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RESEARCH ARTICLE

The uncarboxylated form of osteocalcin is associated with improved glucose tolerance and enhanced β-cell function in middle-aged male subjects

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Abstract

Background Recent human studies support the notion that serum osteocalcin increases β-cell proliferation and insulin secretion, and improves insulin sensitivity. However, no study has examined the effects of serum osteocalcin γ-carboxylation status on these associations or determined the role of uncarboxylated osteocalcin in glucose metabolism in humans.

Methods The aim of this study was to determine the association between uncarboxylated osteocalcin and β-cell function and insulin sensitivity in humans. As many as 199 men, aged 25–60 years (mean age, 47 years), who had never been treated with glucose lowering agents, were enrolled in this cross-sectional study. OGTT was performed and other metabolic parameters, such as, BMI, BP, lipid profiles, and both uncarboxylated and carboxylated osteocalcin plasma levels were measured.

Results When subjects were divided into tertiles by uncarboxylated and carboxylated osteocalcin plasma concentrations, subjects in the upper tertile of each showed lower fasting and post-challenge glucose levels after adjusting for age and BMI ($P < 0.05$). The upper uncarboxylated osteocalcin tertile was associated with higher HOMA-B% levels, which are representative of β-cell function ($P < 0.05$), and the upper carboxylated osteocalcin tertile was associated with lower HOMA-IR values, which are representative of insulin resistance ($P < 0.05$).

Conclusions Elevated levels of both carboxylated and uncarboxylated forms of osteocalcin were associated with improved glucose tolerance. Moreover, the uncarboxylated form of osteocalcin was found to be associated with enhanced β-cell function, and the carboxylated form was associated with improved insulin sensitivity in middle-aged male subjects. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords osteocalcin; glucose tolerance; β-cell function; insulin sensitivity

Abbreviations BMI – body mass index; BP – blood pressure; C – cholesterol; CV – coefficient of variation; FPG – fasting plasma glucose; FPI – fasting plasma insulin; HDL – high-density lipoprotein; HOMA – homeostasis assessment model; hsCRP – high sensitivity C-reactive protein; IL-6 – in-terleukin 6; LDL – low-density lipoprotein; OGTT – oral glucose tolerance test.

Introduction

Recently, Lee *et al*. suggested that bone, like other endocrine organs, secretes the osteoblast-specific protein, osteocalcin, which is known to be involved in glucose metabolism and fat mass. In this previous report, it was elegantly shown that uncarboxylated osteocalcin increases pancreatic β-cell proliferation and insulin secretion, and improves insulin sensitivity by regulating the expression of adiponectin (an insulin-sensitizing adipokine) in adipocytes using osteocalcin-knockout mice and cell-based experiments [1]. Furthermore, Ferron *et al*. found that these results are reproduced in wild-type animals, and suggested that osteocalcin may have therapeutic potential for the treatment of metabolic diseases [2].

More recently, it was reported that serum osteocalcin is negatively associated with BMI, fat mass, and FPG levels, and that serum osteocalcin levels are inversely associated with blood markers of dysmetabolic phenotypes, such as, fasting insulin, hsCRP, and IL-6 levels, and other parameters of atherosclerosis, such as, brachial-ankle pulse wave velocity and intima-media thickness in humans [3–7].

According to the initial observations of Lee *et al*., only the uncarboxylated form of osteocalcin has the ability to induce insulin secretion, and the expression of genes encoding adiponectin and insulin, and as a result, it improves glucose metabolism. In contrast, carboxylated osteocalcin was found to have none of these effects. However, no human study has been undertaken to examine the roles of serum osteocalcin with respect to its γ-carboxylation status or to determine the role of the uncarboxylated form of osteocalcin in glucose metabolism. Therefore, the aim of this study was to determine the association between both uncarboxylated and carboxylated forms of osteocalcin and β-cell insulin secretory function and insulin sensitivity in human subjects.

Materials and methods

Subjects

We recruited study subjects from among those who visited the Kyung Hee East–West Neo Medical Center between December 2006 and July 2008 for the diagnosis, evaluation, or treatment of diabetes. During this period, 1128 subjects (625 men; 503 women) underwent 75-g OGTT, and after obtaining informed consent, plasma samples were stored at $-70\,^{\circ}C$ for future research on cardiovascular disorders and diabetes. Biochemical markers of bone remodelling, including serum osteocalcin are affected by a variety of factors, such as, age, gender, physical activity, diet, and diurnal rhythm; changes with the phase of the menstrual cycle and the season of the year. Therefore, we limited our cohort to middle-aged men, who had never been treated with glucose lowering agents to minimize the change in osteocalcin levels caused by altered bone turnover. The exclusion criteria applied were (1) a history of malignancy; (2) a history of liver or thyroid diseases; (3) hyperparathyroidism; (4) a previous fracture; and (5) the taking of medications known to affect bone or glucose metabolism, such as, glucocorticoids. Finally, 199 men, aged 25–60 years (mean age, 47.0 ± 8.6), were enrolled in this cross-sectional study. According to their OGTT results, subjects were diagnosed with one of these conditions; a normal glucose tolerance, a prediabetes (which included subjects with impaired fasting glucose, impaired glucose tolerance, and both impaired fasting glucose and impaired glucose tolerance), and a diabetes [8].

The study was approved by the ethics committee and the institutional review board of Kyung Hee University Hospital, and complied with the Declaration of Helsinki.

Biochemical measurements

After an overnight fast, 75-g OGTT was started at between 08 : 00 h and 09 : 00 h according to standardized clinical procedures. In brief, after basal blood samples had been drawn (0 min), 75-g glucose (Diasol®; dissolved in 300 mL of water) was consumed within 5 min. Glucose levels in blood were then determined at 30, 60, 90, and 120 min after the glucose had been administered. In addition, plasma insulin and C-peptide levels were also measured at time 0 and 30 min. To estimate insulin sensitivity and β-cell function, HOMA was used based on FPG and FPI concentrations. Insulin resistance was estimated using HOMA-IR, which was defined as [FPI (μU/mL) × FPG (mmol/L)]/22.5. HOMA-B% was calculated using $(20 \times \text{FPI})/(\text{FPG} - 3.5)$, and was viewed to represent β-cell function. In addition, insulinogenic index was defined as the ratio of insulin change to plasma glucose change at 30 min after a 75-g oral glucose load (Δinsulin, 0–30 min/Δplasma glucose, 0–30 min). Insulinogenic index was used to estimate the early-phase insulin secretion.

The plasma glucose levels were determined by the hexokinase method using an auto-analyser (Hitachi, Tokyo, Japan), which had a CV of 1.7%. The plasma insulin (Biosource, Nivelles, Belgium) and C-peptide (Immunotech, Czech Republic) levels were determined using an immuno-radiometric assay, which had intra- and inter-assay CVs of 1.6–2.2 and 6.1–6.5%, and 2.3–3.0 and 3.5–5.1%, respectively. Serum total cholesterol, triglyceride, HDL-C, and LDL-C were determined by the enzymatic (colorimetric) method using an auto-analyser (Hitachi). Plasma uncarboxylated and carboxylated osteocalcin levels were measured by Green Cross Reference Laboratory (South Korea) using a commercial EIA kit (Takara Biomedical Group, Otsu, Shia, Japan), which had intra- and inter-assay CVs of 4.4–6.6 and 5.6–9.8%, and 3.0–4.8 and 0.7–2.4%, respectively. Plasma 25-hydroxyvitamin D levels were measured using an RAI-CT kit (Biosource, Nivelles, Belgium), which had intra- and inter-assay CVs of 2.1–2.7 and 4.4–5.2%.

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Statistical analysis

All data are presented as means ± SDs or proportions, unless otherwise indicated. One-way analysis of variance was used to compare the means between the tertiles of osteocalcin levels, and the linear-by-linear association chi-square test was used for trend analysis between the tertiles. Analysis of covariance was used to control for the effects of other variables. Analysis was performed using SPSS ver. 13.0 (SPSS, IL), and P values of <0.05 were considered significant.

Results

Study subjects were categorized into tertiles according to the plasma levels of uncarboxylated and carboxylated osteocalcin (lower, middle, and upper) and then compared with demographic and metabolic parameters, such as, BMI, BP, and lipid profile results. Regarding uncarboxylated osteocalcin, subjects in the upper tertile were significantly younger and taller than subjects in the lower and middle tertiles, and had higher LDL-C levels, and for carboxylated osteocalcin, the upper tertile was significantly associated with a high BMI. However, no statistical significance was observed between osteocalcin tertiles with respect to BP, total cholesterol, and triglyceride levels (Table 1).

In terms of glucose tolerance, both uncarboxylated and carboxylated osteocalcin were found to be significantly associated with improved glucose tolerance. In particular, as plasma levels of both forms increased, fasting and post-challenge plasma glucose levels significantly decreased. Furthermore, it was noted that decreased glucose levels in subjects in the upper uncarboxylated osteocalcin tertile were significantly associated with a higher HOMA-B% level, and subjects in the upper carboxylated osteocalcin tertile were significantly associated with a lower HOMA-IR level. However, fasting insulin and C-peptide levels were not found to be associated with the uncarboxylated or carboxylated osteocalcin forms. Furthermore, insulinogenic indices showed no association with either form (Table 2).

Similarly, the results obtained indicated a significant linear association between glucose tolerance and uncarboxylated osteocalcin levels, that is, the subjects in the upper tertile were more likely to have been diagnosed with normal glucose tolerance, and less likely to diabetes. However, no linear trend was observed between glucose tolerance and carboxylated osteocalcin levels (Table 3).

Discussion

In the present study, the plasma levels of uncarboxylated osteocalcin were found to be inversely associated with fasting and post-challenge plasma glucose levels in middle-aged men after adjustment for potential confounders, such as, age and BMI. In addition, subjects in the upper uncarboxylated osteocalcin tertile had higher HOMA-B% levels, which suggest that the improved glucose tolerance could be associated with enhanced β-cell function in subjects with elevated uncarboxylated osteocalcin levels.

Although the results of some human studies concur with our results, all the studies have measured total osteocalcin levels, and no previous study has directly measured the levels of the uncarboxylated form of osteocalcin, and determined the role of uncarboxylated osteocalcin in glucose metabolism in humans [3–7]. However, according to Lee *et al.*, only uncarboxylated osteocalcin has the ability to increase insulin secretion by pancreatic β-cells and insulin sensitivity in adipocytes by up-regulating adiponectin [1]. Therefore, to the best of our knowledge, this is the first report to determine the association between the uncarboxylated osteocalcin and glucose tolerance in humans.

In the present study, we measured the levels of the uncarboxylated and carboxylated forms of osteocalcin, and investigated the associations of both with glucose metabolism. In contrast to the results of animal study, it was found that the carboxylated form of osteocalcin

Table 1. Demographics and biochemical measurements by osteocalcin tertiles

	Tertile of uncarboxylated osteocalcin			Tertile of carboxylated osteocalcin		
	Lower ($n = 68$)	Middle ($n = 65$)	Upper ($n = 66$)	Lower ($n = 65$)	Middle ($n = 67$)	Upper ($n = 67$)
Median osteocalcin (range, μg/L)	<0.25	0.42 (0.33–0.64)	1.10 (0.65–11.18)	6.1 (1.1–8.1)	10.6 (8.4–13.4)	15.8 (13.5–48.0)
Age (years)	48.5 ± 7.0	47.7 ± 8.4	43.8 ± 10.1**	45.3 ± 9.2	47.7 ± 8.5	47.0 ± 8.6
Height (cm)	168.2 ± 5.0	169.2 ± 6.2	171.0 ± 5.6*	169.9 ± 5.9	169.0 ± 5.6	169.5 ± 5.6
Body weight (kg)	74.1 ± 9.4	73.9 ± 12.0	75.8 ± 13.9	79.2 ± 12.2	73.0 ± 9.9**	71.7 ± 12.1**
BMI (kg/m^2)	26.1 ± 2.8	25.7 ± 3.7	25.8 ± 3.8	27.3 ± 3.3	25.5 ± 2.9**	24.9 ± 3.6**
Systolic BP (mmHg)	133.3 ± 16.0	133.9 ± 18.0	135.2 ± 16.2	137.2 ± 17.1	133.6 ± 15.9	131.6 ± 16.7
Diastolic BP (mmHg)	83.7 ± 9.4	83.9 ± 10.7	83.7 ± 10.4	85.6 ± 10.6	84.2 ± 9.4	81.5 ± 10.1
Total-C (mmol/L)	5.07 ± 0.98	5.18 ± 1.17	5.03 ± 0.99	5.21 ± 1.08	5.14 ± 1.13	4.92 ± 0.90
LDL-C (mmol/L)	2.86 ± 0.83	3.25 ± 0.99	3.36 ± 1.36#	3.28 ± 1.42	3.11 ± 0.94	3.06 ± 0.82
HDL-C (mmol/L)	1.20 ± 0.33	1.15 ± 0.26	1.16 ± 0.23	1.15 ± 0.21	1.18 ± 0.35	1.18 ± 0.27
Median triglyceride (mmol/L)	2.02 (0.41–14.20)	1.76 (0.49–13.00)	1.84 (0.40–5.13)	2.13 (0.61–12.90)	1.85 (0.41–14.20)	1.76 (0.40–6.74)

Data are presented as means ± SDs, except otherwise indicated. *$P < 0.05$ and **$P < 0.01$, between lower and upper tertiles, #$P < 0.05$, between middle and upper tertiles.

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Table 2. Results of OGTT by osteocalcin tertiles

	Tertile of uncarboxylated osteocalcin			Tertile of carboxylated osteocalcin		
	Lower ($n = 68$)	Middle ($n = 65$)	Upper ($n = 66$)	Lower ($n = 65$)	Middle ($n = 67$)	Upper ($n = 67$)
FPG (mmol/L)	8.7 ± 0.4	$7.5 \pm 0.2^{*}$	$7.5 \pm 0.3^{**}$	8.9 ± 0.4	$7.6 \pm 0.3^{**}$	$7.3 \pm 0.2^{**}$
2-h glucose after OGTT (mmol/L)	15.9 ± 0.7	$13.8 \pm 0.7^{*}$	$13.1 \pm 0.6^{**}$	16.0 ± 0.8	$13.8 \pm 0.7^{*}$	$13.1 \pm 0.6^{**}$
FPI (pmol/L)	81.1 ± 4.3	82.5 ± 4.6	85.9 ± 6.4	93.6 ± 5.9	84.3 ± 5.2	71.9 ± 3.8
Fasting plasma C-peptide (nmol/L)	1.01 ± 0.03	0.94 ± 0.04	0.89 ± 0.05	1.09 ± 0.05	0.91 ± 0.03	0.84 ± 0.03
HOMA-IR	4.28 ± 0.23	4.16 ± 0.37	3.93 ± 0.26	4.93 ± 0.32	4.08 ± 0.31	$3.38 \pm 0.19^{*}$
HOMA-B%	64.7 ± 5.4	68.0 ± 4.3	$81.1 \pm 7.4^{*}$	75.0 ± 7.2	74.3 ± 5.5	64.4 ± 4.8
Insulinogenic index	0.29 ± 0.04	0.39 ± 0.06	0.45 ± 0.07	0.37 ± 0.07	0.39 ± 0.04	0.36 ± 0.06

Data are presented as means $\pm$ SDs.
$^{*}P < 0.05$ and $^{**}P < 0.01$ are compared with lower tertile after adjustment for age and BMI.

Table 3. Category of glucose tolerance by osteocalcin tertiles

	Tertile of uncarboxylated osteocalcin				Tertile of carboxylated osteocalcin			
	Lower ($n = 68$)	Middle ($n = 65$)	Upper ($n = 66$)	P trend	Lower ($n = 65$)	Middle ($n = 67$)	Upper ($n = 67$)	P trend
No. of normal glucose tolerance (%)	1 (12.5)	3 (37.5)	4 (50.0)		1 (12.5)	1 (12.5)	6 (75.0)	
No. of prediabetes (%)	11 (23.9)	16 (34.8)	19 (41.3)	0.02	12 (26.1)	21 (45.7)	13 (28.2)	0.11
No. of diabetes (%)	56 (38.6)	46 (31.7)	43 (29.7)		52 (35.9)	45 (31.0)	48 (33.1)	

Data are presented as numbers of patients (%).

was also inversely associated with plasma glucose level. However, levels of this form were found to be more closely related to the improved insulin sensitivity, as measured by HOMA-IR, rather than to increased β-cell function. Collectively, although both the uncarboxylated and carboxylated forms were found to be inversely associated with glucose tolerance, it could be possible that the principal mechanisms whereby they lower blood glucose levels differ. In a previous study, it was suggested that carboxylated osteocalcin also increases basal and insulin-stimulated glucose transport in cultured rat adipocyte, although these effects of carboxylated osteocalcin were less pronounced than those of the uncarboxylated form [9]. Accordingly, additional study is required to confirm the roles of both osteocalcin forms on glucose metabolism.

Osteocalcin is an osteoblast-specific protein that contains three γ-carboxyglutamic acid residues derived from vitamin K-dependent post-translational modification [10]. It has been reported that the serum levels of uncarboxylated osteocalcin were elevated in elderly women, and to be associated with increased hip fracture risk and reduced bone mineral density [11–13]. Moreover, it is influenced by gender, smoking status, sports activity, dietary intake of the vitamin K source, the time of the year when blood was collected, and especially age [14]. Previous studies have demonstrated that the serum osteocalcin levels were highest in the 20–29 year age group, declined and stabilized, then increased again in the seventh decade in men [15], and serum osteocalcin levels in women showed more dynamic changes, and increased abruptly around the time of menopause [15–17]. Therefore, although we could not adjust for the effects of altered bone turnover on osteocalcin level by measuring bone mineral density or other bone turnover markers, such as, bone-specific alkaline phosphatase or other markers of bone resorption, we did attempt to minimize the effects of other confounders by uniformly enrolling middle-aged men, who were expected to have minimal bone turnover, and who had never been administered glucose lowering agents.

It has been reported that osteocalcin gene promoter activity is up-regulated by 1,25-dihydroxyvitamin D [18,19], and therefore, we measured plasma 25-hydroxyvitamin D levels to determine whether it mediates the glucose lowering effects of osteocalcin. However, no correlations were found between 25-hydroxyvitamin D levels and measures of glucose tolerance, that is, fasting and post-challenge plasma glucose levels, plasma insulin levels, HOMA-IR, HOMA-B%, and insulinogenic indices. Furthermore, no differences were noted between tertiles of uncarboxylated or carboxylated osteocalcin in terms of 25-hydroxyvitamin D levels (data not shown).

This study has several limitations that require consideration. First, this study was based on cross-sectional analysis, and we cannot be certain whether our findings are merely correlations or the osteocalcin has a direct glucose lowering effects in human subjects as is the results of animal and cell-based studies. Second, the sample size was not large enough to reach a definite conclusion. Third, we did not measure serum adiponectin levels, and therefore, we are uncertain whether the associations between plasma osteocalcin levels and improved glucose tolerance were truly mediated by the insulin-sensitizing effects of adiponectin.

In summary, the levels of both carboxylated and uncarboxylated forms of osteocalcin in plasma were found

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to be inversely associated with fasting and post-challenge glucose levels in middle-aged men after adjustment for age and BMI. Furthermore, our findings suggest that the uncarboxylated form of osteocalcin was found to be associated with enhanced β-cell function, and the carboxylated form was associated with improved insulin sensitivity in middle-aged male subjects.

Conflict of interest

None declared.

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