

Negative effect of leptin on bone mass in type 1 diabetes

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Abstract Studies investigating the effect of leptin on bone mass were inconsistent and some related it to the effect of insulin. We intend in this cross-sectional study to investigate the effect of leptin on bone mass in type 1 diabetic patients. We recruited 42 patients with type 1 diabetes for which we determined weight, height, HbA1c, microalbuminuria, serum leptin, bone mineral content (BMC) and density (BMD), and body composition. The patients had an average age of 20.1 ± 0.6 years, an average body mass index (BMI) of 23.6 ± 0.5 kg/cm² and an average duration of diabetes of 9.1 ± 1.0 years. The Z-score was not correlated with HbA1c or duration of the disease, and the average Z-score was not different in patients with microalbuminuria as compared to patients with no reported microalbuminuria. On the other hand, Z-score and BMC correlated negatively with leptin ($r = -0.31$; $p = 0.04$ and -0.60 , $p < 0.01$, respectively). These correlations persisted after adjustment for fat mass. We conclude that not metabolic control of diabetes, but serum leptin has a negative effect on bone density in young patients with type 1 diabetes. This negative effect of leptin on bone density maybe, in part, due to deficiency of endogenous insulin secretion in these patients.

Keywords Type 1 diabetes · Bone · Leptin · Osteoporosis · Bone mineral density

Introduction

The chronic nature of type 1 diabetes and its relatively early onset have always been a source of concern for the medical and psychological well being of the affected patients. The effect of this disease on bone density was studied [1–8]; however, there is still no conclusive evidence that type 1 diabetes is actually associated with a decreased bone density or an increased risk for fractures. Routine screening or initiation of preventive medications for osteoporosis in patients with type 1 or type 2 diabetes is not recommended at the present time [9]. Leptin a product of the *ob* gene was considered to be the link between fat and bone mass because it induces differentiation of the stromal cells toward the osteoblastic lineage [10] and because it affects numerous other endocrine functions such as thyroid and sex hormones [11]. Results of prior human studies investigating the effect of leptin on bone mass were inconsistent. Some studies failed to show any association [12–18], some showed positive association [15, 19–22] and conversely, others showed negative association [23–26].

It has been suggested that the effect of leptin on bone is linked to the effect of insulin [27, 28]. Indeed, the protective effect of leptin against bone loss in obese subjects is lost in the setting of insulin resistance [28]. We therefore hypothesized that the lack of endogenous insulin secretion in the setting of type 1 diabetes mellitus modulates the relationship between leptin and bone mass in these patients. The current study aimed at exploring the influence of leptin on bone density in patients with type 1 diabetes.

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Subjects and methods

The study participants were recruited among patients who were followed up for type 1 diabetes at a referral center in Lebanon (The Chronic Care Center for Diabetes). From 266 patients with type 1 diabetes between the age of 18 and 22 years followed up at that center, only 42 patients (24 females and 18 males) were willing to participate in the study. All subjects were healthy type 1 diabetics, who were free of diseases and not taking medications known to affect bone health. All patients were pubertal with Tanner stage 5 for sexual development. Female patients were all menstruating with no history of prolonged amenorrhea of more than 3 months. At study entry, all subjects underwent physical examination including height (cm) and weight (kg) and body mass index was calculated (kg/m^2). Blood was drawn for glycosylated hemoglobin (HbA1c) and fasting serum leptin levels. Glycosylated hemoglobin was done by the High Performance Liquid Chromatography (HPLC) method using a Variant kit (BIORAD). Plasma samples used for leptin determination were stored at -20°C and were not thawed until the leptin assay was performed. The quantitative detection of leptin levels was performed in duplicate using a commercially available human leptin Radioimmunoassay (RIA) kit (DRG Instruments GmbH, Marburg, Germany). The average was used in the analyses. The presence of microalbuminuria was assessed on a random spot urine sample using Tina-quant[®] Albumin (Roche Diagnostics GmbH, Indiana, USA). Total bone mineral content (BMC), total bone mineral density (BMD), as well as body composition including lean mass and fat mass were measured in the supine position by a dual-energy X-ray absorptiometry using LUNAR device. Total body age and gender specific Z-scores were automatically derived by the densitometer using the Western database provided by the manufacturer.

All patients signed a written informed consent before participating in the study, which was approved by the Institutional Review Board of the American University of Beirut.

Statistical methods

Independent-samples *t* test was used to compare means of continuous variables between two different groups. Correlations between the continuous variables such as BMC, Z-score, duration of the disease, HbA1c, weight, height, lean mass, and fasting serum leptin were assessed using the Pearson correlation coefficient. The correlation between bone parameters and serum leptin were assessed before and after controlling for fat mass. The mean Z-score was compared to zero using the one-sample *t* test, in order to determine whether bone mineral density in the study

population was different from that of their healthy age matched western counterparts. *P* values <0.05 were considered significant. All values are reported as mean \pm standard error of the mean (SEM). Statistical analysis was done using the SPSS version 13.0.

Results

The clinical, biochemical and densitometric characteristics of the study population are shown in Table 1. The mean age was 20.1 ± 0.6 years, the mean BMI was $23.6 \pm 0.5 \text{ kg}/\text{cm}^2$ and the mean duration of diabetes was 9.1 ± 1.0 years and the mean HbA1c was 7.7 ± 0.4 . There was no significant difference between males and females in mean age, BMI, duration of DM or HbA1c. As expected, serum leptin levels and fat mass were higher in females ($p < 0.01$ and 0.05 , respectively), whereas lean mass was higher in males ($p < 0.01$). Males had a higher areal total body BMC and BMD as compared to females ($p < 0.01$). The mean Z-score of the patients was 0.23 ± 1.1 , which by one-sample *t* test, was not significantly different from zero, suggesting that total BMD of the study population was not significantly different from that of their age and gender matched healthy counterparts.

Relationship between bone parameters and diabetes

There was no significant correlation between Z-score and HbA1c or duration of the disease. When the study subjects were divided into two groups according to the presence or absence of albuminuria, the mean Z-score did not differ between the two subgroups. On the other hand, BMC correlated negatively with the duration of diabetes ($R = -0.426$; $p < 0.05$) but not with HbA1c or microalbuminuria (Fig. 1).

Relationship between bone parameters and leptin

There was a significant negative correlation between Z-score and leptin ($r = -0.31$; $p = 0.04$). This correlation persisted after controlling for fat mass ($r = -0.35$; $p = 0.04$).

Similarly, BMC correlated negatively with fasting serum leptin ($R = -0.603$; $p < 0.01$). The negative correlation between BMC and serum leptin persisted after controlling for fat mass ($p < 0.01$). When females and males were analyzed separately, and after controlling for fat mass, leptin correlated with BMC more strongly in females ($R = -0.686$; $p < 0.01$) than in males ($R = -0.575$; $p = 0.051$). The relationship between serum leptin level and total body BMC in the study population is shown in Fig. 2.

Table 1 The clinical, biochemical and densitometric characteristics of the study population

Patients characteristics				
	Total sample	Females	Males	<i>P</i> value of <i>t</i> test*
Number	42	24	18	
Age (years)	20.1 ± 0.6	20.8 ± 0.8	19.1 ± 0.9	NS
BMI (kg/cm ²)	23.6 ± 0.5	24.3 ± 0.7	22.8 ± 0.8	NS
Duration of diabetes (years)	9.1 ± 1.0	10.0 ± 1.3	8.0 ± 1.4	NS
HgA1c (%)	7.7 ± 0.4	8.0 ± 0.6	7.4 ± 0.2	NS
Z-score	0.23 ± 1.1	0.10 ± 1.3	0.40 ± 1.0	NS
BMC (kg)	2.4 ± 0.09	2.1 ± 0.09	2.9 ± 0.09	<0.01
BMD (g/cm ²)	1.1 ± 0.02	1.1 ± 0.02	1.2 ± 0.02	<0.01
Leptin	10.6 ± 1.7	16.0 ± 2.3	3.0 ± 0.4	<0.01
Fat mass (kg)	16.6 ± 1.3	19.1 ± 1.6	12.7 ± 1.7	<0.05
Fat mass (%)	26.6 ± 1.7	32.0 ± 1.6	18.1 ± 1.9	<0.01
Lean mass (kg)	41.1 ± 1.5	35.1 ± 0.7	50.6 ± 1.8	<0.01
Lean mass (%)	67.3 ± 1.6	61.5 ± 1.4	76.3 ± 1.8	<0.01

All values are reported as mean ± standard error of the mean (SEM)

BMI body mass index, *BMC* bone mineral content, *NS* not significant

* Independent-samples *t* test was done to compare the characteristics of the females and males patients

Fig. 1 The relationship between disease duration and total body BMC in the study population: in girls (a) and boys (b)

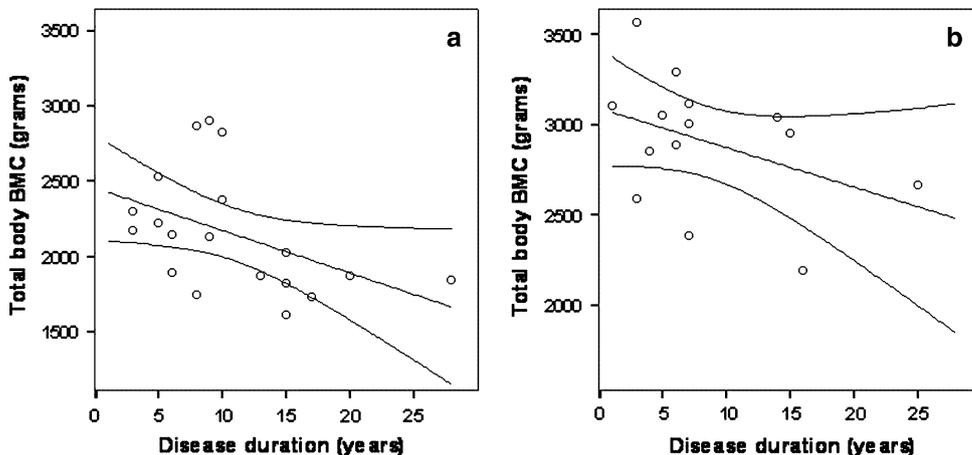
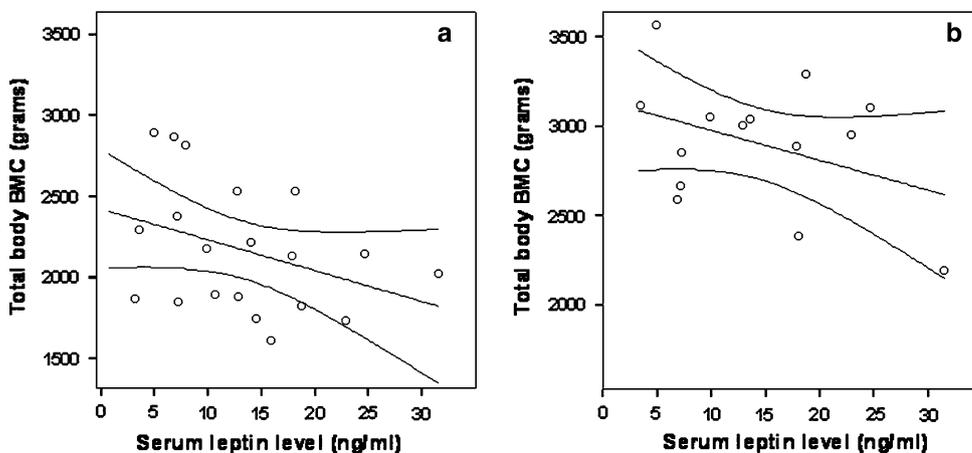


Fig. 2 The relationship between serum leptin level and total body BMC in the study population: in girls (a) and boys (b)



Discussion

This study showed that in patients with type 1 diabetes mellitus, average Z-score was neither significantly affected by the disease nor by the presence of microalbuminuria. Leptin correlated negatively with Z-score in the whole group and with total body BMC in both the genders, the correlation persisted after adjustment for fat mass.

Some studies have suggested that type 1 diabetes is associated with a modest reduction in BMD [1–5, 7], whereas others have found no effect on bone characteristics [4, 6, 7]. In a recent study assessing the prevalence and severity of bone disease in a cohort of young adult male with type 1 diabetic, Alexopoulou et al. [29] found that osteopenia and osteoporosis were present in 40 and 7% of the study population. In our study, BMC was negatively correlated with the duration of diabetes; the average Z-score of our young type 1 diabetic patients was not statistically different from zero. Therefore, a negative effect of type 1 diabetes on bone mass cannot be concluded. This finding is consistent with a study by Rozadilla et al. [6], which showed that bone loss might not represent a major problem in young type 1 diabetic patients with relatively shorter duration of disease. However, the negative correlation with the duration of the disease suggests that there is a risk of bone loss with age and therefore a risk of osteopenia or osteoporosis in adulthood, as it was reported by Alexopoulou et al. This requires further investigation through longitudinal studies. Previous studies also failed to show any association between microalbuminuria [5, 7] or metabolic control [3, 5, 7, 8, 30] and bone mineral density which is also consistent with our findings that neither the presence of microalbuminuria nor HbA1c were correlated with BMC or Z-score.

The effect of leptin on bone mass is still controversial. Few studies have supported the negative effect of leptin on bone density [23–26], whereas other studies showed that it has a positive effect on bone density [15, 19–22]. This effect seems to be different according to the pathway: central versus peripheral [31] and to the insulin level [27, 28]. Indeed, a study analyzing ob/ob mice demonstrated that leptin inhibits bone formation through a hypothalamic relay [31]. A study by Watanabe et al. [32] found that the troglitazone induced decrease in serum leptin is associated with the less bone loss in type 2 diabetes. Moreover, a recent study showed that in the setting of insulin resistance, high leptin level did not result in increased bone density in obese women [28]. Since our patients do not have adequate endogenous insulin secretion, we can hypothesize that the positive effect of leptin on bone might not be mediated by insulin per se but could be mediated by pro-insulin or C-peptide the lack of which resulted in the resistance of bone to leptin; and this is what

caused this negative correlation between BMC and leptin. However, this hypothesis needs to be further investigated. On the other hand, the body composition and body fat content in patients with type 1 diabetes receiving insulin therapy may differ from that of healthy adolescents. Leptin is a polypeptide that is synthesized in adipose tissue. Therefore, insulin therapy may be of importance as a regulator of serum leptin levels and effect. Indeed, it has been previously shown that insulin is a potent stimulator of leptin expression in rodents [33].

Our study has some limitations. The cross-sectional design, the small sample size and the lack of information regarding lifestyle factors, which are important determinants of bone mass in adolescents, such as nutritional factors, calcium intake from dairy products and physical activity.

Nevertheless, we may conclude, based on our results and on all the above-mentioned studies, that metabolic control of diabetes (reflected in this case by HbA1c and microalbuminuria) does not significantly affect bone density in young type 1 diabetic patients and that serum leptin has a negative effect on bone in these patients which is probably in part due to the deficiency of endogenous insulin in these patients.

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