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Circulating osteocalcin is unrelated to glucose homoeostasis in adults with type 1 diabetes

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ABSTRACT

Aims: To assess the relationship between total osteocalcin (tOC), undercarboxylated osteocalcin (ucOC) and a range of markers of glucose homeostasis in type 1 diabetes.
Methods: One hundred and eight community-based Caucasian adults (53 males, 55 females) without a history of osteoporosis and with a mean ± SD age 39.1 ± 15.1 years and median [inter-quartile range] type 1 diabetes duration of 14.3 [6.6–20.4] years participated in a cross-sectional study of bone health. Fasting serum glucose and HbA_{1c}, and serum tOC, ucOC, total adiponectin and procollagen type 1 N-terminal propeptide (P1NP) were measured using validated assays, and daily insulin dose and estimated glucose disposal rate (eGDR) were calculated. Multiple linear regression was used to determine independent associates of markers of glucose homoeostasis (HbA_{1c}, fasting serum glucose, daily insulin dose, eGDR and serum total adiponectin).
Results: In sex-adjusted multivariable regression analyses, ln(serum P1NP) was independently and inversely associated with ln(HbA_{1c}) and ln(serum adiponectin) ($P \le 0.013$). Other associations included those between ln(serum vitamin D) and ln(HbA_{1c}) (inversely), daily insulin dose (inversely) and eGDR (positively) ($P \le 0.035$), as well as an inverse relationship between overweight by waist circumference and ln(serum adiponectin) ($P < 0.001$). Ln(serum tOC) and ln(serum ucOC) were not independently associated with any glucose homoeostasis marker.
Conclusions: These data from well characterized community-based adults with type 1 diabetes do not suggest that there is a role for osteocalcin in the potentially complex interplay between the skeleton and energy homoeostasis in type 1 diabetes.

1. Introduction

Preclinical studies have suggested that osteocalcin (OC), a bone formation marker, may help regulate glucose metabolism through stimulating pancreatic β cell proliferation and insulin expression, enhancing energy expenditure, and increasing expression of adiponectin and thus tissue insulin sensitivity [1,2]. In accord with these findings, a low plasma OC, especially the undercarboxylated form (ucOC), is associated with an increased risk of type 2 diabetes [3] and poor glycemic control after diagnosis [4], but a range of other clinical studies in this context have produced inconsistent results [5].

There are few equivalent data in adults with type 1 diabetes. Although one small-scale study in recently diagnosed young adults found no relationship between total OC (tOC) and aspects of glucose metabolism [6], there is evidence that plasma concentrations of tOC and ucOC are associated inversely with HbA_{1c} and positively with insulin exposure in two larger studies in outpatient clinic samples of patients with type 1 diabetes [7,8]. Whether this is a relationship specific to OC or there is more general bidirectional pathophysiology, with hyperglycemia attenuating bone formation and reduced bone formation further impairing residual insulin secretion and reducing tissue insulin sensitivity [9], is unknown. We have, therefore, assessed this relationship in community-based adults with type 1 diabetes who participated in a cross-sectional study of bone density and turnover [10], determining whether serum concentrations of tOC, ucOC, adiponectin and the bone formation marker procollagen type 1 N-terminal propeptide (P1NP) are associated with glucose homeostasis.

Conflict of Interest Statement: The authors declare that they have no conflict of interest.

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2. Materials and methods

2.1. Patients

We included the 102 patients from the original bone density study [10] and an additional six who were recruited and had blood drawn but did not attend for subsequent densitometry or who were too young for valid T-score calculation (<25 years of age). There was, therefore, a total of 108 white Caucasians (53 males, 55 females) with type 1 diabetes recruited from the community-based Fremantle Diabetes Study cohort [11] and from local outpatient clinics. The patients were unselected but were from sources that would allow identification of most patients with type 1 diabetes resident in the Fremantle area at the time of the study. Inclusion criteria were age ≥ 18 years and a confirmed diagnosis of type 1 diabetes including insulin requirement from first presentation and supportive investigations (islet autoantibodies and serum C-peptide concentrations). Exclusion criteria included osteoporosis (T-score < −2.5) or other conditions/therapies known to affect bone metabolism. The study was approved by the South Metropolitan Area Health Service Human Research Ethics Committee. Written informed consent was obtained from each patient.

2.2. Clinical and laboratory methods

All patients completed a detailed questionnaire relating to diabetes and bone health, and a physical examination was performed. Fasting morning blood samples were collected for measurement of serum glucose, HbA_{1c} and bone turnover markers [10]. All samples were taken on ice and processed promptly with storage of separated sera at −80 °C. Assay of tOC and ucOC was using published methodology [12]. Serum concentrations of tOC were measured by electrochemiluminescent immunoassay using a Modular E170 analyzer (Roche Diagnostics, Australia) with coefficients of variation (CVs) of 3.7% and 2.9% at 18 and 89 μg/L, respectively, and of ucOC by hydroxyapatite binding and precipitation of carboxylated OC followed by assay of the supernatant [12]. Other bone turnover markers including P1NP, carboxy-terminal type I collagen telopeptide (CTx) and 25-hydroxyvitamin D were assayed as described [10]. Total serum adiponectin was measured by sandwich enzyme immunoassay (R&D Systems Inc., Minneapolis, Minnesota) with intra-assay CVs of 2.1–4.3% and inter-assay CVs of 5.4–9.6%.

The estimated glucose disposal rate (eGDR) was calculated as an index of insulin sensitivity (24.4–12.97*WHR- 3.39*AHT-0.60*HbA_{1c}, where WHR is waist-to-hip ratio and AHT a binary variable representing antihypertensive treatment and/or blood pressure ≥ 140/90 mmHg [13]).

2.3. Data analysis

Statistical analyses were performed using SPSS (version 17.0, SPSS Inc., Chicago, IL). Data are presented as proportions, mean ± standard deviation (SD), geometric mean (SD range) or, in the case of variables not conforming to a normal or log-normal distribution, median [interquartile range (IQR)]. Multiple linear regression using forward conditional modeling with entry $P < 0.05$ and removal $P > 0.10$ was used to determine independent associates of markers of glucose homoeostasis from biologically plausible variables with $P < 0.20$ in bivariate analysis. Variables with a logarithmic distribution were natural log-transformed (ln) before analysis. A two-tailed $P < 0.05$ was considered to be significant.

3. Results

3.1. Sample characteristics

The characteristics of the present patients, including bone turnover markers, are summarized in Table 1. Their mean age was 39.1 years and 49.1% were males, with 16.7% of the 55 females being postmenopausal. Their median diabetes duration was 14.3 years and they had a mean body mass index (BMI) of 26.6 kg/m^2. Their geometric mean fasting serum glucose and HbA_{1c} were 9.4 mmol/L and 8.1% (65 mmol/mol), respectively. The mean total daily insulin dose was 0.71 units/kg with 17.6% of the patients managed using continuous subcutaneous insulin infusion via an insulin pump. Sixteen (15.0%) were current smokers and the median alcohol intake was 3.0 g/day. In relation to macrovascular complications, <8% had coronary artery, cerebrovascular or peripheral arterial disease, while the most prevalent microvascular complication was peripheral sensory neuropathy (18.5%).

Table 1
Characteristics of the 108 participants with type 1 diabetes.

Age (years)	39.1 ± 15.1 (18.2–76.1)
Male (%)	49.1
Diabetes duration (years)	14.3 [6.6–20.4]
Body mass index (kg/m^2)	26.6 ± 4.5 (18.7–41.5)
Abdominal obesity by waist circumference* (%)	25.9
Fasting serum glucose (mmol/L)	9.4 (5.5–16.2)
HbA_{1c} (%)	8.1 (6.9–9.4)
HbA_{1c} (mmol/mol)	65 (52–79)
Insulin dose/kg (U/day/kg)	0.71 ± 0.32 (0.05–1.90)
Estimated glucose disposal rate (mg/kg/min)	7.7 ± 2.5
Systolic blood pressure (mm Hg)	121 ± 15 (95–180)
Diastolic blood pressure (mm Hg)	71 ± 10 (20–92)
Total serum cholesterol (mmol/L)	4.7 ± 0.9 (2.6–7.3)
Serum HDL-cholesterol (mmol/L)	1.59 ± 0.4 (0.78–3.12)
Serum LDL-cholesterol (mmol/L)	2.6 ± 0.8 (0.9–5.0)
Serum triglycerides (mmol/L)	0.9 (0.6–1.4)
Serum creatinine (mmol/L)	77 ± 21 (48–186)
Urinary albumin:creatinine ratio (mg/mmol)	2.0 (0.6–6.8)
Serum total osteocalcin (μg/L)	23.2 (15.3–35.2)
Serum undercarboxylated osteocalcin (μg/L)	5.7 (3.9–8.3)
Serum carboxy-terminal type I collagen telopeptide (μg/L)	0.31 (0.17–0.56)
Serum parathormone (pmol/L)	3.9 (2.7–5.7)
Serum procollagen type 1 N-terminal propeptide (μg/L)	46.8 (28.6–76.6)
Serum adiponectin (mg/L)	9.3 (4.8–18.2)
Serum vitamin D (nmol/L)	58.1 (38.1–88.6)

Data are percentages, mean ± SD (range), geometric mean (SD range) or median [interquartile range].
* Using WHO thresholds for Caucasians (≥102 cm in men and ≥88 cm in women).

3.2. Osteocalcin, other bone turnover markers and glucose homoeostasis

We selected HbA_{1c}, fasting serum glucose, insulin dose, eGDR and serum adiponectin as key markers of glucose homeostasis and assessed their bivariate associations (see Table 2). Variables that were significantly (and negatively) associated with ln(HbA_{1c}) comprised male sex, exercise during the previous fortnight, ln(serum vitamin D) and ln(serum P1NP) ($P \le 0.044$). Ln(serum CTx) and ln(serum P1NP) were significantly and negatively associated with ln(fasting serum glucose) ($P \le 0.035$), while there was a positive association with serum creatinine ($P = 0.004$). Age was positively associated, and BMI, waist circumference and serum creatinine negatively associated, with ln(serum adiponectin) ($P \le 0.023$). The only variable associated (inversely) with daily insulin dose was ln(serum vitamin D) ($P = 0.047$). Several variables were associated with eGDR including ln(serum vitamin D) (inversely) and ln(serum parathyroid hormone) (positively) ($P < 0.031$). Neither ln(serum tOC) nor ln(serum ucOC) was associated with any marker of glucose homeostasis.

The results of sex-adjusted multivariable regression analyses are shown in Table 3. Ln(serum P1NP) was independently and inversely associated with ln(HbA_{1c}) and ln(serum adiponectin) ($P \le 0.013$). Ln(serum CTx) was inversely associated with ln(fasting serum

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