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Serum osteocalcin is associated with improved metabolic state via adiponectin in females versus testosterone in males. Gender specific nature of the bone–energy homeostasis axis

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ABSTRACT

Objective: The osteoblast-derived protein osteocalcin (OCN) is known to be involved in glucose metabolism by increasing adiponectin secretion from adipocytes. Recently, OCN was also found to enhance testosterone production in mouse testes, suggesting that OCN effects on energy metabolism may be mediated through testosterone. Our aim was to assess a possible gender difference in the metabolic effect of OCN in humans.
Methods: We included 135 women and 155 men exhibiting changes in glucose tolerance in our study. Oral and intravenous glucose tolerance tests (OGTT and IVGTT, respectively) and a hyperinsulinemic normoglycemic clamp were performed. For clamp indices, whole body (M1) and muscle (M2) glucose uptake values were used. Leptin, adiponectin serum lipid, lipoprotein, total serum OCN and testosterone levels, and body composition were determined.
Results: Higher OCN values were associated with improving metabolic state in both genders. Adiponectin and OCN correlated significantly only in females ($r = +0.254$, $p = 0.0029$), while in men, testosterone and OCN values showed a significant positive correlation ($r = +0.243$, $p = 0.0023$), independent of age, BMI, HbA1c and body composition. In women, adiponectin was confirmed by feature selection analysis as being an independent determinant of OCN, in addition to age and three of the IVGTT glucose values. In men, besides M1, BMI, M2, leptin, body fat percent, and the 90-minute OGTT glucose reading testosterone, but not adiponectin were identified as independent contributors for OCN.
Conclusion: We confirmed the 'classic' adiponectin-mediated insulin-sensitising effect of OCN only in females. In men, a testosterone-mediated OCN metabolic effect is more likely.

Introduction

Lee et al. [1] were the first to describe a novel function of the skeleton on energy metabolism. They showed that the osteoblast-specific protein, osteocalcin (OCN), is involved in glucose metabolism by increasing insulin secretion and cell proliferation in pancreatic β-cells and improving insulin sensitivity by upregulating the expression of the insulin-sensitising adiponectin gene in adipocytes. Further studies in human have confirmed the initial report [2–4]. Collectively, these human studies have shown that serum OCN concentration is negatively associated with the plasma glucose level and body fat mass [3–6], and it is positively associated with insulin secretion [7,8], lower insulin resistance [4,5,9], and higher serum adiponectin concentration [3,9]. In most of this work, the homeostasis model assessment has mainly been used to assess β-cell function, insulin sensitivity and the involvement of osteocalcin on glucose metabolism, although it is well known that fasting indices do not always correlate well with insulin resistance [10]. In a recent study, oral glucose tolerance test (OGTT) indices (Stumvoll and oral glucose insulin sensitivity) were used to confirm these associations [11]. In the latter study, which included both genders, circulating OCN levels correlated positively with serum adiponectin levels, although the association with improved glucose tolerance and insulin secretion and sensitivity was independent of adiponectin.

Recently, it has been demonstrated that osteoblasts are able to induce testosterone production by the testes, though they fail to influence oestrogen production by the ovaries [12]. In addition, undercarboxylated OCN (ucOCN) was found to be positively associated with free testosterone level and negatively associated with luteinizing hormone in type 2 diabetes [13]. In boys aged 11–14 years, free testosterone levels have

Abbreviations: NGT, normal glucose tolerant; GI, glucose intolerant; IFG, impaired fasting glucose; IGT, impaired glucose tolerant; 2DM, type 2 diabetes mellitus; M1, clamp measured whole body glucose uptake; M2, clamp measured muscle glucose uptake; BMI, body mass index; BFP, body fat percent; OGTT, oral glucose tolerance test; IVGTT, intravenous glucose tolerance test; OCN, osteocalcin; IGI, insulogenic index of IVGTT; AIR, acute insulin response of IVGTT; HOMA-IR, homeostasis model assessment of insulin resistance; ucOCN, undercarboxylated osteocalcin; FFA, free fatty acid; AUC, area under the curve; DEXA, dual energy X-ray absorptiometry; WC, waist circumference.

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been found to correlate with OCN and ucOCN levels and periosteal circumference [14]. These findings suggest that ucOCN is involved in testosterone production in male subjects. Testosterone might be also involved in the insulin-sensitising effect of OCN, since hypogonadism has long been associated with metabolic syndrome and insulin resistance in male populations [15–17]. The role of testosterone in the bone–energy homeostasis is presumably gender-specific, as the effects of OCN were only demonstrated in Leydig cells and not in the ovarium; moreover, low testosterone levels are only associated with a metabolic syndrome in men [18]. Gender specific differences were also observed in relation with bone mineral content and insulin resistance, which might suggest the influence of hormonal difference between sexes in the bone–metabolism pathways [19].

Although a beneficial metabolic effect of OCN has been demonstrated in both genders, differences between males and females in the regulation of energy homeostasis by OCN have not been well addressed specifically. Additionally, possible role of testosterone in the metabolic effects of OCN in male subjects has not been examined until now. In our study, we examined the association between OCN, testosterone, adiponectin and metabolic parameters separately in male and female populations in order to assess gender-specific characteristics of the bone–energy homeostasis axis. Insulin sensitivity was measured by the gold standard hyperinsulinemic normoglycemic clamp technique, to eliminate any discrepancy between fasting and/or OGTT indices and true insulin resistance.

Patients and methods

Patients

This study was approved by the Hungarian Central Ethical Committee (A12988-2/2003-1018-EKU). After obtaining signed informed consent, we included 135 women (aged 49 ± 9 years) and 155 men (aged 42 ± 13) in our study, as approved by the ethical committee. Subjects were classified based on results of a standard 75-g OGTT at screening (blood drawn in the 0, 30, 60, 90 and 120 min), according to the American Diabetes Association criteria [20]. We included 47 normal glucose-tolerant (NGT) and 89 glucose-intolerant (GI) subjects in the female group; in the male group, there were 72 NGT and 83 GI subjects. Patients and healthy volunteers were recruited from our own diabetes outpatient clinic and by referral from regional family doctors. All GI patients, which included impaired fasting glucose (IFG), impaired glucose-tolerant (IGT) and type 2 diabetes mellitus (2DM) patients, were drug-naive at the time of the study. Patients receiving any medication influencing bone metabolism or hormone substitution therapy or who suffered from any endocrine disease were excluded from the study.

IVGTT and clamp

All subjects were fasted on the day of the clamp examination. They first underwent a 30-g IVGTT examination to assess insulin secretion (0.3 g/bodyweight iv. glucose injection). Following the IVGTT a hyperinsulinemic euglycemic clamp examination was carried out, described by DeFronzo et al. [21]. During a continuous infusion of insulin ($45\ mU \times min \times m^{-2}$) and glucose (20%), the steady state was set at the constant glucose infusion rate (earliest from the 120 min of clamp), where blood sugar level stayed between 5.0 and 5.9 mM/l for at least 30 min after the beginning of steady state. Glucose and insulin levels were measured from venous blood at 3, 5, 10, 20, 30, 40, 50, and 60 min samples of IVGTT, before the beginning and the 0, 10, 20, and 30 min samples of the steady sate of clamp. Insulin secretion was determined from IVGTT by the insulogenic index [Δ(insulin 5 min − insulin 3 min) / Δ(glucose 5 min − glucose 3 min)] and the AIR [(insulin 5 min + insulin 3 min) / (2 − insulin 0 min)], both being sensitive indicators of the 1st phase insulin response, and hence the real beta cell function. Glucose and insulin AUC values were calculated using the trapezoidal rule, both from OGTT and IVGTT. We used whole body and lean body (muscle) adjusted glucose uptake (M1 and M2 values, mg/min/kg) calculated from the glucose infusion rates during clamp, to measure insulin sensitivity. Body composition was determined by dual-energy X-ray absorptiometry (DPX-MD+, GE-Lunar, USA, Florida).

Biochemical measurements

Total OCN, total testosterone, FSH and serum insulin levels were measured with Elecsys 2010 immunohistochemical automat (Roche Diagnostic kits, Germany). CV for osteocalcin test varies between 1.8 and 6.5% respectively for the kits used in our study. Serum leptin and adiponectin levels were measured by an enzyme-linked immunosorbent method (Quantikine DLP00 and Quantikine DRP300 kits; R&D Systems, Minneapolis, MN, USA). Free fatty acid (FFA), glucose and conventional lipid parameters were measured on a Hitachi Laboratory Automat (Cobas Mira 912; Roche Diagnostics, Germany). HbA1c levels were measured by the IFCC reference method. Lipid fractionation was done by the Lipoprint System (Quantimetrix, USA). The VLDL, IDL-A, -B, and -C, and LDL1–4 subfractions, total LDL and HDL were separated by electrophoresis and examined in relation to the OCN levels.

Statistics

All statistical analyses were performed with R Statistical Software (version 2.15.0). Data points are expressed as mean ± standard deviation. The Wilcoxon test was used to assess group differences of biochemical and anthropometric parameters. Spearman's correlation coefficients were calculated to test the association between OCN and biochemical variables, since non-linearity characterised these associations. Partial correlation coefficients were used to assess the influence of possible confounding factors such as age, body mass index (BMI), body fat percent (BFP), and HbA1c levels (as being a mixed diabetic and non-diabetic population). Further adjustment with adiponectin and FSH in females and testosterone in males was done to exclude the effect of menopausal state in women, and the possible role of adiponectin/testosterone in mediating the metabolic effects of OCN in males/females. A p value of ≤ 0.05 was considered significant.

Feature selection (the Boruta algorithm) was used to find the most important attributes that are independently associated with OCN (Figs. 1 and 2). This algorithm is a wrapper built around the randomForest classification algorithm implemented in the R package randomForest [22]. The randomForest gives a numerical estimate of the importance of a feature, and a Z score is used as the importance measure since it takes into account the fluctuations of the mean accuracy loss amongst trees in the forest. To avoid random fluctuations in determining the importance of any given attribute, a reference set of 'shadow attributes' are used for deciding which attributes are truly important, since the importance of a shadow attribute can be nonzero only due to random fluctuations [23]. With this method we were able to clearly identify those parameters which have the potential to determine the value of OCN, independently of each other.

Results

Baseline characteristics

General metabolic and other characteristics of male and female subjects in the study are summarised in Table 1. The NGT and GI subjects are presented separately. Although OCN mean values were slightly higher in the NGT than in the GI groups in both genders, no significant difference was observed in OCN levels between NGT and GI subjects. In women this is the result of the effect of varying ages, that masks the positive association between OCN and glycemic state, in men however (see later) OCN is mainly associated with indicators of insulin