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Original article

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

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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/m^2). 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.

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

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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 75 g 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 $\geq$ 7.0 mmol/L and/or 2hPG $\geq$ 11.1 mmol/L) [17]. Total plasma osteocalcin was measured using an ELISA method (N-Mid Osteocalcin ELISA; Immunodiagnostic Systems Ltd, Boldon, Tyne & Wear, UK). For the present analysis, participants with renal failure, recent fractures ($\leq$ 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-yearfollow-up are presented in Table 1. The study population, which included 154 men (50.3%), was middle-aged (mean age 54.4 $\pm$ 10.2 years) and nearly obese, with a mean body mass index (BMI) score at baseline of 29.5 $\pm$ 4.9 kg/m^2. Their risk for future development of type 2 diabetes was high (mean FINDRISC: 12.9 $\pm$ 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