcifications. Serum osteocalcin levels were also determined by radioimmunoassay.

Results. – The patients’ mean age was 57.8 ± 6.4 years (duration of diabetes: 13.4 years; mean HbA1c level: 8.01 %), and 37.2 % had coronary heart disease, 56 % had an abnormal intima–media thickness, 26.9 % had carotid plaques and 32.1 % had aortic calcifications. Coronary heart disease was associated with higher levels of osteocalcin in male vs female patients (1.95 ± 1.36 vs 0.93 ± 0.86 ng/mL, respectively; P = 0.006). Also, higher concentrations of osteocalcin were found in female patients with vs without abnormal intima–media thicknesses (2.17 ± 1.84 vs 1.25 ± 0.67 ng/mL, respectively; P = 0.042), carotid plaques (2.86 ± 2.10 vs 1.43 ± 1.09 ng/mL, respectively; P = 0.03) and aortic calcifications (2.85 ± 1.97 vs 1.26 ± 0.83 ng/mL, respectively; P = 0.002). Serum osteocalcin levels were associated with coronary heart disease on multivariate logistic regression (odds ratio: 2.27, 95 % confidence interval: 1.21–4.25; P = 0.01).

Conclusion. – In T2DM patients, serum osteocalcin levels were associated with parameters of atherosclerosis, suggesting that osteocalcin is involved not only in bone metabolism, but also in atherosclerotic disease.

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Keywords: Type 2 diabetes; Osteocalcin; Atherosclerosis

Résumé

Relation entre les concentrations d’ostéocalcine sérique et l’athérome dans le diabète de type 2.

Objectif. – Analyser les relations éventuelles entre les concentrations d’ostéocalcine sérique et les marqueurs de l’athérome chez des patients atteints de diabète de type 2 (DT2).

Méthodes. – Étude transversale incluant 78 patients atteints de DT2. La présence d’une atteinte coronarienne clinique, l’épaisseur intima-média, les plaques d’athérome des carotides et les calcifications aortiques ont été évaluées. Les concentrations sériques d’ostéocalcine ont été dosées par méthode RIA.

Résultats. – En moyenne, l’âge était de 57.8 ± 6.4 ans, la durée de diabète de 13.4 ans et l’HbA1c de 8.01 %. Parmi les patients, 37.2 % présentaient une atteinte coronarienne, 56 % une épaisseur intima-média anormale, 29.6 % des plaques d’athérome carotidien et 32.1 % calcifications aortiques. L’atteinte coronarienne était associée avec des concentrations élevées d’ostéocalcine chez les hommes (1.95 ± 1.36 vs. 0.93 ± 0.86 ng/mL,

Abbreviations: baPWV, brachial–ankle pulse wave velocity; CHD, coronary heart disease; EPCS, endothelial precursors; FPG, fasting plasma glucose; IMT, intima–media thickness; OC, osteocalcin; T2DM, type 2 diabetes; TG, triglycerides.

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1. Introduction

Over the past few years, cross-sectional and longitudinal studies have suggested a relationship between osteoporosis and atherosclerosis [1–5]. In animal models and in humans, the presence of proteins associated with bone formation in calcified lesions has suggested that the process is regulated similarly to mineralization in bone [6,7]. For this reason, whether serum OC secreted from osteoblasts in bone is able to influence atherosclerosis and whether osteoblast-like cells present in vessels modulate the process, or not, need to be clarified.

The relationship between OC and atherosclerosis parameters in humans has been explored in recent papers, but with conflicting results. In healthy postmenopausal women, there was an increased prevalence of carotid atherosclerosis in women with OC levels above the median and low bone mineral density [8]. Patients with coronary atherosclerosis have a higher percentage of EPCs expressing OC compared with those with normal endothelial function and no structural coronary artery disease [9]. In addition, serum OC has been negatively associated with IMT and baPWV in men [10], but not in women, with T2DM. Other authors have described low [11] or increased [12] serum OC levels in patients with severe atherosclerosis, although these studies did not include T2DM patients.

There have also been convincing data to support reciprocal regulation between energy and bone metabolism. OC-knockout mice have both increased bone mass and abnormal amounts of visceral fat, and are also glucose-intolerant [13]. Also, in animal (mouse) models, OC administration has been shown to regulate gene expression in both beta cells and adipocytes, thereby affecting the development of obesity, T2DM and other metabolic diseases [14]. Mice null for exp, a gene involved in gamma-carboxylglutamic acid (GLA) carboxylation of OC, have higher OC bioactivity influencing insulin and adiponectin regulation, and express severe hypoglycaemia, but are protected against diet-induced obesity and diabetes [13]. Also, in humans, OC levels are inversely related to fat mass and plasma glucose in elderly men [15], and undercarboxylated OC is negatively correlated with body mass index (BMI) in overweight and obese men [16], suggesting a link between adipose tissue and OC in the regulation of bone metabolism in humans.

The aim of the present study was to analyze the relationship between serum levels of OC and atherosclerotic disease in patients (males and females) with T2DM. Also assessed were any correlations between OC, glucose metabolism and BMI in this population.

2. Methods

2.1. Study subjects

The present cross-sectional study included 78 patients with T2DM (43 men and 35 women, mean ages 57 ± 7 and 59 ± 6 years, respectively) with a diagnosis of diabetes according to American Diabetes Association criteria (2005). Of the female patients, 30 were postmenopausal. From January 2006 to December 2007, patients who visited the endocrinology unit at San Cecilio University Hospital were consecutively recruited for evaluation of T2DM. All of our study participants were Caucasian and ambulatory, with normal values of serum calcium and phosphorus, and none had either renal, hepatic, gastrointestinal or thyroid disease. In addition, none had been treated with calcium supplements, vitamin D preparations, hormone therapy, antiresorptive therapy, thiazides, steroids or other medications that might affect bone mass. Patients treated with glitazones were excluded.

Detailed medical records were taken to identify data related to the development of diabetes and other comorbidities, such as hypertension, dyslipidaemia and CHD. CHD was defined as previous myocardial infarction, a diagnosis of stable or unstable angina, or previous coronary revascularization surgery. Current use of insulin, sulphonylurea, metformin, antihypertensive drugs and statins were also recorded.

The study was conducted with the approval of the ethics committee of the San Cecilio University Hospital, and conformed to the relevant ethical guidelines for human and animal research as per the Helsinki Declaration. Written informed consent was obtained from all study participants.

2.2. Carotid intima–media thickness and aortic calcification measurements

Ultrasonographic examination of the carotid arteries was performed with patients in supine position using Doppler ultrasonography (Toshiba PowerVision 6000). The maximum IMT at the carotid bifurcation (BIF) was determined between the near and far walls of the carotid bifurcation on the right and left sides. Each part was measured from views of both longitudinal and vertical sections at the bifurcation. If a discrepancy was observed in the measured values between longitudinal and vertical sections, the smaller value was selected to avoid overestimation. The BIF–IMT was defined as the mean of the measurements from the right and left sides. The value for each side was obtained from the mean of 10 wall measurements. A single trained
sonographer (M.J.L.V.) performed the ultrasonographic study in all subjects. The BIF–IMT, measured in millimeters (mm), was considered pathological if it was ≥ 0.9 mm, and was considered to be carotid atherosclerosis if the BIF–IMT was ≥ 1.2 mm or 50% greater than the BIF–IMT in the adjacent area. Plaques were identified as calcified by findings of bright white echoes on sonography.

The presence of aortic calcification was evaluated on lateral-view conventional X-rays of the thoracic and lumbar spine (T4–L5). Severity of anterior and posterior aortic calcifications was graded individually on a 0- to 3-point scale for each lumbar segment, and the results summarized to obtain three composite scores:

- affected segments (range: 0–4);
- anterior and posterior affected (range: 0–8);
- anteroposterior severity (range: 0–24) [17].

Patients with calcification in one or more lumbar segments were considered to have aortic calcification.

### 2.3. Biochemical measurements

Serum was collected after an overnight fast. Biochemical parameters, serum total, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol and TGs were measured, using standard biochemical methods. Dyslipidaemia was defined according to Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP-III) criteria as follows: LDL ≥ 100 mg/dL; TGs ≥ 150 mg/dL; HDL < 40 mg/dL in women and <50 mg/dL in men; and/or current dyslipidaemia treatment. HbA1c was determined by high-performance liquid chromatography (HPLC). OC was measured by radioimmunoassay (RIA; DiaSorin, Stillwater, MN, USA) according to the manufacturer’s instructions. The RIA required the simultaneous addition of samples of rabbit antibovine OC antibody and 125I bovine OC, followed by overnight incubation at 2–8 °C. Phase separation was accomplished by the addition of a complex of goat antirabbit serum, carrier rabbit serum and polyethylene glycol. The intra- and interassay coefficient of variation (CV) was 5.3% and 8.6%, respectively.

### 2.4. Other parameters

Height, weight and waist circumference were measured at baseline according to standard procedures. Weight was measured to the nearest 100 g, using digital electronic scales. Height and waist circumference were measured to the nearest 1 mm, using a stadiometer and a metal anthropometric tape, respectively. BMI was calculated as weight divided by the square of height in meters (kg/m²).

Blood pressure was also measured in a standardized manner twice, using a standard mercury sphygmomanometer (12 cm wide and 35 cm long). The mean of the two values was used in the analysis. Hypertension was defined as values > 140/90 mmHg and/or the use of antihypertensive treatment.

### 2.5. Statistical analysis

Data were recorded and analyzed using SPSS version 15.0 software (SPSS Inc, Chicago, IL, USA). Data were expressed as means ± standard deviation (SD). A P value <0.05 was considered significant. A normal distribution of variables was determined by the Kolmogorov–Smirnov test.

Mean values in groups were compared by parametric statistics (Student’s t test and ANOVA) or non-parametric statistics (Mann–Whitney and Kruskal–Wallis tests), depending on the distribution of the variable of interest. Pearson’s standard linear-regression analysis (normal distribution or Spearman’s test (non-normal distribution) were used for the correlation studies. Multivariate logistic regression was performed to evaluate the association between OC and CHD. The initial model was age-adjusted, whereas the second model was adjusted for established cardiovascular risk factors (age, BMI, diabetes duration, hypertension, smoking, dyslipidaemia). The full model was adjusted for all variables included in the second model, with additional adjustments for surrogate markers of cardiovascular disease (abnormal IMT, carotid plaques and aortic calcifications).

### 3. Results

BMI and the percentage of patients with hypertension were significantly higher in female than in male diabetic patients (P < 0.05). A larger number of men had abnormal IMTs (P < 0.05; Table 1). Serum OC levels were higher in women compared with men, although the difference was not significant (1.62 ± 1.33 vs 1.35 ± 1.19 ng/mL, respectively; P = 0.365).

In women, serum OC levels were higher in those with vs those without abnormal IMTs (2.17 ± 1.84 vs 1.25 ± 0.67 ng/mL, respectively; P = 0.042), carotid plaques (2.86 ± 2.10 vs 1.43 ± 1.09 ng/mL, respectively; P = 0.03) and aortic calcifications (2.85 ± 1.97 vs 1.26 ± 0.83 ng/mL, respectively; P = 0.002; Fig. 1). CHD was associated with higher levels of OC in men vs women (1.95 ± 1.36 vs 0.93 ± 0.86 ng/mL, respectively; P = 0.006; Fig. 2).

In the logistic-regression analysis, serum OC levels were independent predictors of CHD after adjusting for age, cardiovascular risk factors and surrogate markers of cardiovascular disease (full-model odds ratio [OR]: 2.27, 95% confidence interval [CI]: 1.21–4.25; P = 0.01) (Table 2).

There was no relationship between serum OC, FPG, HbA1c, BMI or atherosclerosis-related parameters in the entire cohort, nor when women and men were analyzed separately (Table S1; see supplementary material associated with this article online).

### 4. Discussion

In the present study, serum OC levels were higher in diabetic men with CHD and in diabetic women with abnormal IMTs, aortic calcifications and carotid plaques. In addition, after adjusting for various confounders, serum OC levels were significantly related to CHD in the entire cohort. Previous studies had explored the relationship between OC and atherosclerosis, but
found conflicting results. In a study by Braam et al. [12], serum OC levels were higher in patients with advanced atherosclerosis compared with healthy controls, although “advanced atherosclerosis” was not specifically defined. However, no relationship was observed between OC levels and IMT. Kanazawa et al. [10] found that OC was negatively associated with IMT and baPWV in T2DM men, but not women. Pennisi et al. [11] described low OC levels in patients with atherosclerosis of peripheral vessels, but their study did not include patients with either T2DM or coronary disease. Gender-related and ethnic differences among the subjects might explain in part these conflicting data. Furthermore, the presence of T2DM and the location/type of vascular disease might also have influenced the results. It has even been suggested that there is an association between OC and adiponectin secretion [14,18]. In humans, baseline OC has been associated with circulating adiponectin [19]. There have been reports of gender-related differences in adiponectin serum levels [20], and adiponectin concentrations are significantly higher in Europeans compared with other ethnic groups [21]. Gender- and ethnic-related differences in adiponectin levels may explain in part such discordant results. However, as there were no measurements of adiponectin for the present cohort, this hypothesis could not be tested in our study. Nevertheless, there is evidence to show the influence of bone proteins on cardiovascular disease. During atherogenesis, bone matrix proteins, including OC, may have a regulatory role in the atherosclerotic calcification process [7]. Patients with coronary atherosclerosis had a higher percentage of EPCs expressing OC and osteonectin compared with subjects who had normal endothelial function and no structural CHD. It has been

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of study subjects.</th>
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<tbody>
<tr>
<td></td>
<td>All</td>
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<tr>
<td>Number of subjects</td>
<td>78</td>
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<tr>
<td>Age (years)</td>
<td>57.8 ± 6.4</td>
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<tr>
<td>Duration of diabetes (years)</td>
<td>13.4 ± 7.5</td>
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<tr>
<td>Body weight (kg)</td>
<td>81.8 ± 15.8</td>
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<tr>
<td>Height (cm)</td>
<td>159.9 ± 20.1</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>31.2 ± 5.6</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>135.2 ± 20.0</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79.6 ± 12</td>
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<tr>
<td>Smoker</td>
<td>14 (18%)</td>
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<tr>
<td>Antihypertensives</td>
<td>55 (70.5%)</td>
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<td>Statins</td>
<td>54 (69.2%)</td>
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<tr>
<td>Insulin</td>
<td>50 (64.1%)</td>
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<tr>
<td>OADs</td>
<td>59 (75.6%)</td>
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<tr>
<td>Hypertension</td>
<td>62 (79.5%)</td>
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<tr>
<td>Dislipidaemia</td>
<td>71 (92%)</td>
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<tr>
<td>Coronary heart disease</td>
<td>29 (37.2%)</td>
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<tr>
<td>Abnormal IMT</td>
<td>44 (56.4%)</td>
</tr>
<tr>
<td>Carotid plaques</td>
<td>21 (26.9%)</td>
</tr>
<tr>
<td>Aortic calcifications</td>
<td>25 (32.1%)</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.84 ± 0.19</td>
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<tr>
<td>FPG (mg/dL)</td>
<td>173.3 ± 62.9</td>
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<tr>
<td>Hba1c (%)</td>
<td>8.0 ± 0.19</td>
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<tr>
<td>LDL-C (mg/dL)</td>
<td>97.0 ± 33.9</td>
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<tr>
<td>HDL-C (mg/dL)</td>
<td>50.2 ± 17.1</td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)</td>
<td>1.48 ± 1.26</td>
</tr>
</tbody>
</table>

Data are expressed as n, n (%) or means ± SD; unpaired t test: *P < 0.05 for men vs women; χ² test for comparisons of categorical variables: **P < 0.05 for men vs women.

BMI: body mass index; OADs: oral antidiabetic drugs; IMT: intima–media thickness; FPG: fasting plasma glucose; LDL/HDL-C: low-density/high-density lipoprotein cholesterol.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Multivariate regression analysis of the relationship between osteocalcin and coronary heart disease.</th>
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<tbody>
<tr>
<td></td>
<td>OR</td>
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<tr>
<td>Coronary heart disease</td>
<td></td>
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<tr>
<td>Crude</td>
<td>1.79</td>
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<tr>
<td>Age-adjusted</td>
<td>1.75</td>
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<tr>
<td>Adjusted for cardiovascular risk factors (age, gender, BMI, hypertension, smoking, diabetes duration)</td>
<td>2.07</td>
</tr>
<tr>
<td>Full-model (CV risk factors, aortic calcifications, abnormal IMT, carotid plaques)</td>
<td>2.27</td>
</tr>
</tbody>
</table>

OR: odds ratio; BMI: body mass index; CV: cardiovascular; IMT: intima–media thickness.
suggested that the expression of OC in EPCs might constitute a marker of early disease in coronary atherosclerosis [9]. In addition, the same proinflammatory factors involved in the pathogenesis of osteoporosis may lead to the expression of an osteogenic phenotype through endothelial lineage cells, providing a potential mechanism for a link between osteoporosis and vascular calcifications. Such experimental and clinical data could explain our present results showing a relationship between OC levels and atherosclerotic disease and, more specifically, that serum OC levels may be independent predictors of CHD after adjusting for several confounders.

Recently, some new metabolic roles have been proposed for OC. There have been reports of an inverse relationship between OC and insulin resistance [18,19], and a negative correlation between OC and fat mass in elderly non-diabetic men [14] and in men with T2DM [10]. However, we could find no relationship between serum OC and BMI in our present diabetic patients. Again, gender-related and ethnic differences in subjects from different populations may explain these different results.

Studies investigating the correlation between OC and glucose levels in humans have also shown discordant results. There have been reports of an inverse relationship between OC and fasting glucose in elderly non-diabetic men [15] and in a study of healthy volunteers, of whom 5% had diabetes [18]. In obese men, undercarboxylated OC was negatively correlated with fasting glucose [16]. In T2DM, OC was negatively correlated with fasting glucose and HbA1c in men, but not women [10]. In the present study, we could find no relationship between serum OC
and either FPG or HbA1c. The tiny magnitude of this association and limited sample size may explain this discrepancy in results. It has also been proposed that hyperglycaemia suppresses OC expression in osteoblasts and its secretion into the circulation. Better metabolic control in our study population, considering the results of Kanazawa et al. [20], may also have influenced the results. Another factor explaining discordant data may be that the relationship between OC and glucose is influenced by the presence of diabetes, diabetes progression and the severity of atherosclerosis.

The present study had several limitations. First, the sample was not large enough to arrive at any definite conclusions. Second, we enrolled into the study only those patients who were referred to San Cecilio University Hospital for evaluation and treatment of diabetes and, thus, the patients included in our study may have had relatively more severe diabetes and may not have been representative of the general diabetic population. Third, the design of our study was cross-sectional and, consequently, the assessment of larger number of patients is required to determine the role of OC in T2DM patients with atherosclerosis.

On the other hand, the strengths of our present study were the inclusion of well-characterized T2DM patients and an exhaustive evaluation of the presence of vascular disease.

5. Conclusion

In patients with T2DM, serum OC levels were associated with atherosclerotic parameters and CHD, suggesting that OC is important not only for bone metabolism, but also in the development of atherosclerosis. Our present data suggest that OC is involved in atherosclerosis in patients with T2DM, although its role as a biomarker of vascular disease still remains to be ascertained.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgements

This work was supported, in part, by grants from the Health Research Fund (Carlos III Institute of Health, PI081302) and RETICEF (RD06/0013/1014).

Appendix A. Supplementary data

Supplementary material (Table S1) associated with this article can be found at http://www.sciencedirect.com, at doi: 10.1016/j.diabet.2011.07.008.

References


