Leptin is an Independent Determinant of Bone Mineral Density in Men with Type 2 Diabetes

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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 Belarussian men aged 45 to 65 years. Plasma total cholesterol (TC), high-density lipoprotein cholesterol, triglyceride concentrations were assessed and low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol (LDL-C) were calculated. Hemoglobin A1c, 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 level positively or negatively [6] correlates with BMD. Leptin has at least two different effects on bone metabolism, i.e., an indirect inhibitory effect on osteoclastogenesis and a direct stimulatory effect on bone formation [7]. This effect seems to be different according to the pathway: central versus peripheral [8] and to the insulin level [9-11]. Indeed, a study analyzing ob/ob mice demonstrated that leptin inhibits bone formation through a hypothalamic relay [8, 12]. A study by Watanabe et al. [13] found that the troglitazone induced decrease in serum leptin is associated with the less bone loss in 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.
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 Belarussian men with T2DM who consecutively visited this institute. Patients aged 45 to 60 years with T2DM, with body mass index (BMI) values between 18.5 and 40 kg/m\(^2\) were included in this study.

Patients with thyroid diseases and liver cirrhosis were excluded from the study. The inclusion criteria were as follows: written informed consent, T2DM, 45-60 years old, body mass index >18.5 and <40.0 kg/m\(^2\). All patients were treated with diet, oral antidiabetic drugs, and/or insulin.

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 Friedwald equation. Hemoglobin A1c (HbA1c) 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 simply 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 HbA1c 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/cm².

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 were 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, HbA1c, 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 (β=0.058, p=0.001) (Table 3). When dividing patients into two groups according to the treatment of diabetes, correlation between BMD and log leptin was attenuated, probably due to the insufficient number of patients (data not shown). Also, when we aimed to patients with BMI more than 30 kg/m², correlation between BMD and log leptin was attenuated, but remained significant (β=0.032, p=0.02).
Discussion

Osteoporosis is the most common metabolic bone disease, characterised 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 is a peptide hormone secreted by adipocyte, which controls body weight by regulating appetite and energy metabolism [18]. Leptin, the product of the ob gene, is a singlechain proteohormone with a molecular mass of 16 kDa that 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. Data in vitro suggest that leptin stimulates bone formation, possibly by acting on human marrow stromal cells to enhance osteoblast 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) - mediator of mineral metabolism [21]. These mediators, such as OPG and RANKL [22], work in a concerted manner to maintain normal mineral homeostasis in the bone [23]. OPG is a potent inhibitor of bone loss by its virtue of serving as a decoy receptor for RANKL; and thus minimising its effect in terms of bone resorption. An imbalance in the OPG to RANKL ratio may potentially impact upon these processes. Therefore, reduced levels of leptin may result in both reduced bone formation and increased bone resorption.

Thomas et al. 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 [24]. On the other hand, Morberg et al. showed an inverse association in men [25] 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 have supported the current findings in women.

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 [35-37] and/or fat mass [38, 39].

Two studies found that leptin was inversely associated with BMD in women after controlling for insulin [9] and weight [40]. Because women have 2- to 3-fold higher leptin levels than men [41], 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. Analyses combining men and women showed a statistically significant leptin–sex interaction for BMD at the radius and for NTX, supporting the observed sex differences. 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 and unexplained.

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

Several limitations need to be acknowledged and addressed regarding the present study. First, the sample size is small. We could not measure fat mass and free fat mass, and evaluate physical activity. Also, we could 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 [42]. 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.
Acknowledgment

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References


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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>54.1 ± 4.8</td>
</tr>
<tr>
<td>Duration of diabetes, y</td>
<td>7.0 (3.0-12.0)</td>
</tr>
<tr>
<td>Smoking, none/current</td>
<td>104/62</td>
</tr>
<tr>
<td>Current treatment, diet/OHA/insulin/OHA and insulin</td>
<td>2/85/55/26</td>
</tr>
<tr>
<td>Waist hip ratio</td>
<td>0.98 ± 0.07</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29.9 ± 5.5</td>
</tr>
<tr>
<td>Hemoglobin A₁c, %</td>
<td>8.2 (7.0-9.7)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.2 ± 1.4</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.8 (1.2-2.6)</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol, mmol/L</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol, mmol/L</td>
<td>2.5 (1.8-3.5)</td>
</tr>
<tr>
<td>Estradiol, IU/L</td>
<td>0.16 (0.10-0.20)</td>
</tr>
<tr>
<td>Luteinizing hormone, IU/L</td>
<td>4.5 (2.9-6.1)</td>
</tr>
<tr>
<td>Follicle-stimulating hormone, IU/L</td>
<td>2.4 (1.2-4.6)</td>
</tr>
<tr>
<td>Immune-reactive insulin, mU/L</td>
<td>9.2 (6.0-17.3)</td>
</tr>
<tr>
<td>Leptin, g/L</td>
<td>7.8 (3.4-16.1)</td>
</tr>
<tr>
<td>Bone mineral density of lumbar spine, g/cm²</td>
<td>1.16 ± 0.19</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation or median (25th - 75th percentile).
**Table 2.** Univariate correlation between BMD and other variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.04</td>
<td>0.57</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>0.28</td>
<td>0.72</td>
</tr>
<tr>
<td>Waist hip ratio</td>
<td>-0.04</td>
<td>0.57</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.23</td>
<td>0.002</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.18</td>
<td>0.81</td>
</tr>
<tr>
<td>Luteinizing hormone</td>
<td>-0.002</td>
<td>0.98</td>
</tr>
<tr>
<td>Follicle-stimulating hormone</td>
<td>-0.006</td>
<td>0.94</td>
</tr>
<tr>
<td>Immune-reactive insulin</td>
<td>0.15</td>
<td>0.062</td>
</tr>
<tr>
<td>Hemoglobin A1c</td>
<td>-0.001</td>
<td>0.99</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.013</td>
<td>0.87</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.14</td>
<td>0.07</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol</td>
<td>0.06</td>
<td>0.58</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol</td>
<td>0.10</td>
<td>0.90</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.21</td>
<td>0.006</td>
</tr>
</tbody>
</table>

r: correlation coefficients


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

<table>
<thead>
<tr>
<th>Variables</th>
<th>β</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index</td>
<td>0.003</td>
<td>-0.002, 0.008</td>
<td>0.23</td>
</tr>
<tr>
<td>Log immune-reactive insulin</td>
<td>0.010</td>
<td>-0.057, 0.077</td>
<td>0.08</td>
</tr>
<tr>
<td>Log triglyceride</td>
<td>0.054</td>
<td>-0.016, 0.124</td>
<td>0.13</td>
</tr>
<tr>
<td>Log leptin</td>
<td>0.058</td>
<td>0.021, 0.105</td>
<td>0.001</td>
</tr>
</tbody>
</table>

R² = 0.52

All variants are adjusted for the analysis. β: standardized regression coefficient; CI: confidence interval.