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

Mellitus

Olga Vasilkova^{1,2}, Tatiana Mokhort³, Tamara Sharshakova¹, Naomi Hayashida⁴ and Noboru Takamura⁴

¹Department of Public Health, Gomel State Medical University, Gomel, Belarus, ²The Republican Research Centre for Radiation Medicine and Human Ecology, Gomel, Belarus and ³Department of Internal Medicine, Belarussian State Medical University, Minsk, Belarus

⁴Department of Radiation Epidemiology and Public Health, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Correspondence to: Noboru Takamura, M.D., Ph.D.

Professor, Department of Radiation Epidemiology

Nagasaki University Graduate School of Biomedical Sciences

1-12-4 Sakamoto, Nagasaki 8528523, Japan

Tel: +81-95-819-7170; Fax: +81-95-819-7172

E-mail: takamura@nagasaki-u.ac.jp

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 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 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/m² 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/m². 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 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 $\pm$ 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 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/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, 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 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 ($\beta=0.032$, $p=0.02$).

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- κ B (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 IBMI, 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

This work was supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan through the Nagasaki University Global COE program. We would also like to thank Miho Yoshida for her technical assistance.

References

1. Vestergaard P, Rejnmark L, Mosekilde L (2005) Relative fracture risk in patients with diabetes mellitus, and the impact of insulin and oral antidiabetic medication on relative fracture risk. *Diabetologia* 48:1292-1299
2. Isaia G, Bodrato L, Carlevatto V, Mussetta M, Salamano G, Molinatti GM (1987) Osteoporosis in type II diabetes. *Acta Diabetol Lat* 24:305-310
3. Gregorio F, Cristallini S, Santeusanio F, Filipponi P, Fumelli P (1994) Osteopenia associated with non-insulin-dependent diabetes mellitus: what are the causes? *Diabetes Res Clin Pract* 23:43-54
4. Tuominen JT, Impivaara O, Puukka P, Rönnemaa T (1999) Bone mineral density in patients with type 1 and type 2 diabetes. *Diabetes Care* 1999;22:1196-1200
5. Barrett-Connor E, Holbrook TL (1992) Sex differences in osteoporosis in older adults with non-insulin-dependent diabetes mellitus. *JAMA* 267:3333-3337
6. Yamauchi M, Sugimoto T, Yamaguchi T, Nakaoka D, Kanzawa M, Yano S, Ozuru R, Sugishita T, Chihara K (2001) Plasma leptin concentrations are associated with bone mineral density and the presence of vertebral fractures in postmenopausal women. *Clin Endocrinol* 55:341-347
7. Steppan CM, Crawford DT, Chidsey-Frink KL, Ke H, Swick AG (2000) Leptin is a potent stimulator of bone growth in ob/ob mice. *Regul Pept* 92:73-78
8. Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, Shen J, Vinson C, Rueger JM, Karsenty G (2000) Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell* 100:197-207

9. Kontogianni M, Dafni U, Routsias J, Skopouli F (2004) Blood leptin and adiponectin as possible mediators of the relation between fat mass and BMD in perimenopausal women. *J Bone Miner Res* 19:546–551
10. Abou Samra R, Baba N, Torbay N, Dib L, Fuleihan GE (2005) High plasma leptin is not associated with higher bone mineral density in insulin-resistant premenopausal obese women. *J Clin Endocrinol Metab* 90:2588–2594
11. Hania S. Kassem et al (2008) Negative effect of leptin on bone mass in type 1 diabetes. *Acta Diabetol* 45:237-241
12. A. Nar et al (2009) The effect of metformin on leptin in obese patients with type 2 diabetes mellitus and nonalcoholic fatty liver disease. *Acta Diabetol* 46:113-118
13. Watanabe S, Takeuchi Y, Fukumoto S, Fujita H, Nakano T, Fujita T (2003) Decrease in serum leptin by troglitazone is associated with preventing bone loss in type 2 diabetic patients. *J Bone Miner Metab* 21(3):166–171
14. Anon (1993) Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. *Am J Med* 94:646–650
15. H. Safaei et al (2007) Lovastatin effects on bone mineral density in postmenopausal women with type 2 diabetes mellitus. *Acta Diabetol* 44:76-82
16. Barrett-Connor E, Kritz-Silverstein D (1996) Does hyperinsulinemia preserve bone? *Diabetes Care*. 19:1388–1392
17. Rondinone CM (2006) Adipocyte-derived hormones, cytokines, and mediators. *Endocrine* 29:81–90
18. Friedman JM, Halaas JL (1998) Leptin and the regulation of body weight in mammals. *Nature* 395:763–770
19. Jong Suk Park et al (2010) Visceral adiposity and leptin are independently associated with C-reactive protein in Korean type 2 diabetic patients. *Acta Diabetol* 47:113-118

20. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL (1999) Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. *Endocrinology* 140:1630-1638
21. Reid IR (2008) Relationships between fat and bone. *Osteoporos Int* 19:595–606
22. Dhruv K. Singh et al, Low serum osteoprotegerin levels in normoalbuminuric type 1 diabetes mellitus, *Acta Diabetol*
23. Schoppet M, Preissner KT, Hofbauer LC (2002) RANK ligand and osteoprotegerin: paracrine regulators of bone metabolism and vascular function. *Arterioscler Thromb Vasc Biol* 22(4):549–553
24. Thomas T, Burguera B, Melton LJ 3rd, Atkinson EJ, O'Fallon WM, Riggs BL, Khosla S (2001) Role of serum leptin, insulin, and estrogen levels as potential mediators of the relationship between fat mass and bone mineral density in men versus women. *Bone* 29:114–120
25. Morberg CM, Tetens I, Black E, Toubro S, Soerensen TI, Pedersen O, Astrup A (2003) Leptin and bone mineral density: A cross-sectional study in obese and nonobese men. *J Clin Endocrinol Metab* 88:5795-5800
26. Sun AJ, Jing T, Heymsfield SB, Phillips GB (2003) Relationship of leptin and sex hormones to bone mineral density in men. *Acta Diabetol* 40:101-105
27. Sato M, Takeda N, Sarui H, Takami R, Takami K, Hayashi M, Sasaki A, Kawachi S, Yoshino K, Yasuda K (2001) Association between serum leptin concentrations and bone mineral density, and biochemical markers of bone turnover in adult men. *J Clin Endocrinol Metab* 86:5273-5276
28. Ruhl CE, Everhart JE (2002) Relationship of serum leptin concentration with bone mineral density in the United States population. *J Bone Miner Res* 17:1896-1903
29. Dennison EM, Syddall HE, Fall CH, Javaid MK, Arden NK, Phillips DI, Cooper C (2004) Plasma leptin concentration and change in bone density among elderly men and women: The Hertfordshire Cohort Study. *Calcif Tissue Int* 74:401-406

30. Zoico E, Zamboni M, Adami S, Vettor R, Mazzali G, Tosoni P, Bissoli L, Bosello O (2003) Relationship between leptin levels and bone mineral density in the elderly. *Clin Endocrinol (Oxf)* 59:97-103
31. Oh KW, Lee WY, Rhee EJ, Baek KH, Yoon KH, Kang MI, Yun EJ, Park CY, Ihm SH, Choi MG, Yoo HJ, Park SW (2005) The relationship between serum resistin, leptin, adiponectin, ghrelin levels and bone mineral density in middle-aged men. *Clin Endocrinol (Oxf)* 63:131-138
32. Pasco JA, Henry MJ, Kotowicz MA, Collier GR, Ball MJ, Ugoni AM, Nicholson GC (2001) Serum leptin levels are associated with bone mass in nonobese women. *J Clin Endocrinol Metab* 86:1884–1887
33. Blain H, Vuillemin A, Guillemin F, Durant R, Hanesse B, de Talance N, Doucet B, Jeandel C (2002) Serum leptin level is a predictor of bone mineral density in postmenopausal women. *J Clin Endocrinol Metab* 87:1030–1035.
34. Roux C, Arabi A, Porcher R, Garnero P (2003) Serum leptin as a determinant of bone resorption in healthy postmenopausal women. *Bone* 33:847–852.
35. Iwamoto I, Douchi T, Kosha S, Murakami M, Fujino T, Nagata Y (2000) Relationships between serum leptin level and regional bone mineral density, bone metabolic markers in healthy women. *Acta Obstet Gynecol Scand* 79:1060–1064.
36. Martini G, Valenti R, Giovani S, Franci B, Campagna S, Nuti R (2001) Influence of insulin-like growth factor-1 and leptin on bone mass in healthy postmenopausal women. *Bone* 28:113–117.
37. Zoico E, Zamboni M, Adami S, Vettor R, Mazzali G, Tosoni P, Bissoli L, Bosello O (2003) Relationship between leptin levels and bone mineral density in the elderly. *Clin Endocrinol (Oxf)* 59:97–103.

38. Thomas T, Burguera B, Melton LJ III, Atkinson EJ, O'Fallon WM, Riggs BL, Khosla S (2001) Role of serum leptin, insulin, and estrogen levels as potential mediators of the relationship between fat mass and bone mineral density in men versus women. *Bone* 29:114–120.
39. Goulding A, Taylor RW (1998) Plasma leptin values in relation to bone mass and density and to dynamic biochemical markers of bone resorption and formation in postmenopausal women. *Calcif Tissue Int* 63:456–458
40. Blum M, Harris SS, Must A, Naumova EN, Phillips SM, Rand WM, Dawson-Hughes B (2003) Leptin, body composition and bone mineral density in premenopausal women. *Calcif Tissue Int* 73:27–32.
41. Thomas T, Burguera B (2002) Is leptin the link between fat and bone mass? *J Bone Miner Res* 17:1563–1569
42. Iacone R, Russo O, Russo P, Venezia A, Varriale V, Gerardi MC, Strazzullo P (2002) Plasma leptin measurements in epidemiological investigation: Comparison of two commonly used assays and estimate of regression dilution bias. *Nutr Metab Cardiovasc Dis* 12:71-79

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

Age, yrs	54.1±4.8
Duration of diabetes, y	7.0 (3.0-12.0)
Smoking, none/current	104/62
Current treatment, diet/OHA/insulin/OHA and insulin	2/85/55/26
Waist 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 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 A1c	-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