Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 62; Numer/Number 5/2011
ISSN 0423–104X

Relation of leptin, adiponectin and insulin resistance to bone mineral density in type 2 diabetic postmenopausal women

Zależność między stężeniami leptyny, adiponektyny, insulinoopornością a gęstością mineralną u kobiet z cukrzycą typu 2 w okresie pomenopauzalnym

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Abstract

Background: Our aim is to identify the relation of leptin, adiponectin and insulin resistance to bone mineral density (BMD) in type 2 diabetic postmenopausal women and compare it with that experienced by nondiabetics.

Material and methods: Seventy six postmenopausal female patients were included in the study. Postmenopausal type 2 diabetic (n = 19) and nondiabetic patients (n = 19) with spine and/or hip BMD T score lower than -2 were included in the study, and postmenopausal type 2 diabetic (n = 20) and nondiabetic women (n = 18) with normal BMD (T score > –1) were selected as control groups. Those receiving therapy for osteoporosis, over the age of 65, those who had a disease and were taking a medication that could affect bone metabolism were excluded. Biochemical tests, as well as leptin, adiponectin and insulin levels, were measured and insulin resistance was calculated using the HOMA test.

Results: There was no correlation between low BMD and leptin, adiponectin and insulin resistance. There was only a negative correlation between leptin and femur Ward's triangle BMD.

Conclusion: Further large-scale studies must to be performed in order to analyse the effects of leptin, adiponectin and insulin resistance on bone metabolism in type 2 diabetic patients. **(Pol J Endocrinol 2011; 62 (5): 429–435)**

Key words: *leptin, adinopectin, insulin resistance, bone mineral density*

Streszczenie

Wstęp: Celem badania było porównanie zależności między stężeniami leptyny i adiponektyny oraz insulinoopornością a gęstością mineralną kości (BMD) u kobiet po menopauzie chorujących na cukrzycę typu 2 i u osób z niechorującej na cukrzycę grupy kontrolnej.

Materiał i metody: Do badania zrekrutowano łącznie 76 kobiet po menopauzie, które podzielono na grupy obejmujące kobiety chorujące na cukrzycę typu 2 (n = 19) i bez cukrzycy (n = 19), u których wartość T score dla BMD kręgosłupa i/lub bliższego odcinka kości udowej wynosiło mniej niż -2 oraz chore na cukrzycę typu 2 (n = 20) i niechorujące na cukrzycę (n = 18) z prawidłowymi wartościami BMD (T score > -1). Kryteria wykluczenia obejmowały stosowanie leków przeciwosteoporotycznych, wiek ponad 65 lat, choroby lub terapie mogące wpływać na metabolizm kostny. U uczestniczek badania wykonano badania biochemiczne, oznaczono stężenia leptyny, adiponektyny i insuliny oraz oszacowano stopień insulinooporności HOMA.

Wyniki: Nie zaobserwowano korelacji między niskimi wartościami BMD a stężeniami leptyny i adiponektyny oraz insulinoopornością. Stwierdzono jedynie odwrotną zależność między stężeniem leptyny a BMD kości udowej w obrębie trójkąta Warda.

Wnioski: Konieczne są dalsze badania obejmujące dużą grupę chorych, aby ustalić wpływ stężeń leptyny i adiponektyny oraz insulinooporności na metabolizm kostny u chorych na cukrzycę typu 2. **(Endokrynol Pol 2011; 62 (5): 429–435)**

Słowa kluczowe: *leptyna, adiponektyna, insulinooporność, gęstośćć mineralna kości*

Introduction

Osteoporosis is a systemic disease of the skeleton characterised by low bone mass and disruption of the microarchitecture of the bone tissue, which is followed by an increase in bone fragility and a tendency to fracture [1]. Since bone mineral density (BMD) increases, and the fracture risk decreases, in obese people, it has been argued that there is a positive relation between body weight and bone mass [2, 3]. Mechanical effects

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associated with increased weight elevate BMD. Increased aromatisation of androgens into oestrogen in the fat tissues, and high free sex steroid levels which are associated with a decrease of sex hormone binding globulin in obese people also increase bone mass [4]. Similarly in the obese, hyperinsulinaemia due to insulin resistance shows a direct mitogenic effect and increases bone formation [4].

Bone is a continuously regenerated tissue. The bone remodelling unit is composed of osteoclasts for bone resorption, and osteoblasts for bone formation. Osteoclasts are composed of haematopoietic precursors in circulation and in bone marrow. Osteoblasts and adipocytes derive from mesenchymal stem cells [5, 6]. Hormones such as leptin and adiponectin, and proinflammatory cytokines such as tumour necrosis factor and interleukin-6 may also affect bone remodelling.

The relationship between leptin and BMD has been much debated. There are studies in the literature reporting a positive correlation between leptin and BMD [7–9], others reporting no significant relation [10–13], and yet others reporting a negative correlation [4, 14]. Blain et al. [7] reported a significant positive correlation between the full body, femur neck and lumbar vertebrae BMD, and serum leptin levels and suggested that leptin was an important predictor of BMD in postmenopausal women. Goulding et al. [8] and Yamauchi et al. [9] found similar results. The above mentioned studies point out the positive correlation between leptin and bone metabolism. This situation can be explained by the direct stimulatory effect of leptin on osteoblast differentiation, which is one of the effects of leptin on bone metabolism [6]. There are also some other studies which demonstrate that locally secreted leptin from adipocytes in the bone marrow increases bone formation [15]. This fact suggests local production of leptin could have a role in bone metabolism [12].

The relationship between diabetes and BMD has been investigated in many studies. While the relationship between osteopenia and type 1 diabetes has been well defined [16], the effects of type 2 diabetes on bone metabolism have not yet been clearly identified. In this study, our aim was to investigate whether there was a difference between postmenopausal women with and without low BMD in terms of adipocytokines such as leptin, adiponectin and insulin resistance; and to identify the differences between the same parameters according to the level of BMD in type 2 diabetic patients.

Material and methods

The study patients were postmenopausal women who were referred to our hospital for measurement of BMD. Postmenopausal was defined as not having had a menstrual period for at least one year [17]. We excluded patients who had rheumatic diseases, chronic hepatitis and renal diseases, endocrinological disorders such as pituitary and thyroid diseases, patients over the age of 65 years and patients receiving glucocorticoids, calcium, vitamin D, bisphosphonates and hormone replacement therapy. Postmenopausal type 2 diabetic (n = 19) and nondiabetic patients (n = 19) with spine and/or hip BMD T score lower than –2 were included in the study, and postmenopausal type 2 diabetic (n = 20) and nondiabetic women (n = 18) with normal BMD (T score > –1) were selected as control groups. All diabetic women were diagnosed as diabetes mellitus after the age of 35. Women were matched by age and length of the postmenopausal period. There was no statistically significant difference between diabetics and nondiabetics according to age or length of postmenopausal period.

The diabetic patients' group included five patients who used insulin, 29 patients taking oral anti-diabetic drugs, two patients who used oral anti-diabetic and insulin together, two patients who did not take any medication, but rather controlled their blood glucose by diet, and one patient who had recently been diagnosed as diabetic. Patients' body weight, height and waist circumference were measured and then BMI was calculated according to the formula. Blood sampling was performed after an overnight fast. The blood samples were centrifuged for four minutes at 3,500 rpm and serum samples were obtained. Biochemical markers (plasma glucose, creatinine, lipid profiles, alkaline phosphatase, calcium, phosphorus) were measured with a biochemistry autoanalyser (Aeroset®, Abbott, Abbott Park, IL, USA) by standard biochemical methods. Osteocalcin levels were measured using the electrochemiluminescent method (Elecsys 1010®, Roche, Weinheim, Germany). Deoxy-pyridinoline levels were calculated using the chemiluminescent immunometric assay method (Immulite 2000®, DPC, Los Angeles, CA,USA) in 24-hour urine test. Frozen plasma samples were thawed at room temperature and then serum adiponectin levels were identified by means of a commercial kit (Human Adiponectin ELISA KIT®, Linco Research, St Charles, MO, USA) and EL_x 808 IU Ultra Microplate instrument (Bio-Tek Instruments Inc, Winooski, VT, USA). Serum leptin levels were assessed using a commercial kit (Biosource LEPTIN EASIA KIT®, Nivelles Belgium) and EL_x 808 IU Ultra Microplate instrument (Bio-Tek Instruments Inc). Serum insulin levels were assessed by means of a commercial kit (Biosource INS- EASIA KIT® Nivelles Belgium) and EL_x 808 IU Ultra Microplate instrument (Bio-Tek Instruments Inc.).

Insulin resistance was calculated by the HOMA-IR method, which is frequently used in practice. HOMA-IR score was calculated by the following formula defined

by Matthews [18]. HOMA-IR = [fasting serum insulin (μIU/mL) x serum glucose (mmol/dL)]/22.5. According to this method, a high HOMA-IR score indicates low insulin sensitivity. This method is less complicated, cheaper and more practical in detecting insulin sensitivity compared to the glucose clamp technique.

BMD measurement was performed using the DEXA method (Norland XR-46®, Norland Corp, Fort Atkinson, WI, USA) on lumbar vertebrae and proximal femur areas. L2-L4 vertebrae, femur neck, femur trochanter and Ward's triangle bone mineral densities were assessed in gram/cm^2; lumbar vertebrae and femur neck T and Z scores were defined. Fat mass, lean mass and total fat percentages were assessed by means of full body scan performed by the same instrument. Lean mass and fat mass were calculated in grams.

Statistical analysis

Computer software was used for the statistical analysis (SPSS ver. 13, Chicago, IL, USA). Quantitative data was expressed in average ± standard deviation. In the analysis of demographic data of the patients one-way analysis of variance (one way ANOVA) was used. Pearson's correlation coefficient was used in testing the relation between the parameters. Kruskal-Wallis test was used in testing whether there was a statistically significant difference between the groups or not. Mann-Whitney U test was used to test the significance of the difference between two groups. In the comparison of diabetic and non-diabetic patients and patients with and without osteoporosis, student-t test was employed. Statistical significance was accepted when $p < 0.05$.

Results

Demographic characteristics of the patients analysed under four groups are set out in Table I. The average age of the patients in group 4 was significantly lower than the average age of those in group 1. Similarly, menopause period was found to be significantly shorter in group 4 compared to group 1 and group 3.

Fasting blood glucose and HbA_{1c} levels of the diabetic group (groups 1 and 2) were detected to be significantly high in comparison with the non-diabetic group (groups 3 and 4). Triglyceride levels of the patients in group 1 were found to be higher compared to all other groups. No significant difference was defined between the groups in terms of biochemical parameters, insulin, leptin, or adinopectin levels, except for insulin resistance (Table II). Spine and hip BMD values of each group are shown in Table III.

Discussion

In this study, our aim was to detect whether there was a relationship between leptin, adiponectin, insulin resistance, and BMD in postmenopausal women with and without osteoporosis and to define the differences in the same parameters of type 2 diabetic women with and without osteoporosis. At the end of the study, we could not identify any correlation between leptin, adiponectin and insulin resistance, and bone mass in diabetic and nondiabetic patients with and without low BMD.

In recent clinical studies, it has been reported that adult patients with congenital leptin deficiency have low BMD despite their increased fat mass [19]. A child with congenital leptin deficiency responded well to recombinant leptin injection, and an increase in the BMD alongside a decrease in the fat mass was observed [20]. Takeda et al. explained that the sympathetic nervous system was the mediator to the intracerebroventricular effects of leptin. Osteoblasts express adrenergic receptor and adrenergic agonist application inhibits bone formation which leads to a decrease in the trabecular bone mass [21]. Recent data indicates that elevated

Table I. *Demographic characteristics of the groups*
Tabela I. *Charakterystyka demograficzna badanych*

	Group 1 DM & low BMD (n = 19)	Group 2 DM & normal BMD (n = 20)	Group 3 NonDM & low BMD (n = 19)	Group 4 NonDM & normal BMD (n = 18)
Age (years)	58.00 ± 4.35 *	55.26 ± 4.38	54.89 ± 4.86	52.83 ± 3.60
Postmenopausal period (years)	10.16 ± 6.71*	7.42 ± 5.92	8.68 ± 5.09 *	4.76 ± 3.99
Body weight [kg]	84.21 ± 14.30	78.05 ± 15.48	78.15 ± 17.01	79.72 ± 15.04
BMI [kg/m^2]	36.17±6.41	33.35±6.06	33.76±7.60	34.09±6.48
Waist circumference [cm]	106.36 ± 27.74	102.95 ± 13.39	103.36 ± 14.65	104.94 ± 13.44

*compared to group 4, $p < 0.05$

Table II. *Biochemical evaluation and insulin, insulin resistance, leptin, and adiponectin values of the groups*
Tabela II. *Parametry biochemiczne, insulinooporność oraz stężenia insuliny , leptyny i adiponektyny w poszczególnych grupach*

	Group 1 DM & low BMD (n = 19)	Group 2 DM & normal BMD (n = 20)	Group 3 NonDM & low BMD (n = 19)	Group 4 NonDM & normal BMD (n = 18)
Fasting blood glucose [mg/dL]	154.52 ± 59.78*	171.40 ± 67.49*	96.63 ± 8.10	98.05 ± 7.80
HbA_{1c} (%)	7.17 ± 1.76*	7.46 ± 1.40*	5.24 ± 0.38	5.46 ± 0.64
Triglyceride [mg/dL]	201.94 ± 74.89**	127.80 ±66.92	143.00 ± 98.27	141.05 ± 68.93
Cholesterol [mg/dL]	199.36 ± 62.63	184.60 ± 66.92	143.00 ± 98.27	141.05 ± 68.93
HDL-cholesterol [mg/dL]	55.21 ± 14.64	53.70 ± 13.74	55.16 ± 15.91	60.11 ± 19.39
Calcium [mg/dL]	9.69 ± 0.29	9.68 ± 0.52	9.74 ± 0.38	9.87 ± 0.37
Phosphorus [mg/dL]	3.97 ± 0.68	3.82 ± 0.45	3.65 ± 0.76	3.80 ± 0.49
Alkaline phosphatase [U/L]	117.68 ± 66.79	107.85 ±64.29	110.01 ± 58.33	106.16 ± 46.95
Osteocalcin [ng/mL]	20.20 ± 14.61*	18.50 ± 6.52*	29.66 ± 8.47	26.36 ± 8.97
Deoxypyridinoline [nmol/mg creatynine]	8.57 ± 10.38	6.04 ± 1.91	7.11 ± 2.26	6.91 ± 2.43
Insulin [μU/mL]	18.94 ± 11.52	17.54 ± 12.70	17.48 ± 10.51	14.24 ± 5.35
Insulin resistance (%)	6.97 ± 4.09*	8.34 ± 9.44 *	4.15 ± 2.47	3.49 ± 1.47
Leptin [ng/mL]	10.09 ± 6.77	9.12 ± 6.31	10.82 ± 7.55	10.34 ± 7.27
Adiponectin [μg/mL]	7.31 ± 6.45	6.44 ± 3.16	7.68 ± 3.30	7.58 ± 3.23

*compared to groups 3 and 4, $p < 0.05$; **compared to groups 2, 3 and 4, $p < 0.05$

Table III. *Bone mineral measurements of the groups*
Tabela III. *Gęstość mineralna kości w poszczególnych grupach*

	Group 1 DM & low BMD (n = 19)	Group 2 DM & normal BMD (n = 20)	Group 3 NonDM & low BMD (n = 19)	Group 4 NonDM & normal BMD (n = 18)
Lumbar spine BMD [g/cm^2]	0.82 ± 0.76*	1.13 ± 0.16	0.81 ± 0.11*	1.10 ± 0.77
Femoral neck BMD [g/cm^2]	0.68 ± 0.12*	0.91 ± 0.97	0.71 ± 0.85*	0.89 ± 0.78
Trochanter BMD [g/cm^2]	0.61 ± 0.93*	0.74 ± 0.93	0.59 ± 0.56*	0.76 ± 0.10
Wards triangle BMD [g/cm^2]	0.47 ± 0.12*	0.70 ± 0.11	0.46 ± 0.77*	0.69 ± 0.80

*compared to groups 2 and 4, $p < 0.05$

sympathetic activity level increases bone resorption [22]. On the contrary, subcutaneous infusion of leptin to ob/ob mouse led to an increase in bone mineral content and decreased the adipose tissue quantity in the bone marrow [23]. These findings indicated that there was a positive correlation between endogenous leptin levels and BMD. The results of these studies suggest that the effect mechanism of leptin in circulation on the bone might be independent from the fat mass [9].

Goulding et al. found significant correlation between serum leptin level and deoxy-pyridinoline and hydroxy-pyridinoline, bone resorption biomarkers, and osteocalcin in postmenopausal women [8]. On the other hand, Yamauchi et al. did not detect any significant correlation between plasma leptin levels and urinary deoxy-pyridinoline and osteocalcin in postmenopausal women [9].

In our study, we did not find any significant relation between serum leptin levels and bone formation and resorption biomarkers such as osteocalcin, and deoxy-pyridinoline. As a result, plasma leptin levels do not have a correlation with the biochemical markers of either osteoclastic or osteoblastic activity, which suggests that leptin does not play a direct role in bone metabolism [8]. Kontogianni et al. defined a negative correlation between leptin and L2–L4 vertebrae BMD in perimenopausal women [4]. We found a negative correlation between femur Ward's triangle and leptin in

our study, while we could not report such an association for L2–L4 vertebrae BMD. Femur Ward's triangle is rich in trabecular bone, just like the vertebrae. Degenerative changes in vertebrae bones, which are associated with obesity, may lead to false high levels of vertebrae BMD. Isparta is an endemic region for fluorosis and fluorosis-induced osteosclerosis. So women may have higher BMD than normal [24, 25]. This could be the reason why we could not detect a negative relation between leptin and vertebra BMD in this study.

Serum leptin levels are higher in obese patients. Nevertheless, the relation between leptin and obesity associated diabetes is not very well known [26]. Kontogianni et al. found a significant positive correlation between leptin levels and BMI, fat percentage, free fat mass and lean body mass in their study [4].

Adiponectin levels are low in obese, hyperinsulinaemic and type 2 diabetic patients and adiponectin levels also increase with age [27]. In our study, adiponectin levels were lower in type 2 diabetic patients compared to the non-diabetics, however the difference was not statistically significant. Although there wasn't a statistically significant difference in age between type 2 diabetic and non diabetic groups when considered as a whole group, type 2 diabetic patients' groups in our study had a higher average age than the non-diabetic groups and the smaller number of patients in these groups might be the reason why we did not find any significant difference between adiponectin levels. Although there is a common stem cell in the bone marrow for adipocytes and osteoblasts, and in spite of the information available on the synthesis of various adipocytokines in the osteoblasts, the relationship between leptin, adiponectin and BMD is controversial. We did not find any difference between the postmenopausal female patient groups with and without osteoporosis in terms of leptin or adiponectin levels. The low number of patients might have prevented any significant correlation. However, there are different conclusions in the literature as well. When we categorised the diabetic patients into two groups as those with and without osteoporosis, we also did not find a difference in leptin or adiponectin levels. The interesting correlation indicating the interaction between adipocytes and bone metabolism should be clarified in further studies.

Adipocytes express adiponectin abundantly and specifically, and it has been reported that adiponectin receptors are detected in cells forming the bone [27]. Lenchik et al. analysed the possible relation between adiponectin levels and bone mass in their study which was conducted on 42 male and 38 female (86% of the women were postmenopausal) patients [28]. They defined an inverse proportion between the adiponectin levels and BMD in all skeletal areas for all patients. Adiponectin has structural similarities with tumour necrosis factor-alpha (TNF-α) family members, which have an important role in the regulation of osteoclastogenesis, and receptor activator nuclear factor kappa B ligand (RANKL) and osteoprotegerin. It has been demonstrated that adiponectin affects nuclear transcription factor kappa B (NFKB), which is a critical transcription factor for osteoclastogenesis [29, 30] and it is suggested to be one of the mechanisms of adiponectin to affect the bone. Giving that recombinant adiponectin prevents adipogenesis at the preadipocytes which stem from bone marrow, this indicates that adiponectin has an impact on the bone marrow environment [31].

It is argued that insulin accelerates the synthesis of the bone matrix by stimulating the function of osteoblasts. Some researchers are of the opinion that insulin is necessary for normal bone mineralisation. There is also an argument that BMD increasing effect of obesity can be due to hyperinsulinaemia which is mostly seen with obesity, together [32]. Oner et al., in their study of 30 non-obese and 30 obese postmenopausal women, suggested that higher BMD levels of the obese postmenopausal women compared to the non-obese could be associated with insulin rather than weight stress and the effects of sex hormones [33]. Connor et al. [32] and Albala et al. [34] argue that insulin indirectly stimulates the function of osteoblasts as it increases the production of insulin like growth factor (IGF-1) in the liver and it is bound to the IGF-1 receptors since it is structurally similar to IGF-1. Then insulin could have a positive impact on BMD by increasing bone collagen synthesis and replication of preosteoblastic cells.

In this study, we could not identify any correlation between serum insulin levels and femur and L2–L4 vertebrae BMD.

The relationship between diabetes and BMD had been analysed in many studies. Although the relation between osteopenia and type 1 diabetes had been well defined before [16], the effects of type 2 diabetes on bone metabolism have yet to be clearly explained. There are studies referring to higher [35], similar [36] or lower [37] bone mass in type 2 diabetic patients compared to normal controls. Isaia et al. [38] measured lumbar vertebrae BMD of 49 postmenopausal diabetic and 28 non-diabetic women, and femoral (neck, intertrochanter, Ward's) and total BMD of 65 diabetic and 42 non-diabetic women. There was no significant difference between diabetic and non-diabetic groups for L2–L4 vertebrae BMD. At the femoral level, however, type 2 diabetic patients had higher BMD in areas other than the Ward's triangle. They argued that higher BMD might be associated with the anabolic effect of hyperinsulinaemia, which increases osteoblastic activity. The

different lumbar vertebrae and femur BMD values in the diabetic group can be explained by the fact that cortical bone might have been protected more [38]. Another reason for high BMD in diabetic patients is the negative effect of insulin on the sex hormone binding globulin. Low sex hormone binding globulin level coexists with high serum oestradiol levels [39]. In our study, we could not identify a significant difference between diabetic and non-diabetic patients in terms of femur and L2–L4 vertebrae BMD.

Cakatay et al. compared osteocalcin, total alkaline phosphatase, and urinary deoxy-pyridinoline levels in their study, which included 35 type 2 diabetic and 35 non-diabetic patients. They identified significantly low osteocalcin levels in diabetic patients compared to the control group. No significant difference was reported for the other parameters [40].

In our study, when we compared the diabetic groups with the non-diabetics, we did not identify any significant difference between serum calcium, phosphorus, urine calcium, alkaline phosphatase, or urine deoxy-pyridinoline. On the other hand, we observed lower osteocalcin levels in diabetic patients.

Our study has some limitations. Firstly, the groups were not large enough or homogenous enough to make definite conclusions. Secondly, insulin or antidiabetic drugs such as glitazones that diabetic patients use were not estimated. Finally, vertebral fractures were excluded from consideration.

Conclusion

Although the majority of epidemiologic studies have highlighted the existence of the interaction between adiposity and bone, results of these studies have contrasting conclusions. In our cross-sectional study, we conclude that leptin adiponectin levels are not correlated with BMD in either type 2 diabetics or nondiabetics. However, we believe that further research involving more participants and the investigation of local leptin and adiponectin levels in bony tissue are necessary to clarify the exact role of these adipocytokines in the bone metabolism.

Conflict of interest statement

The authors have no conflict of interest to declare.

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