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ORIGINAL ARTICLE

Leptin is an independent determinant of bone mineral density in men with type 2 diabetes mellitus

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Abstract To investigate the possible relationship of leptin to bone mineral density (BMD) in men with type 2 diabetes mellitus (T2DM), we screened 168 Belarusian men aged 45–65 years. Plasma total cholesterol (TC), high-density lipoprotein cholesterol, and triglyceride concentrations were assessed, and low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol (LDL-C) were calculated. Hemoglobin A_{1c}, immune-reactive insulin (IRI), serum total testosterone, and sex hormone-binding globulin were also evaluated. BMD was evaluated using dual-energy X-ray absorptiometry. By univariate linear regression analysis, BMD was significantly correlated with body mass index ($r = 0.23$, $P = 0.002$) and leptin ($r = 0.21$, $P = 0.006$). By multivariate regression analysis adjusting for confounding factors, log leptin was independently correlated with BMD ($\beta = 0.058$, $P = 0.001$). Our study revealed that leptin is an independent determinant of BMD in patients with T2DM. Further research is necessary to confirm this association and to develop ways to correct abnormalities of bone metabolism in patients with T2DM.

Keywords Type 2 diabetes mellitus (T2DM) · Leptin · Bone mineral density (BMD)

Introduction

Although several reports have demonstrated that diabetic patients have an increased risk for bone fracture [1], changes in bone metabolism in patients with type 2 diabetes mellitus (T2DM) are still controversial [2–4]. T2DM is typically associated with obesity, which has itself been associated with higher BMD and may protect against osteoporosis and fractures [5]. The protective effect on BMD in obese subjects may be mediated through increased muscle mass and fat mass.

On the other hand, the contribution of adipocytokines such as leptin to BMD in patients with T2DM is also controversial. Previous studies have shown that serum leptin levels positively or negatively [6] correlate with BMD. Leptin has at least two different effects on bone metabolism, that is, an indirect inhibitory effect on osteoclastogenesis and a direct stimulatory effect on bone formation [7]. This effect seems to differ in the central versus peripheral pathway [8] and based on insulin levels [9–11]. Indeed, a study analyzing ob/ob mice demonstrated that leptin inhibited bone formation through a hypothalamic relay [8, 12]. A study by Watanabe et al. [13] found that the troglitazone-induced decreases in serum leptin levels are associated with less bone loss in patients with type 2 diabetes.

Taking these findings into consideration, the aim of this study was to investigate the possible relationship of leptin to BMD in patients with T2DM.

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Subjects and methods

Subjects

Prior to this study, ethical approval was obtained from the special committee of The Republican Research Centre for Radiation Medicine and Human Ecology (Gomel, Republic of Belarus). We investigated 168 Belarusian men with T2DM who consecutively visited this institute. The inclusion criteria were as follows: written informed consent, T2DM, 45–60 years old, and body mass index (BMI) >18.5 and <40.0 kg/m^2. All patients were treated with diet, oral antidiabetic drugs, and/or insulin. Patients with thyroid disease and liver cirrhosis were excluded.

Demographic parameters were collected, height and weight were measured, and BMI was calculated. Data related to the duration of diabetes and medications were also collected. BMD was evaluated using dual-energy X-ray absorptiometry (DXA) (GE Lunar Prodigy Advance, New York, NY, USA). BMD was measured at the left femur.

Biochemical measurements

After informed consent was obtained, fasting blood samples were collected. Plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) concentrations were assessed using standard enzymatic methods, and low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were calculated by the Friedewald equation. Hemoglobin A_{1c} (HbA_{1c}) was assayed using high-performance liquid chromatography. Immune-reactive insulin (IRI) was measured by radioimmunoassay (RIA) using DSL 10-1600 (ACTIV, Diagnostic Systems Laboratories, Inc., Webster, TX, USA). Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by chemiluminescent immunometric assays.

Statistical analysis

Data are presented as mean ± standard deviation or median (25th–75th percentile). Spearman rank correlation analysis was performed to evaluate leptin and other existing parameters. Multivariate linear regression analysis was also performed to evaluate BMD and other existing parameters adjusted for BMI, log IRI, log TG, and log leptin. A P value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS v11.0 software (SPSS Japan, Tokyo, Japan).

Results

Characteristics of the study participants are shown in Table 1. The mean age was 54.1 ± 4.8 years, duration of diabetes was 7.0 (3.0–12.0) years, and HbA_{1c} was 8.2 (7.0–9.7) %. Concentrations of LH, FSH, and PRL were 4.5 (2.9–6.1) IU/L, 4.5 (3.2–6.8) IU/L, and 8.1 (5.9–11.6) IU/L, respectively. Mean BMD of the left femur was 1.114 ± 0.137 g/cm^2.

By univariate linear regression analysis, BMD was significantly correlated with BMI ($r = 0.23$, $P < 0.002$ and leptin ($r = 0.21$, $P = 0.006$). BMD was relatively, but not significantly, correlated with IRI and TG ($r = 0.15$, $P = 0.062$ and $r = 0.14$, $P = 0.07$, respectively). On the other hand, age, duration of diabetes, estradiol, LH, FSH, HbA_{1c}, TC, HDL-C, and LDL-C were not significantly correlated with BMD (Table 2).

By multiple regression analysis, BMD was significantly correlated with log leptin after adjustment for BMI, WHR, log HOMA-IR, and log TG ($\beta = 0.058$, $P = 0.001$) (Table 3). When we divided patients into two groups according to treatment of diabetes (oral therapy versus insulin), the correlation between BMD and log leptin was attenuated, probably due to the insufficient number of patients (data not shown). In addition, when we performed a subgroup analysis of patients with a BMI > 30 kg/m^2, the correlation between BMD and log leptin was attenuated, but remained significant ($\beta = 0.032$, $P = 0.02$).

Table 1 Clinical characteristics of men with type 2 diabetes mellitus ($n = 168$)

Age, years	54.1 ± 4.8
Duration of diabetes, years	7.0 (3.0–12.0)
Smoking, none/current	104/62
Current treatment, diet/OHA/insulin/OHA, and insulin	2/85/55/26
Waist-to-hip ratio	0.98 ± 0.07
Body mass index, kg/m^2	29.9 ± 5.5
Hemoglobin A_{1c}, %	8.2 (7.0–9.7)
Total cholesterol, mmol/L	5.2 ± 1.4
Triglyceride, mmol/L	1.8 (1.2–2.6)
High-density lipoprotein cholesterol, mmol/L	1.3 ± 0.6
Low-density lipoprotein cholesterol, mmol/L	2.5 (1.8–3.5)
Estradiol, IU/L	0.16 (0.10–0.20)
Luteinizing hormone, IU/L	4.5 (2.9–6.1)
Follicle-stimulating hormone, IU/L	2.4 (1.2–4.6)
Immune-reactive insulin, mU/L	9.2 (6.0–17.3)
Leptin, g/L	7.8 (3.4–16.1)
Bone mineral density of lumbar spine, g/cm^2	1.16 ± 0.19

Values are mean ± standard deviation or median (25th–75th percentile)

Table 2 Univariate correlation between BMD and other variables

	r	*P*
Age	−0.04	0.57
Duration of diabetes	0.28	0.72
Waist-to-hip ratio	−0.04	0.57
Body mass index	0.23	0.002
Estradiol	0.18	0.81
Luteinizing hormone	−0.002	0.98
Follicle-stimulating hormone	−0.006	0.94
Immune-reactive insulin	0.15	0.062
Hemoglobin A_{1c}	−0.001	0.99
Total cholesterol	0.013	0.87
Triglyceride	0.14	0.07
High-density lipoprotein cholesterol	0.06	0.58
Low-density lipoprotein cholesterol	0.10	0.90
Leptin	0.21	0.006

r correlation coefficients

Table 3 Multivariate linear regression analysis of BMD with relevant factors adjusted for confounding factors

Variables	β	95% CI	*P*
Body mass index	0.003	−0.002, 0.008	0.23
Log immune-reactive insulin	0.010	−0.057, 0.077	0.08
Log triglyceride	0.054	−0.016, 0.124	0.13
Log leptin	0.058	0.021, 0.105	0.001

$R^2 = 0.52$

All variants are adjusted for the analysis. β standardized regression coefficient, *CI* confidence interval

Discussion

Osteoporosis is the most common metabolic bone disease, characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and increased susceptibility to fractures [14, 15]. The relationship between body weight and BMD in patients with T2DM is complex and not completely understood. Possible explanations for the protective bone effects of increased body weight include increased aromatization of androgens to estrogen in adipose tissue, mechanical loading, lower levels of sex hormone-binding globulin, and increased bone formation due to high circulating insulin levels [16].

Adipose tissue produces and releases a variety of proinflammatory and anti-inflammatory factors including TNF-α, leptin, adiponectin, and resistin [17]. Leptin, a peptide hormone that is the product of the ob gene, is a single chain proteohormone with a molecular mass of 16 kDa. Leptin is secreted by adipocyte that controls body weight by regulating appetite and energy metabolism [18]. Leptin is thought to play a key role in the regulation of body weight [19].

The role of leptin in bone turnover and osteoporosis is not completely understood. In vitro data suggest that leptin stimulates bone formation, possibly by acting on human marrow stromal cells to enhance osteoblasts and inhibit adipocyte differentiation [20]. Leptin also inhibits osteoclastogenesis by decreasing the receptor activator of nuclear factor-κB (RANK) and its ligand (RANKL) and increasing the production of osteoprotegerin (OPG), a mediator of mineral metabolism [21]. OPG and RANKL [22] work together to maintain normal mineral homeostasis in the bone [23]. An imbalance in the OPG to RANKL ratio may potentially affect the process of bone formation and resorption. Therefore, a reduction in leptin, which affects both of these mediators, may result in both reduced bone formation and increased bone resorption.

Thomas et al. [24] first published the hypothesis of an association between serum leptin levels and BMD in men and concluded that fat mass and leptin are weakly and inconsistently predictive of BMD in men. On the other hand, Morberg et al. [25] showed an inverse association in men and others reported no association [26–29]. Another study found that leptin was positively associated with total body BMD in 92 older men, but the association disappeared after BMI was added to the regression model [30]. In another study, leptin was negatively correlated with BMD at the lumbar spine in 80 Korean men 42–70 years of age after adjusting for BMI [31]. Some other studies in women have supported the current findings. Pasco et al. [32] found that leptin was associated with BMD at the spine and hip independent of fat mass and weight in 214 pre- and postmenopausal women. Two studies in postmenopausal women found that leptin was associated with BMD at the femoral neck and total body in models adjusted for percent body fat [6] or fat mass [33]. Others reported that leptin was associated with decreased bone resorption in postmenopausal women after adjustment for body fat or BMI [34]. However, several other studies in women found that leptin was not associated with BMD or markers of bone turnover after adjusting for BMI [30, 35, 36] and/or fat mass [37, 38].

Two studies found that leptin was inversely associated with BMD in women after controlling for insulin [9] and weight [39]. Because women have 2- to 3-fold higher leptin levels than men [40], higher levels may be necessary for beneficial leptin effects or the association in women may be more evident because of their broader range of leptin levels compared with men. Men and women had similar rates of annual bone loss over the 4 years so this did not explain baseline sex differences [41]. Analyses combining men and women showed a statistically significant leptin–sex interaction for BMD at the radius and for cross-linked

N-telopeptides of type I collagen (NTX) [42]. Previous studies that have investigated the association between leptin and bone in men and women have performed sex-specific analyses [28, 29]. We believe the sex differences are real, but no clear explanation has yet been determined to explain them.

Our study clearly showed that BMD was positively associated with leptin in male patients with T2DM. This association remained significant even after adjusting for BMI, log IRI, and log TG. These results indicate that the influence of leptin on BMD does not depend on insulin secretion.

Our study had several limitations. First, the sample size was small. In addition, we did not measure specific body composition values, such as waist-to-thigh ratio, fat mass, or free fat mass. Future studies should take these values into account to clarify whether the correlation we observed is maintained by controlling for increased weight and/or obesity. In addition, we did not evaluate physical activity. Because physical activity is negatively associated with leptin levels independent of age, sex, smoking, and body adiposity [43] while it is positively associated with BMD, future studies should control for this factor. Furthermore, we did not evaluate bone formation markers, 25-hydroxyvitamin D levels, and used a single leptin assay. However, it has been shown that a single morning fasting leptin measurement, as used here, can characterize usual leptin levels for an individual within a population [44]. Further studies are needed to clarify the influence of leptin level on BMD in patients with T2DM.

In conclusion, our study revealed that BMD of the femur was positively associated with leptin in male patients with T2DM. Further research is necessary to confirm this association and to develop ways to correct abnormalities of bone metabolism in patients with T2DM.

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