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Relationship between plasma osteocalcin, glycaemic control and components of metabolic syndrome in adult Nigerians with type 2 diabetes mellitus

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Abstract

Aims: To determine the levels of plasma osteocalcin (OC) in Nigerians with type 2 diabetes mellitus (DM) and compare these to levels in non-diabetic controls (NDM). To assess the relationship of OC to glycaemic control and parameters of metabolic syndrome (MetS) and compare its levels in Nigerians with and without MetS.

Methods: The waist circumference (WC), body mass index (BMI) and blood pressure of 200 study participants were taken. Plasma osteocalcin, fasting glucose (FPG), glycated haemoglobin (HbA1c), high density lipoprotein cholesterol (HDL-c) and triglyceride (TG) levels were determined. Metabolic syndrome was defined by the International Diabetes Federation criteria. Statistical significance was set at 0.05.

Results: Osteocalcin levels were lower in the DM group ($p = 0.002$) and inversely related to FPG ($r = -0.198$, $p = 0.003$), HbA1c ($r = -0.313$, $p < 0.001$), BMI ($r = -0.331$, $p < 0.001$), WC ($r = -0.339$, $p < 0.001$) and TG ($r = -0.145$, $p = 0.040$), but directly related to HDL-c levels ($r = 0.166$, $p = 0.019$). Osteocalcin was higher in participants without MetS (Median 8.75 ng/mL IQR[5.48–12.68]ng/mL) than in those with MetS (Median 4.74 ng/mL, IQR[2.80–9.12]ng/mL), $p < 0.001$.

Conclusions: Plasma osteocalcin levels are inversely associated with good glycaemic control and components of MetS and are lower in individuals with DM and in those with MetS. These findings support a vital role of the bone, in the regulation of glucose and energy metabolism, in Nigerians. Further extensive studies are required to explore the potentials of OC in the management of DM and MetS.

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Conflict statement

The authors state that there is no conflict of interest.

Keywords

Osteocalcin; Diabetes mellitus; Metabolic syndrome; Glycaemic control; Obesity

1. Introduction

The age-long ascribed role for the bone is that of structural support and locomotion. Biochemically, its known function had been in the regulation of calcium and phosphate homeostasis [1]. However, in the past decade, animal studies [2,3] and studies in humans of non-African origin [4,5] have suggested an endocrine function for the bone in the regulation of glucose and energy metabolism via the bone-derived protein – osteocalcin (OC).

Diabetes mellitus (DM), a disorder of glucose and lipid metabolism has been considered a looming epidemic in Nigeria [6]. About 10 to 18.5% of medical ward admissions in Nigeria are diabetes-related, with case-fatality rate of 23% [7,8]. Previous studies have shown a high prevalence of metabolic syndrome in patients with type 2 diabetes mellitus [9,10]. Diabetes mellitus and metabolic syndrome (MetS) are two conditions which separately, predispose an individual to a high risk for cardiovascular disease. When these two conditions occur together in an individual, the risk for cardiovascular disease becomes even higher [11]. The burden of the two conditions and the associated complications has continued to rise in Nigeria, despite the use of diet, lifestyle modifications and conventional pharmacotherapy. Therefore, there is a need for more research into the characteristics of these two conditions. We performed a study among adult Nigerians with type 2 DM to determine the levels of plasma OC and compare these to levels in non-diabetic controls (NDM). Furthermore, we assessed the relationship of this bone protein to glycaemic control and parameters of MetS and we compared its levels in individuals with and without MetS.

2. Materials and methods

2.1. Study background

A cross-sectional study of adults with type 2 DM receiving care at the Diabetes Outpatient Department of the Lagos University Teaching hospital was carried out over a three-month period. Nondiabetic controls were recruited from the staff and retirees of the hospital and its College of Medicine. The Lagos University Teaching Hospital is a 761-bed tertiary healthcare facility with a 60–70% bed occupancy, located in the South-Western part of Nigeria. Approval for the study was obtained from the Health Research Ethics Committee of the hospital. The study was performed according to the Declarations of Helsinki (2013).

2.2. Participant selection

Every fifth individual attending the clinic, who consented to participate in the study and who was receiving care by diet and/or oral hypoglycaemic agents was recruited until calculated sample size was achieved. Patients receiving thiazolidinediones and medications known to affect bone and calcium metabolism such as vitamin D, bisphosphonates, calcitonin, oestrogen, tamoxifen or corticosteroids were excluded from the study. Pregnant women, non-fasting patients, patients in renal failure and those with haemoglobinopathies or a

history of malignancy, hyperparathyroidism, thyroid dysfunction, prolonged immobility or fracture in the preceding six months were also excluded from the study.

2.3. Data collection

Study participants were requested to respond to an interviewer-administered questionnaire after a 12-h overnight fast, subsequently, their blood pressure readings and anthropometric measurements were taken using standard protocols.

Blood pressure (BP) readings were taken twice from the participants' right arms using an automated sphygmomanometer (Omron Medical, United Kingdom), after they had been seated for ten minutes. The average of two readings was recorded to the nearest 1.0 mmHg.

Weight was measured using a mechanical lever scale (Taylor, USA). Each participant was weighed twice and each reading was taken after nulling the scale to zero reading. The measurements were taken with participants standing barefooted, in light clothing with pockets emptied and their arms hanging naturally by the sides, while looking forwards. The weight was taken as the average of two readings, calculated to the nearest 0.1 kg. Participants' heights were determined using a stadiometer (QuickMedical, USA). They were requested to take off their footwear, headgears and excessive hair ornaments and then asked to stand with shoulders relaxed, arms by the sides, legs straight and feet together. Measurement was then taken to the nearest centimetre, with the buttocks, upper back or head touching the measuring surface of the stadiometer rule [12]. As a measure of generalized obesity, each participant's BMI was computed by dividing the weight in kilograms, by the square of the height in metres (kg/m^2).

To determine abdominal obesity, measurement of the WC was taken using a stretch-resistant tape (HTS, China). Each participant's WC was taken from the zero mark on the tape rule. The tape rule was wrapped snugly, but not constrictingly, around the participant at a level parallel to the floor, midpoint between the top of the iliac crest and the lower margin of the last palpable rib in the mid axillary line. The reading was taken to the nearest centimetre, at the end of expiration, after a few consecutive natural breaths [13].

2.4. Specimen collection

Venous blood was collected from the antecubital fossa using a multisample needle, after antiseptic preparation of the venepuncture site. Specimen for plasma OC was collected into ethylene diamine tetra acetic acid-aprotinin (EDTA-aprotinin) vacutainers and placed on ice, while specimen for glycated haemoglobin (HbA1c) assay was collected into EDTA vacutainers, that for fasting plasma glucose (FPG) assay was collected into fluoride oxalate vacutainers and that for serum triglycerides (TG) and high density lipoprotein cholesterol (HDL-c) was collected into plain vacutainers and allowed to clot. All specimens except that for HbA1c were centrifuged at 4000 rpm for five minutes and the supernatant collected and all except the fluoride oxalate specimen were stored at -80°C for a maximum of three months, prior to laboratory analyses. Glycated haemoglobin assay was performed immediately after specimen collection. Fasting plasma glucose assay was also performed immediately, following centrifugation and separation of plasma.

2.5. Laboratory analyses

A solid phase sandwich enzyme linked immunosorbent assay (GenWay Biotech, USA), was used for the quantitative determination of OC in plasma, and read out using Acurex plate read (Acurex Diagnostics, USA). Low and normal level controls were assayed in duplicate during each run.

Glycated haemoglobin was determined using an automated boronate affinity chromatography method which is National Glycohaemoglobin Standardization Program certified (Infopia, Korea).

Plasma glucose was determined by the glucose oxidase method. Serum TG assay was performed using standard enzymatic methods. The HDL-c was determined by a two-step method using a precipitant to isolate the non-HDL-c components in the serum in the first step, and subsequent quantitative determination of the HDL-c by standard enzymatic methods for cholesterol determination. All three assays for FPG, TG and HDL-c were performed on the Sinnowa® Chemistry Analyzer using reagents from Biolabo, France and quality control sera from Randox Laboratories, UK.

2.6. Definition of metabolic syndrome

Metabolic syndrome was defined according to the International Diabetes Federation (IDF) criteria as three or more of abdominal obesity (waist circumference ≥ 80 cm in a female or ≥ 94 cm in a male); hyperglycaemia (fasting plasma glucose ≥ 5.6 mmol/L or a known diagnosis of diabetes); hypertension (systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 85 mmHg or use of anti-hypertensive therapy); and dyslipidaemia (fasting plasma TG ≥ 1.7 mmol/L or treatment for hypertriglyceridaemia and/or fasting HDL-c < 1.3 mmol/L in a female and < 1.03 mmol/L in a male or treatment for hypo alpha lipoproteinaemia) [14].

2.7. Statistical analysis

Data were analysed using IBM SPSS version 22 for Windows (Chicago IL). Normality of distribution of continuous variables was assessed using the Kolmogorov-Smirnov test. Thereafter, measures of location and variability were computed for the continuous variables. Normally distributed variables were reported as Mean (standard deviation) while the variables with non-parametric distribution were reported as median (interquartile range). Differences in the distribution of continuous variables in the DM and NDM groups, and the participants with MetS and those without MetS were evaluated using the Mann-Whitney *U* test. The age and gender-adjusted Spearman's correlation coefficient was employed to assess the relationship between osteocalcin levels and markers of glycaemic control (glucose and HbA1c), adiposity (BMI and waist circumference) and other components of MetS (TG, HDL-c and BP). P value < 0.05 was considered to be statistically significant.

3. Results

One hundred and eight participants with DM (54 males and 54 females) and 92 non-diabetic (NDM) controls (39 males and 53 females) aged 34–70 years were studied. The plasma OC

levels were significantly lower in the DM group than in the NDM group (Fig. 1). The distribution of plasma OC levels and parameters of MetS in the DM and NDM groups are shown in Table 1. Plasma OC had an inverse relationship with fasting glucose ($\rho = -0.197$, $p = 0.012$), glycated haemoglobin ($\rho = -0.343$, $p < 0.001$), BMI ($\rho = -0.313$, $p < 0.001$), waist circumference ($\rho = -0.367$, $p < 0.001$), diastolic blood pressure ($\rho = -0.086$, $p = 0.230$), systolic blood pressure ($\rho = -0.145$, $p = 0.042$) and triglycerides ($r = -0.145$, $p = 0.040$), and directly related to HDL-c levels ($r = 0.166$, $p = 0.019$). Table 2 shows the details of these relationships. Hypoalbuminaemia was prevalent in 180 (90.0%) participants, hypertension was prevalent in 148 (74.0%) participants and 145 (72.5%) participants had abdominal obesity. Hyperglycaemia was prevalent in 117 (58.5%) participants while hypertriglyceridaemia was prevalent in 93 (46.5%) participants. Metabolic syndrome was present in 105 (97.2%) participants with DM and 34 (37.0%) of the NDM participants. Metabolic syndrome was prevalent in 139 (69.5%) study participants.

Plasma OC was higher in participants without MetS (Median 8.75ng/mL IQR[5.48–12.68]ng/mL) than in those with MetS (Median 4.74 ng/mL, IQR[2.80–9.12]ng/mL), $p < 0.001$ (Fig. 2).

4. Discussion

Metabolic syndrome is a cluster of clinical and biochemical abnormalities associated with insulin resistance and it increases the risk for cardiovascular disease and type 2 DM. Type 2 DM is a metabolic disorder associated with chronic hyperglycaemia and characterized by insulin resistance and relative insulin deficiency. In this study, the finding of significantly lower levels of plasma osteocalcin in patients with type 2 DM when compared to the nondiabetics is in line with reports from China [15], South Korea [16] and Sweden [17]. A number of mechanisms may be involved in these findings. It has been suggested that hyperglycaemia induces a low turnover of bone with osteoblast dysfunction resulting in suppression of bone formation and plasma osteocalcin level [16,18]. Hyperglycaemia and advanced glycation end products induce osteoblast apoptosis and also restrain osteoblastic differentiation [19,20]; hence the reduction in osteocalcin. The osteoblast expresses the insulin receptor, a tyrosine kinase which requires tight regulation since its activation can occur in the absence of a ligand [21]. It has been shown that insulin signalling in osteoblasts is necessary for whole-body glucose homeostasis because it increases OC activity. Research in animal models and cell cultures showed that circulating osteocalcin stimulates the production of insulin by exerting a direct effect on pancreatic β cells [22]. Expression of insulin target genes is reduced in the skeletal muscle and liver of OC knockout mice, in addition to the decrease in insulin secretion and sensitivity and the glucose intolerance observed in them. When these OC-knockout mice were fed with osteocalcin, an improvement in insulin secretion and sensitivity occurred [2,22].

Although diabetes has been inversely associated with bone turnover markers [23], a study that has concomitantly measured other bone turnover marker reported that the observed association between osteocalcin and dysmetabolic phenotype is not confounded by measures of bone turnover [24]. It is also important to note that osteocalcin is synthesized by the bone gamma-carboxyglutamate protein (*BGLAP*) gene located in the well-replicated region of

type 2 diabetes linkage on chromosome 1q22. A report from a large case-control study in African Americans, in which the *BGLAP* gene was sequenced in individuals with type 2 diabetes has suggested that coding variants at residue 94, in exon 4 of the gene, situated near the γ -carboxylation site, may modify glucose homeostasis traits [25].

This study found significant inverse relationship between HbA1c and plasma osteocalcin, similar to previous studies in China which reported significant inverse relationship of serum osteocalcin with HbA1c in different populations of Chinese adults with type 2 diabetes [15,26]. The inverse relationship observed between fasting plasma glucose and osteocalcin from most previous studies [15,17,26] was also significant in this study. In some studies the uncarboxylated form of osteocalcin appeared to mediate the effects of osteocalcin on metabolic phenotype [22,27]. Other studies have however suggested that both ncarboxylated and carboxylated forms of osteocalcin increase basal and insulin-stimulated glucose transport although the effect of the carboxylated form was less robust. Specifically, the carboxylated form has been found to be more closely related to improved insulin sensitivity, while the uncarboxylated osteocalcin is closely related to insulin secretion and beta cell proliferation [28]. Collectively, the two forms consist the total osteocalcin and have been found to be inversely associated with glucose intolerance, although the chief mechanisms through which each lowers blood glucose level may vary.

In this study, the findings of a persistent inverse relationship of plasma osteocalcin with measures of obesity are in line with reports from the United States [24], Sweden [17] and studies in cell cultures and animals [3]. Adipose tissue modulates metabolism through the release of non-esterified fatty acids, glycerol and hormones, leptin and adiponectin inclusive [29]. The production of many of these substances is altered in obesity. Adiponectin acts as an insulin sensitizer, nevertheless, the distribution of body fat is a crucial determinant of insulin sensitivity. Generalized obesity is typically associated with insulin resistance, while insulin sensitivity varies with differences in body fat distribution [30]. Individuals who are lean, with a more peripheral distribution of fat are usually more insulin sensitive than their lean peers who have a preponderance of centrally distributed fat. The secretion of adiponectin by omental adipocytes is strongly and negatively correlated with BMI [31]. Fat mass influences bone metabolism through the adipocytokines secreted from adipocytes [2]. Osteocalcin derived from the bone, on the other hand, regulates fat mass and glucose metabolism [22]. Adiponectin influences bone metabolism by acting on its osteoblast-situated receptor to cause proliferation, differentiation and mineralization of osteoblasts [32,33]. Likewise, osteocalcin acts on adipocytes to stimulate adiponectin production and action. Adiponectin subsequently promotes insulin sensitivity. Insulin on the other hand, acts directly on osteoblasts, thus, an interactive relationship exists between bone and energy metabolism. Also, worthy of mention is the origin of the osteoblast and the adipocyte, these two cells develop from a common precursor; the mesenchymal stem cell [34]. Their common origin may be the link between the osteoblast-derived protein- osteocalcin and the obesity – associated hormones. In various animal models of obesity, osteocalcin was protective against type 2 diabetes and obesity. Mice which received a high fat diet and recombinant osteocalcin gained significantly less weight and had significantly smaller fat pads than mice not given osteocalcin. The osteocalcin-treated mice exhibited higher insulin sensitivity and possessed better glucose-tolerance than their untreated counterparts [22].

These benefits appeared to occur through an upregulation of genes responsible for energy expenditure.

The present study observed lower osteocalcin levels in individuals with MetS than in those without MetS. Furthermore, osteocalcin was inversely related to TG and directly related to HDL-c levels. A group of researchers in Australia reported an association of lower osteocalcin with increased risk of MetS and TG levels but no relationship with HDL-c [35]. Kanazawa and colleagues also reported evidence of a significant inverse relationship of osteocalcin with TG levels [32]. Amongst a population of blacks and non-Hispanic whites, a negative relationship between osteocalcin and the presence of MetS was reported [36]. However, a study among post-menopausal women in Korea found no correlations between osteocalcin and lipid profile [16]. Fernandez-Real et al. reported that TG was not associated with baseline serum osteocalcin in obese non-diabetic females, nonetheless following a dietary and resistance-training intervention, the serum osteocalcin became inversely related to the TG levels [4]. Although evidence from animal studies show a negative relationship between osteocalcin and TG levels [3,22], results from human studies are few and conflicting. The variations in the findings from the different studies in humans suggest a need for more research with regards to osteocalcin and lipid metabolism in humans, in order to establish the pathophysiologic role of osteocalcin in lipid metabolism.

Some limitations to the present study include its crosssectional nature, therefore the relationships reported are only associative, and hence conclusions regarding causality cannot be drawn. Additionally, vitamin D, parathyroid hormone, and adiponectin levels were not assessed in the study participants. These hormones play a role in bone and energy metabolism. Despite these limitations, the study involved a sizeable population that is representative of the Nigerian adults. Fasting morning blood samples were also utilized and this minimized the effect of meals and diurnal variation on osteocalcin levels. Furthermore, participants with conditions known to affect bone and energy metabolism were excluded from the study.

In conclusion, the findings from this study are suggestive of an endocrine role of the bone in glucose and energy metabolism in the Nigerian population. These findings indicate a need for more extensive research into the role of osteocalcin in the development, progression and management of MetS and type 2 DM.

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Fig. 1.
Distribution of Plasma Osteocalcin among Participants with Diabetes and Controls.

Fig. 2. Distribution of Plasma Osteocalcin among Participants, in the Presence and Absence of Metabolic Syndrome. Non-MetS- participants without metabolic syndrome; MetS- participants with metabolic syndrome.

Table 1

Biophysical and Biochemical Profile of Study Participants.

Variable	Whole group	Diabetics	Non-diabetics	p value
Age in yrs (Median (IQR))	61.0 (54.0–66.0)	61.0 (55.0–66.0)	60.0 (53.0–65.0)	0.436
BMI in kg/m ² (Median (IQR))	25.98 (23.05–29.01)	26.51 (23.90–29.60)	25.10 (22.60–28.48)	0.075
WC in cm (Median (IQR))	94 (86–102)	98 (93–104)	83 (82–97)	0.000 ^a
SBP in mmHg (Median (IQR))	130 (120–140)	130 (120–140)	120 (113–130)	0.006 ^a
DBP in mmHg (Median (IQR))	80 (70–80)	80 (70–80)	80 (70–80)	0.176
FPG in mmol/l (Median (IQR))	5.4 (4.9–7.4)	7.1 (5.5–9.1)	5.0 (4.7–5.3)	0.000 ^a
HbA1c% (Median (IQR))	6.3 (5.8–7.9)	7.7 (6.6–9.6)	5.8 (5.6–6.0)	0.000 ^a
HDL-C in mmol/l (Median (IQR))	0.90 (0.79–1.06)	0.85 (0.71–1.12)	0.98 (0.85–1.03)	0.145
TG in mmol/l (Median (IQR))	0.71 (0.59–0.89)	0.76 (0.62–1.04)	0.70 (0.56–0.82)	0.013 ^a
OC in ng/ml (Median (IQR))	5.63 (3.06–9.99)	4.98 (2.83–9.09)	8.03 (3.42–12.63)	0.002 ^a

BMI—Body Mass Index, OC—Osteocalcin, WC—Waist circumference, SBP—Systolic blood pressure, DBP—Diastolic blood pressure, FPG—Fasting plasma glucose, HbA1c—glycated haemoglobin, HDL-c—High density lipoprotein cholesterol, TG—triglycerides, OC—osteocalcin.

^a Statistically significant.

Table 2

Age and gender-Adjusted Spearman's Correlation of Osteocalcin with Variables.

Variables	RS	p value
BMI	−0.313	0.000 ^a
WC	−0.367	0.000 ^a
SBP	−0.145	0.042 ^a
DBP	−0.086	0.230
FPG	−0.197	0.012 ^a
HbA1C	−0.343	0.000 ^a
HDL-C	0.193	0.006 ^a
TC	−0.144	0.043 ^a

BMI—Body Mass Index, OC—Osteocalcin, WC—Waist circumference, SBP—Systolic blood pressure, DBP—Diastolic blood pressure, FPG—Fasting plasma glucose, HbA1c—glycated haemoglobin, HDL-c—High density lipoprotein cholesterol, TG—triglycerides, OC—osteocalcin.

^a Statistically significant.