baseline osteocalcin levels and incident diabetes in a 3-year prospective study of high-risk individuals

S. Liatis a,*, P.P. Sfikakis a, A. Tsiakou a, C. Stathi a, E. Terpos b, N. Katsilambros a, K. Makrilakis a

a First Department of Propaedeutic Medicine & Diabetes Center, Athens University Medical School, Laiko General Hospital, 17 Ag. Thoma str, 11527 Athens, Greece
b Department of Clinical Therapeutics, Athens University Medical School, Athens, Greece

Received 20 November 2013; received in revised form 14 January 2014; accepted 14 January 2014
Available online 12 February 2014

Abstract

Aim. – Experimental evidence suggests that osteocalcin is a key messenger that affects both adipocytes and insulin-producing β cells. Epidemiological cross-sectional studies have shown a negative association between plasma levels of osteocalcin and glucose. For this reason, the hypothesis that lower baseline osteocalcin plasma levels are associated with diabetes was prospectively tested.

Methods. – The study population consisted of individuals at high risk for type 2 diabetes who were screened for participation in the Greek arm of a European type 2 diabetes prevention study (the DE-PLAN study). All participants were free of diabetes at baseline and underwent a second evaluation 3 years later. Diabetes status was defined according to an oral glucose tolerance test.

Results. – A total of 307 subjects were included in the present analysis. The population, including 154 men (50.3%), was middle-aged (54.4 ± 10.2 years) and overweight (BMI: 29.5 ± 4.9 kg/m²). At baseline, mean total plasma osteocalcin was lower in those with impaired fasting glucose and/or impaired glucose tolerance compared with those with normal glucose tolerance (6.0 ± 3.1 ng/mL vs. 7.3 ± 4.0 ng/mL, respectively; P = 0.01). After 3 years, 36 subjects had developed diabetes. In the prospective evaluation, there was no association between baseline osteocalcin levels and diabetes (OR: 1.04 per 1 ng/mL, 95% CI: 0.93–1.15; P = 0.49) on multivariable logistic regression analysis, nor was there any correlation with changes in plasma glucose after 3 years (r = 0.09, P = 0.38).

Conclusion. – Our prospective results show that lower levels of circulating osteocalcin do not predict future diabetes development and, in contrast to most cross-sectional published data so far, suggest that this molecule may not be playing a major role in glucose homoeostasis in humans.

© 2014 Elsevier Masson SAS. All rights reserved.

Keywords: Osteocalcin; Bone density; Type 2 diabetes

1. Introduction

Type 2 diabetes is a major global health threat, with rapidly increasing incidence rates in most nations [1]. Its pathogenesis is characterized by the complex interplay between increased insulin resistance and decreased insulin secretion by β cells. Several aspects, however, of the mechanisms linking these two fundamental pathogenetic components remain unclear.

In recent years, a feedback loop has been proposed between the energy-control system and bone metabolism, with osteocalcin, a low-molecular-weight osteoblast-derived protein, playing the role of a bone messenger that affects both adipocytes and insulin-producing β cells [2]. Indeed, it has been shown in mice and cell cultures that osteocalcin increases the production of adiponectin, an adipocyte-derived protein (adipokine) that potently increases insulin sensitivity [2,3]. It has also been shown that osteocalcin promotes insulin secretion by pancreatic β cells [2,3]. On the other hand, it has been found that insulin itself exerts stimulatory effects on osteocalcin secretion by osteoblasts [4,5], suggesting the presence of a signalling circuit between the skeletal system and the glucose homoeostasis system. Osteocalcin undergoes gamma carboxylation in a vitamin K-dependent process prior to its secretion, and it is the uncarboxylated form of the protein that is thought to be metabolically active [2].

Recent cross-sectional epidemiological studies [6–10] have examined the relationship between plasma osteocalcin and several aspects of glucose homoeostasis in humans, and
demonstrated a negative relationship between plasma osteocalcin levels and insulin-resistance indices as well as with plasma glucose levels. Furthermore, it has been demonstrated that, in patients with type 2 diabetes, serum osteocalcin levels are associated with parameters of atherosclerosis, suggesting that osteocalcin is involved not only in bone metabolism, but also in atherosclerotic disease [11]. However, the hypothesis that plasma osteocalcin influences glucose homoeostasis parameters and, more importantly, the incidence of diabetes has not been assessed prospectively. Therefore, the present prospective study aimed to examine the relationship between plasma osteocalcin levels and the future development of diabetes in a cohort of middle-aged individuals who had been screened by oral glucose tolerance test (OGTT) before participating in a diabetes prevention programme.

2. Methods

2.1. Study population

The study included individuals who had participated in the Greek arm of the DE-PLAN (diabetes in Europe – prevention using lifestyle, physical activity and nutritional intervention) study, a non-intensive, community-based, diabetes prevention programme [12]. The project was approved by the participating hospital’s ethics committee and the Greek National Drug Organization. All participants gave their informed consent according to the general recommendations of the Declaration of Helsinki.

To recruit participants for the study, screening for type 2 diabetes risk was performed using the Finnish Diabetes Risk Score (FINDRISC) questionnaire, a validated tool [13]. In the Greek arm of the study, all individuals were further invited to undergo an OGTT to identify those with unknown diabetes and to validate the FINDRISC questionnaire in the Greek population [14]. The lifestyle intervention included those with a FINDRISC score ≥ 15, and constituted six-hour-long sessions held by a registered dietitian at the participants’ place of residence or work; these sessions have been described in detail elsewhere [15]. In addition, all those who underwent an OGTT at baseline were invited to attend a complete follow-up examination, including another OGTT, 3 years later.

At both the baseline and follow-up examinations, a standard OGTT (with 75g of glucose) was given to all participants. Plasma glucose levels were measured at a central accredited university research laboratory using an enzyme assay. Fasting plasma insulin was measured by enzyme-linked immunosorbent assay (ELISA). Homoeostasis model assessment for insulin resistance (HOMA–IR) and insulin secretion (HOMA–β) indices were calculated according to formulas previously described elsewhere [16].

Based on their OGTT results, participants were classified as having either normal glucose tolerance [NGT; fasting plasma glucose (FPG)<6.1 mmol/L and 2-h plasma glucose (2hPG)<7.8 mmol/L], impaired fasting glucose (IFG; FPG 6.1–6.9 mmol/L), impaired glucose tolerance (IGT; 2hPG 7.8–11.0 mmol/L) or diabetes (FPG ≥ 7.0 mmol/L and/or 2hPG ≥ 11.1 mmol/L) [17]. Total plasma osteocalcin was measured using an ELISA method (N-Mid Osteocalcin ELISA; Immunodiagnostics Systems Ltd, Boldon, Tyne & Wear, UK).

For the present analysis, participants with renal failure, recent fractures (<6 months) and/or receiving medications that could possibly interfere with bone metabolism (such as calcium/vitamin D supplements, bisphosphonates, glucocorticoids, oestrogens, warfarin or testosterone) were excluded.

Out of 3240 completed FINDRISC questionnaires, 869 respondents agreed to undergo the initial OGTT. A total of 94 were found to have unknown (screening-detected) diabetes and so were excluded. Of the remaining 775 subjects, 368 had a second OGTT 3 years later, 348 did not wish to participate further and 59 were lost to follow-up. Thus, 307 participants were included in the present analysis, after excluding 61 who fulfilled the exclusion criteria described above. Of these participants, 113 had received the DE-PLAN lifestyle intervention during the first year of the follow-up period.

2.2. Statistical analysis

Statistical analyses were performed using the statistical software package IBM-SPSS Statistics, version 20.0. For cross-sectional analyses, comparisons between groups of normally distributed data were performed using the independent samples Student’s t test or an analysis of variance (ANOVA). All P values were adjusted for multiple comparisons using the Bonferroni correction. Log-transformation of the data was applied where appropriate. Analysis of covariance (ANCOVA) was used to adjust the compared means for confounders. For non-normally distributed data, the Mann–Whitney U test (two independent samples) or Kruskal–Wallis H test was performed. For simple correlations, Pearson (normally distributed variables) or Spearman (non-normally distributed variables) correlation tests were used. With the proviso that glucose homoeostasis status (and hence the presence of diabetes) was assessed at two separate examinations separated by 3 years, multivariable stepwise logistic regression analysis was used to assess the independent contribution of osteocalcin and other variables possibly associated with the development of diabetes at the follow-up examination. P values (two-tailed)<0.05 were considered statistically significant.

3. Results

The demographic and clinical characteristics of the study participants at baseline and at the 3-year follow-up are presented in Table 1. The study population, which included 154 men (50.3%), was middle-aged (mean age 54.4 ± 10.2 years) and nearly obese, with a mean body mass index (BMI) score at baseline of 29.5 ± 4.9 kg/m². Their risk for future development of type 2 diabetes was high (mean FINDRISC: 12.9 ± 4.9), although most of the subjects (74.5%) had NGT at baseline. As shown in Table 1, mean weight, BMI, waist circumference (WC), and systolic and diastolic blood pressure (SBP and DBP, respectively) did not change significantly over 3 years. On the other hand, the mean FINDRISC had increased significantly by 0.7 units and
Table 1
Demographic and clinical characteristics of the study participants at baseline and after 3 years.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After 3 years</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men (%)</strong></td>
<td>154 (50.2)</td>
<td>154 (50.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>54.4 [53.3–55.5]</td>
<td>57.5 [56.4–58.6]</td>
<td></td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>83.2 [81.5–84.8]</td>
<td>83.5 [81.7–85.2]</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>29.4 [29.0–30.1]</td>
<td>29.5 [29.0–30.1]</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>WC (men)</strong></td>
<td>102.3 [100.4–104.7]</td>
<td>103.3 [101.2–105.4]</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>WC (women)</strong></td>
<td>93.4 [91.9–94.5]</td>
<td>93.5 [90.5–96.2]</td>
<td>0.93</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>126.1 [124.1–128.1]</td>
<td>127.7 [125.4–130.0]</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>78.0 [76.7–79.2]</td>
<td>76.5 [74.8–78.1]</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>FINDRISC</strong></td>
<td>12.9 [12.4–13.5]</td>
<td>13.7 [12.9–14.4]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>FPG (mmol/L)</strong></td>
<td>5.5 [5.4–5.6]</td>
<td>5.9 [5.8–6.0]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>NGT (%)</strong></td>
<td>228 (74.3)</td>
<td>176 (57.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>IFG/IGT (%)</strong></td>
<td>79 (25.7)</td>
<td>95 (30.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Diabetes (%)</strong></td>
<td>0 (0)</td>
<td>36 (11.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Total osteocalcin</strong></td>
<td>6.9 [6.5–7.4]</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Plasma insulin</strong></td>
<td>6.7 [3.8–10.9]</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>HOMA-IR</strong>*</td>
<td>1.6 [1.0–2.8]</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Data are presented as n (%), means [95% confidence interval] or medians [interquartile range]; *medians; BMI: body mass index; WC: waist circumference; SBP/DBP: systolic/diastolic blood pressure; FINDRISC: Finnish Diabetes Risk Score; FPG: fasting plasma glucose; NGT: normal glucose tolerance; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; NA: not available; HOMA-IR: homeostasis model assessment for insulin resistance.

respectively), with associations remaining non-significant after all appropriate adjustments. When plasma osteocalcin levels were stratified by quartiles, no significant difference was observed in plasma glucose (fasting and post-load) across these categories, although there was a trend towards lower BMI and lower 2hPG in the higher vs. lower quartiles (Table 2).

In the prospective evaluation by univariable analysis, total osteocalcin levels at baseline did not differ between those who developed diabetes and those who did not (7.0 ± 3.8 ng/mL vs. 6.9 ± 3.8 ng/mL, respectively; OR: 1.0, 95% CI: 0.9–1.1; P = 0.93). Also, there was no correlation between baseline plasma osteocalcin levels and plasma glucose at 3 years (whether fasting or 2-h post-load). Subjects who developed diabetes were, as expected, older with significantly higher values for BMI, WC, SBP, DBP, FPG, 2hPG, HOMA-IR, HOMA-β and FINDRISC at baseline.

Multivariable logistic regression analysis found no association between baseline osteocalcin levels and incident diabetes, whichever model was applied. In the fully adjusted model (Table 3), incident diabetes was independently associated with higher values for FINDRISC and the presence of IFG/IGT at baseline. Participation in the lifestyle intervention and weight loss during follow-up were independently associated with lower diabetes incidence (Table 3), whereas plasma osteocalcin was not related to diabetes incidence (OR: 1.04, 95% CI: 0.93–1.15; P = 0.49). In the same model, when plasma osteocalcin (as a continuous variable) was substituted by osteocalcin quartiles, no difference in diabetes incidence was observed between the lowest and highest quartiles (OR for highest vs. lowest: 1.88, 95% CI: 0.59–6.0; P = 0.28). In addition, no association was found.

Table 3
Multivariable logistic regression model* for incident diabetes after 3 years of follow-up.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFG/IGT</td>
<td></td>
<td>5.88</td>
<td>2.45–14.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FINDRISC</td>
<td>Unit</td>
<td>1.28</td>
<td>1.13–1.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intervention</td>
<td></td>
<td>0.19</td>
<td>0.07–0.53</td>
<td>0.002</td>
</tr>
<tr>
<td>Weight loss</td>
<td>%</td>
<td>0.94</td>
<td>0.89–0.99</td>
<td>0.03</td>
</tr>
<tr>
<td>Plasma osteocalcin</td>
<td>ng/mL</td>
<td>1.04</td>
<td>0.93–1.15</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*Also included (but found to be non-significant) were age, gender and baseline values for body mass index, blood pressure, fasting plasma glucose, fasting plasma insulin and HOMA-IR; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; FINDRISC: Finnish Diabetes Risk Score.

Table 2
Selected baseline characteristics of the study participants stratified by plasma osteocalcin quartiles.

<table>
<thead>
<tr>
<th>Quartile</th>
<th>1st quartile</th>
<th>2nd quartile</th>
<th>3rd quartile</th>
<th>4th quartile</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Osteocalcin (ng/mL)</strong></td>
<td>3.3 [3.1–3.5]</td>
<td>5.3 [5.1–5.4]</td>
<td>7.2 [7.0–7.4]</td>
<td>11.9 [11.1–12.9]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>54.2 [51.8–56.6]</td>
<td>54.7 [52.2–57.3]</td>
<td>52.7 [50.4–55.0]</td>
<td>55.8 [53.7–57.9]</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Male gender (%)</strong></td>
<td>53.2</td>
<td>53.9</td>
<td>49.4</td>
<td>44.7</td>
<td>0.60</td>
</tr>
<tr>
<td><strong>FPG (mmol/L)</strong></td>
<td>5.5 [5.4–5.7]</td>
<td>5.5 [5.4–5.6]</td>
<td>5.6 [5.4–5.7]</td>
<td>5.3 [5.2–5.6]</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>2hPG (mmol/L)</strong></td>
<td>6.2 [5.8–6.6]</td>
<td>5.8 [5.4–6.2]</td>
<td>5.5 [5.1–5.8]</td>
<td>5.5 [5.2–5.9]</td>
<td>0.11</td>
</tr>
</tbody>
</table>

BMI: body mass index; FPG: fasting plasma glucose; 2hPG: 2-h post-load plasma glucose; FINDRISC: Finnish Diabetes Risk Score.
between baseline plasma osteocalcin and changes in plasma glucose 3 years later \( r = 0.09, P = 0.38 \).

4. Discussion

The present study has shown that total plasma osteocalcin levels are not associated with incident diabetes after 3 years of follow-up in a cohort of middle-aged, overweight Caucasians with an increased risk for future type 2 diabetes. To our knowledge, this is the first full-cohort prospective observational study to examine this association.

The mechanistic rationale for exploring this relationship was raised several years ago when studies by Lee et al. [2] in mice and *ex vivo* demonstrated that osteocalcin acts on adipocytes and pancreatic β cells by promoting the secretion of adiponectin and insulin, respectively. Subsequently, this led to an hypothesis proposing that the skeletal system is actively involved in the regulation of energy balance and that osteocalcin as a major mediator in the process. However, the actual existence and, more important, the significance of such a link in humans remained unclear.

Previous cross-sectional studies have consistently shown a relationship between plasma osteocalcin levels and various parameters associated with glucose metabolism, including fat mass, FPG/2hPG, insulin, adiponectin and insulin-resistance/insulin-secretion indices [6–10]. Indeed, in all these studies, plasma osteocalcin was inversely related to FPG and insulin resistance. Two of the studies [7,10] involved exclusively diabetic patients.

Interestingly, however, a recent cross-sectional, family-based study across three generations demonstrated only a weak, age-specific association between osteocalcin and glucose metabolism in women without diabetes, irrespective of the fraction of osteocalcin measured [18]. The present study results confirm this cross-sectional association, as plasma osteocalcin levels were lower in subjects with IFG and/or IGT compared with those with NGT, even after adjusting for possible confounders, while the association between plasma osteocalcin and FPG was weak.

Yet, despite the favourable cross-sectional associations, higher plasma osteocalcin levels did not protect against the development of diabetes. One study of 1229 non-diabetic Koreans, with findings similar to ours and a retrospective follow-up design, found that despite positive baseline associations between serum osteocalcin and favourable metabolic parameters, there was no association between total plasma osteocalcin and incident diabetes (90 identified cases) after 8.4 years [19]. On the other hand, a recent nested case – control study from Spain (153 cases and 306 controls) and a small nested case – control study of Thai men (63 cases and 63 controls) showed that total baseline osteocalcin levels (but not uncarboxylated osteocalcin) were independently associated with the development of diabetes after 5 and 10 years of follow-up, respectively [20,21]. Moreover, Pittas et al. [6] found a prospective inverse relationship between mean osteocalcin concentrations (based on three measurements taken before and during follow-up) and changes in FPG over a 3-year period. That study was performed in a population at low risk for type 2 diabetes although no other clinical endpoints were examined.

It is difficult to explain the contradictory results produced by the above-mentioned epidemiological studies. As osteocalcin is released from the bone matrix during bone resorption, and despite the fact that plasma osteocalcin is traditionally considered a bone-formation marker, circulating osteocalcin reflects both bone formation and resorption [22]. This means that osteocalcin levels might be influenced by several parameters associated with bone metabolism, including age, gender, menopausal status, physical activity, smoking, renal function and the use of a number of medications. In patients with type 2 diabetes, although bone mineral density is usually preserved or even increased, their fracture risk is nevertheless still higher than in age-, gender- and BMI-matched individuals [23]. Thus, it is likely that such a complex network of interactions might produce several cross-sectional associations of minor or no physiological importance. It has been shown that chronic exposure to high glucose levels inhibits cell growth of an osteoblast-like cell line in humans in a dose-dependent manner, while it decreases osteocalcin mRNA levels in mouse osteoblasts [24,25]. In the light of such a finding, it could be argued that the lower osteocalcin levels observed in patients with diabetes or even pre-diabetes in cross-sectional studies may perhaps be a consequence, rather than a cause, of hyperglycaemia.

The lack of an association between plasma osteocalcin levels and the future development of diabetes supports the notion that osteocalcin may have minimal effects on glucose homoeostasis in humans [18,19]. This notion is also in accordance with the absence of any evidence showing an increased risk for incident diabetes related to drugs known to reduce circulating levels of osteocalcin, such as bisphosphonates, raloxifene, strontium ranelate and the RANK ligand antibody denosumab [26–29]. In addition, there are no reports of a lower type 2 diabetes risk in patients receiving coumarin derivatives, although a protective effect might be expected as warfarin increases uncarboxylated osteocalcin [30].

The main strength of the present study is its prospective design. The study population was balanced in terms of gender, and the moderately high diabetes risk allowed for a significant number of diabetes cases. Also, diabetes was not self-reported, but diagnosed by OGTT. In contrast, among the limitations that need to be taken into consideration is the fact that, although total plasma osteocalcin was measured, data for the uncarboxylated form of the protein were not available. Because it has been postulated that the latter is metabolically active, and in spite of the very good correlation between total and uncarboxylated osteocalcin, some possible associations between the latter form and metabolic parameters, both cross-sectionally and prospectively, may have been missed. Another limitation is that, apart from plasma osteocalcin, other bone turnover markers were not measured, making it difficult to separate the putative role of osteocalcin in glucose metabolism from its role as a factor for bone formation. Furthermore, a significant proportion (52.5%) of participants in the Greek arm of the DE-PLAN study declined to further participate and undergo a second OGTT examination or were lost to follow-up. The main reason stated
for discontinuation was discomfort with the OGTT procedure [14]. This resulted in a reduction in the size of the study population and, consequently, a smaller number of observed incident diabetes cases, a fact that reduced the study’s power. Nevertheless, the absence of any trend associating baseline plasma osteocalcin and incident diabetes does not imply a lack of statistical power as a possible explanation for our results.

In conclusion, our present prospective results show that lower osteocalcin plasma levels are not associated with the development of type 2 diabetes over a 3-year follow-up in high-risk individuals and, in contrast to most of the cross-sectional published data so far, also suggest that this molecule may not be playing a major role in human glucose homeostasis. Further prospective studies in larger cohorts and for longer periods of time are now needed to clarify the presence of any such association.

5. Funding source

This project was funded by the Commission of the European Communities, Directorate C–Public Health, grant agreement No. 2004310. Under the rules of the agreement, it was also co-funded by the private sector and, in this case, supported by an unrestricted educational grant from Bristol-Myers Squibb Greece and a grant by the Hellenic Society of Lipidology, Atherosclerosis and Vascular Disease.

Disclosure of interest

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

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.diabet.2014.01.001.

References


