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Osteocalcin, adipokines and their associations with glucose metabolism in type 1 diabetes ☆

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ABSTRACT

To determine osteocalcin (OC) and adipokines in type 1 diabetes (T1D) and healthy controls, and to explore possible associations between glucose and bone metabolism, body composition and adipokines.

Serum levels of total OC, undercarboxylated (UC-OC), leptin, adiponectin, and other parameters of glucose and bone metabolism were measured in 128 patients with T1D (mean duration 21.2 years) and in 77 healthy controls, matched for gender, age, and body mass index (BMI). Partial correlations (adjusted for age and gender) with parameters of body composition (BMI, fat body mass [derived from bone mineral density scans]), glycaemic control (hemoglobin A1c (HbA1c), daily insulin dose in T1D), skeletal homeostasis (osteoprotegerin (OPG), receptor activator of NF-κB ligand (RANKL), all measured in serum), and serum insulin-like growth factor 1 (IGF-1) were also examined. Independent predictors of total and UC-OC were then explored.

Total OC was lower in males with T1D (16.3 ± 6.4 vs. 22.2 ± 9.9 ng/ml; $p = 0.001$), whereas UC-OC did not show group differences. Adiponectin was higher in T1D patients, both for males and females (8.9 ± 6.6 vs. 5.7 ± 2.5 μg/ml; $p = 0.004$ and 13.8 ± 6.4 vs. 8.8 ± 4.0 μg/ml; $p < 0.001$). IGF-1 was lower only in females with T1D (146.6 ± 68.8 vs. 203.0 ± 74.4 ng/ml; $p < 0.001$). BMI and fat body mass were similar in T1D and controls.

In T1D patients, total OC was inversely correlated with BMI and HbA1c, and UC-OC inversely correlated with HbA1c. In T1D patients, leptin positively correlated with BMI, fat body mass and daily insulin dose, while adiponectin inversely correlated with BMI and daily insulin dose.

Multivariate regression modelling showed that determinants of higher total OC levels were male gender ($p = 0.04$, ß-coefficient = 2.865) and lower HbA1c ($p = 0.04$, ß-coefficient = −0.117), whereas determinants of UC-OC levels were T1D ($p = 0.016$, ß-coefficient = 2.015), higher IGF-1 ($p = 0.004$, ß-coefficient = 0.011) and lower HbA1c ($p = 0.011$, ß-coefficient = − 0.061).

Total OC and UC-OC are associated with good glycaemic control in T1D, with gender-specific differences for total-OC. The association of leptin and adiponectin with glycaemic control, as observed in controls, does not seem to be a feature in T1D, although both adipokines appear to be related to the insulin demand.

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Introduction

Type 1 diabetes (T1D) is a condition with hyperglycaemia and absolute insulin deficiency, as a result of cellular-mediated autoimmune destruction of the β-cells of the pancreas [1]. The risk of fractures in patients with T1D is significantly higher than in the general population [2]. Impaired bone quality, as a result of increased formation of advance glycation end products (AGEs) and direct effects of hyperglycaemia and insulin deficiency on osteoblasts, is believed to contribute to the risk of fracture in patients with T1D [3].

Energy metabolism is known to be closely linked to the activity of bone cells [4]. Insulin binds to specific receptors on osteoblasts, thereby inhibiting the expression of osteoprotegerin (OPG) and increasing the activity of osteoclasts. Leptin, a key signalling hormone of appetite regulation, regulates osteoblast function through a hypothalamic relay [5]. A feedback loop between osteoblast activity and energy metabolism has also been recently discovered [6].

Osteocalcin (OC), a hormone secreted by osteoblasts, undergoes carboxylation during post-translational modification and is stored in

Abbreviations: BMD, bone mineral density; HbA1c, glycated hemoglobin A1c; SD, standard deviation; OC, osteocalcin; UC-OC, undercarboxylated OC.

☆ This study was conducted according to the principles of the Declaration of Helsinki.

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the bone extracellular matrix. Preclinical studies have shown that undercarboxylated osteocalcin (UC-OC) stimulates ß-cell proliferation and insulin production [6,7]. The circulating levels of OC are known to increase with improved glucose control in type 2 diabetes (T2D) [8], whereas reduced levels of carboxylated and undercarboxylated OC are associated with the risk of developing T2D [9].

Insulin-like growth factor-1 (IGF-1) mediates osteoanabolic actions within the skeletal tissue [10]. IGF-1 is mainly produced in the liver but is also highly expressed in the bone tissue [11]. Serum levels of IGF-1 are associated with bone mass [12], although this association is probably specific to females [13]. Deficiency of IGF-1, as has been demonstrated in T1D, may therefore contribute to skeletal abnormalities in T1D. Indeed, substitution of IGF-1 in a streptozotocin (STZ)-induced mouse model of T1D induces new bone formation and improves the biomechanical properties of bone [14].

It is of great interest to elucidate the complex interrelationship between bone and energy metabolism. In healthy controls leptin is strongly associated with insulin resistance sensitivity, whereas adiponectin and OC are associated with insulin resistance [15]. In general, the investigation of glucose metabolism in T1D is complex because the endogenous production of insulin ceases, and insulin resistance can only be estimated from the insulin dosage required to maintain glucose homeostasis. In a cohort of adolescents and young adults with T1D, Thrailkill et al. have already shown that insulin exposure positively correlates with UC-OC [16].

The present study was conducted to investigate several parameters of bone and energy metabolism in a well-defined cohort of patients with long-standing T1D. The data were further analysed to explore for more complex associations between glucose, bone, and energy metabolism in T1D.

Research design and methods

This single-centre cross-sectional study was initially performed to assess bone mineral density and prevalent fractures in patients with T1D and healthy controls [17]. The T1D study cohort was consecutively recruited at the outpatient clinic of the Division of Metabolic Diseases, University Hospital, Jena, Germany. The study was approved by the local ethics committee and was performed according to the principles of the Helsinki declaration. All subjects provided written informed consent before participation. The primary aim of the study was to investigate bone mineral density in T1D and to analyse associations with glucose metabolism. The details of the study have been published elsewhere [17].

Study population

Patients with T1D aged between 20 and 70 years were eligible for inclusion in the study. They were excluded if any of the following criteria applied: T1D duration <3 years, previous (>3 months) or current treatment with corticosteroids, menopause or no menstrual period within the last 12 months, pregnancy, inflammatory disease, malnutrition, renal failure with a glomerular filtration rate (GFR) (MDRD formula) <30 ml/min, specific osteoporosis medication, and severe mental or somatic disease. Controls without T1D were recruited from the university staff and referrals from general practitioners. The participants were matched by gender, age (blocks of 5 years) and body mass index (BMI, blocks of 5 kg/m^2), with the aim to obtain a sample ratio of 2:1 (T1D: healthy controls). The 2:1 scheme was chosen in order to include a sufficient number of female and male patients in the T1D group. To avoid a bias of a different power in T1D patients and controls, the variable "group" was incorporated in multivariate analyses (see below).

With the exception of T1D-related criteria, the same inclusion and exclusion criteria were applied to controls.

All participants underwent a physical examination and a standardized interview on demographic data, medical history and lifestyle behaviour. BMI was calculated from measured body weight and body size as body weight (kilograms)/height (metres^2). Daily insulin dose (basal and post prandial) was derived from the patients' clinical records.

Laboratory analyses

Blood samples were drawn between 8:00 and 10:00 a.m. after an overnight fast. The following laboratory parameters were measured: HbA1c (normal 24.6–41.0 mmol/mol) by high-performance liquid chromatography (HPLC) (Tosoh-Glykohemoglobin-Analyzer-HLC-723-GHbV, Tosoh Corporation, Tokyo, Japan) and total OC by TRACE technology on a Kryptor automated immunofluorescence system (BRAHMS, Berlin, Germany). Enzyme immunoassays were used for the analysis of UC-OC (Glu-OC EIA Kit, Takara Bio Inc., Otsu, Japan), of IGF-1, adiponectin and leptin (Quantikine ELISA kits, R&D Systems Inc., Minneapolis, USA), as well as of osteoprotegerin and sRANKL (osteoprotegerin and total sRANKL ELISA Kits, Immundiagnostik AG, Bensheim, Germany).

Body composition

Body composition was measured by dual-energy x-ray absorptiometry (Prodigy Advance, General Electric Medical Systems Lunar, Diegem, Belgium). Body fat content was determined by GE Lunar body composition software, with provision of values for total body percentage fat. Regular scans of a body-composition phantom were performed to control for inter-day variations (variation coefficient 0.46%).

Statistical analysis

Results were expressed as mean ± standard deviation unless otherwise noted, separately for males and females. The differences between groups were statistically evaluated by independent two-sample *t*-test. Partial correlation coefficients, adjusted for age and gender, were assessed to estimate the correlation between variables of interest. Multivariate linear regression models were fitted to evaluate the influence of different variables on total OC and UC-OC. For all analyses, a statistically significant difference was accepted for $p < 0.05$.

Results

Study population characteristics

Table 1 summarizes the characteristics of the 128 patients with T1D and the 77 healthy controls. Gender, age, and BMI were similarly distributed in both groups. Patients with T1D had significantly higher HbA1c, as expected. The mean duration of diabetes was 21.2 years and the mean daily insulin dose was 0.63 U kg^{-1} day^{-1}. Body fat content and fat distribution did not differ between T1D and controls (data not shown).

Parameters of skeletal and endocrine homeostasis

Comparisons between T1D patients and controls were carried out separately for males and females, as shown in Table 2, and also irrespective of gender for some parameters (Fig. 1).

Total OC was significantly lower in males with T1D – but not in females with T1D – compared with controls (Fig. 2). Healthy males had higher total OC levels compared to healthy females ($p = 0.011$), whereas no gender differences were found in T1D ($p = 0.391$). The levels of UC-OC did not significantly differ in males or females with T1D compared with controls, nor did they show relevant gender-specific differences in controls or T1D ($p = 0.596$ and $p = 0.247$, respectively). The levels of OPG were significantly higher in males and females with T1D compared with controls. The levels of sRANKL were very similar in all groups (Table 2).

The levels of leptin did not differ between T1D patients and healthy controls, but this parameter showed a clear gender-specific difference, with females showing higher levels than males in controls and T1D

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