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ORIGINAL ARTICLE

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The influence of ghrelin, adiponectin, and leptin on bone mineral density in healthy postmenopausal women

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Abstract The association of body fat mass (FM) with bone mineral mass (BMC) and bone mineral density (BMD) has been attributed to a mechanical load exerted on the skeleton by FM and by the effect of different hormones. The aim of the present study was to determine whether there is a relationship between ghrelin, adiponectin, and leptin with BMC and BMD in healthy postmenopausal women ($n = 88$; age, 68.9 ± 6.8 years; body mass index, 27.4 ± 3.6 kg/m^2). Body composition, BMC, and BMD were derived by dual-energy X-ray absorptiometry. Waist-to-hip (WHR) and waist-to-thigh (WTR) ratios were also obtained. Ghrelin was associated with total BMC (β = –0.945; $P = 0.0001$), total BMD (β = –0.959; $P = 0.0001$), lumbar spine BMD (β = –0.945; $P = 0.0001$), and femoral neck BMD (β = –0.957; $P = 0.0001$), and remained associated ($P < 0.041$) in different analyses that controlled for measured body composition and hormonal and insulin resistance values. However, the associations between ghrelin and measured bone mineral values were no longer significant ($P > 0.149$) when adjusted for body fat distribution values (WHR, WTR). Adiponectin was significantly related to total BMC (β = –0.931; $P = 0.0001$), total BMD (β = –0.940; $P = 0.0001$), lumbar spine BMD (β = –0.937; $P = 0.0001$), and femoral neck BMD (β = –0.940; $P = 0.0001$) values, and these relationships remained significant ($P < 0.019$) after adjusting for measured body fat, hormonal, and insulin resistance values but not when adjusted for fat-free mass (FFM; $P > 0.106$). In addition, significant associations of leptin with total BMC (β = 0.912; $P = 0.0001$), total BMD (β = 0.907; $P = 0.0001$), lumbar spine BMD (β = 0.899; $P = 0.0001$), and femoral neck BMD (β = 0.906; $P = 0.0001$) were found. These associations remained significant ($P < 0.010$) in different analyses that controlled for hormonal and insulin resistance values, but the associations between leptin and bone mineral values were no longer significant ($P > 0.145$) when adjusted for specific body composition values (WHR, WTR, FM, and FFM). In conclusion, it appears that the influence of plasma ghrelin, adiponectin, and leptin levels on BMC and BMD values is mediated or confounded by the specific body composition parameters in healthy postmenopausal women.

Key words bone mineral density · ghrelin · adiponectin · leptin · postmenopausal women

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Introduction

Body mass has been considered as one of the strongest predictors of bone mineral mass (BMC) and bone mineral density (BMD) [1,2], and is inversely associated with postmenopausal bone loss and bone turnover [3,4]. Body mass depends on fat mass (FM) and fat-free mass (FFM), and a number of cross-sectional studies have established a strong relationship between FM and BMD in women [2,5], especially after menopause [6,7]. Excessive FM, consistent with obesity, induces a greater mechanical loading on the skeleton, which contributes to the total body mass and BMD relationship [8]. Accordingly, the association of FM with BMC and BMD has been attributed to a combination of mechanical load exerted on the skeleton by FM and the effect of hormones produced by fat cells [5]. For example, leptin, the product of the *LEP* gene, has emerged as a potential candidate for explaining the protective effect of FM on bone [9–13]. However, cross-sectional studies performed on women to assess the role of leptin in bone metabolism have demonstrated contradictive results [2,11], and the relationship between leptin concentration and BMD has not been made clear in women at different ages [10,14].

Another adipose-modulated biochemical signal that may explain some of the association between FM and BMD is adiponectin, a polypeptide hormone expressed

specifically and abundantly in adipose tissue [15] and produced in visceral, subcutaneous, and bone marrow fat depots [16]. Circulating adiponectin concentrations are negatively regulated in obesity, diabetes, and cardiovascular disease, and increase after weight loss and negative energy balance [17,18]. The exact relationship between adiponectin and bone has not yet been elucidated. Some of the cross-sectional studies observed no correlation between circulating adiponectin and BMD at several sites of the skeleton in middle-aged men [19] and women [11], while other studies found such a relationship in middle-aged women [10,20]. In addition to adiponectin, ghrelin may also play a role in the FM and BMD relationship in women at different ages as there is a report on inverse association of plasma ghrelin concentration and BMD in healthy adolescent girls [21]. Ghrelin is an endogenous ligand of the growth hormone secretagogue receptor and the principal site of ghrelin synthesis is the stomach [22]. Ghrelin transfers information from the stomach to the hypothalamus and influences growth hormone release in response to changes in energy homeostasis [23]. Ghrelin has been reported to increase FM by stimulating appetite and reducing fat use [23,24]. Plasma ghrelin levels are reduced in obese individuals [25] and substantially elevated in anorexic subjects [21,26].

Leptin, adiponectin, and ghrelin seem to play an important role in the regulation of body composition throughout life, but the mechanisms are not yet well understood [27]. Furthermore, to our knowledge, no studies have been performed to examine the possible associations of adiponectin and ghrelin with bone tissue in healthy postmenopausal women. Therefore, we examined a group of healthy postmenopausal women to determine the relationship of leptin, adiponectin, and ghrelin with bone mineral values. In addition, we evaluated different body composition and hormone factors that are known to affect bone metabolism.

Subjects and methods

Participants

Eighty-eight postmenopausal women between the ages of 58 to 80 years were recruited for this study. All subjects signed an informed consent that was approved by the Medical Ethics Committee of the University of Tartu, Tartu, Estonia. Prior to study enrollment, volunteers completed medical and physical activity questionnaires [2,10]. They were excluded from the study if they reported current or previous conditions that might have interfered with bone metabolism (such as heart disease, long-term corticosteroid use, smoking, or alcoholism as well as long-term high physical activity). At the time of the study, no participants were receiving treatments such as calcium, vitamin D, calcitonin, bisphosphonates, and diuretics, which could influence bone mineral values [2,10,11]. All participants had a body mass index (BMI; kg/m^2) of less than 30 kg/m^2 and were matched for their level of mean daily energy expenditure [2,10].

All women were asked to come for two visits to complete the testing. On the first visit, the anthropometric parameters of the subjects were measured and a venous blood sample was taken in the morning after a 10-h fast. The second measurement session consisted of body composition and bone mineral assessments by dual-energy X-ray absorptiometry (DXA). Measurement sessions were separated by approximately 1 week, dependent on the participant's schedule and DXA availability. In addition, all participants completed a 3-day energy expenditure questionnaire [28].

Body composition and bone mineral measurements

Height was measured to the nearest 0.1 cm by means of a Martin metal anthropometer, and body mass was measured to the nearest 0.05 kg by means of a medical electronic scale (A&D Instruments, Abingdon, UK). The BMI was calculated. Body fat distribution was defined by waist-to-hip ratio (WHR) [10]; waist circumference was obtained as the minimum value between the iliac crest and the lateral costal margin, and hip circumference as the maximum value area of the buttocks. The waist-to-thigh circumference ratio (WTR) was also calculated [10].

Whole-body fat and lean and bone mineral mass were measured by DXA using a DPX–IQ densitometer (Lunar Corporation, Madison, WI, USA) equipped with adult, proprietary software, version 3.6. Participants were scanned in light clothing while lying flat on their backs with arms at their sides. The fast scan mode and standard participant positioning were used for total body measurements and analyzed using the extended analysis option. Bone mineral density values were determined as the total body BMD and at the sites of posteroanterior spine (L2–L4) [10,20,29] and femoral neck [2,20,30].

Blood analysis

A 10-ml blood sample was obtained from the antecubital vein with the participant in the upright position in the morning (0700–0800) after an overnight fast. Plasma was separated and frozen at –20°C for later analysis. Total ghrelin concentration was determined in duplicate using a commercially available radioimmunoassay (RIA) kit (Linco Research, St. Charles, MO, USA). The sensitivity of this kit was 93 pg/ml, and the intra- and interassay coefficients of variation (CV) were <10% and <14.7%, respectively. Total adiponectin concentration was assessed in duplicate using a commercially available RIA kit (HADP-61HK; Linco Research, St. Charles, MO, USA). The intra- and interassay CVs were <7%. Leptin concentration was also determined in duplicate by RIA (Mediagnost, Reutlingen, Germany). This assay has the intra- and interassay CVs <5%. Insulin-like growth factor-I (IGF-I) and insulin concentrations were analyzed in duplicate on an Immulite 2000 (DPC, Los Angeles, CA, USA). The intra- and interassay CVs for insulin were 4.5% and 12.2%, respectively, at an insulin concentration of 6.6 μIU/ml, and the intra- and interassay

CVs for IGF-I were <7%. Glucose concentration was measured using the hexokinase/glucose-6-phosphate dehydrogenase method with a commercial kit (Boehringer, Mannheim, Germany). The insulin resistance index from fasting plasma insulin and plasma glucose levels was estimated using the homeostasis model assessment (HOMA): fasting plasma insulin (μIU/ml) × fasting plasma glucose (mmol/l)/22.5 [31]. The greater the HOMA value, the greater the level of insulin resistance.

Statistical analysis

Statistical analysis was performed with SPSS 11.0 for Windows (Chicago, IL, USA), and means and standard deviations were determined. Pearson correlation coefficients were computed to explore the relation between bone mineral and other measured variables. Regression analysis models and backward elimination procedures were also used to evaluate potential associations of ghrelin, adiponectin, leptin, or measured bone mineral indices with several independent variables [20]. Significance was set at $P < 0.05$.

Results

The mean (±SD), minimum, and maximum values of measured characteristics for study population are presented in Table 1. Mean daily energy expenditure averaged at 1953.1 ± 655.4 kcal. Total BMC was significantly related to height, body mass, BMI, WHR, FM, FFM, ghrelin, leptin, and HOMA values (Table 2). Total BMD was associated with height, body mass, BMI, WHR, FM, FFM, leptin, insulin, and HOMA parameters. Lumbar spine BMD was significantly related to height, body mass, BMI, FM, FFM, ghrelin, and adiponectin values. Femoral neck BMD was significantly related to body mass, BMI, WHR, FM, FFM, leptin, insulin, and HOMA values.

In separate regression models, negative associations of plasma ghrelin concentration with total BMC (β = –0.945; $P = 0.0001$), total BMD (β = –0.959; $P = 0.0001$), lumbar spine BMD (β = –0.945; $P = 0.0001$), and femoral neck BMD (β = –0.957; $P = 0.0001$) were observed. These associations remained significant ($P < 0.041$) after controlling for adiponectin, leptin, IGF-I, insulin, glucose, HOMA, BMI, FM, and FFM, but not when adjusting for WHR and WTR ($P > 0.149$). Adiponectin was significantly related to total BMC (β = –0.931; $P = 0.0001$), total BMD (β = –0.940; $P = 0.0001$), lumbar spine BMD (β = –0.937; $P = 0.0001$), and femoral neck BMD (β = –0.940; $P = 0.0001$) values. These relationships remained significant ($P < 0.019$) after adjusting for ghrelin, leptin, IGF-I, insulin, glucose, HOMA, BMI, WHR, WTR, and FM, but not when adjusting for FFM ($P > 0.106$). In addition, significant associations of leptin with total BMC (β = 0.912; $P = 0.0001$), total BMD (β = 0.907; $P = 0.0001$), lumbar spine BMD (β = 0.899; $P =$

Table 1. Mean (±SD) subject characteristics of study population ($n = 88$)

Parameter	Mean ± SD	Range
Age (years)	68.9 ± 6.8	58–80
Height (cm)	159.7 ± 5.3	148.3–173.7
Body mass (kg)	72.7 ± 13.3	46.3–89.8
BMI (kg/m^2)	27.4 ± 3.6	20.1–29.7
WHR	0.82 ± 0.07	0.69–1.00
WTR	1.54 ± 0.16	1.20–2.01
%FM	38.8 ± 6.6	23.1–48.7
FM (kg)	27.6 ± 8.0	6.8–40.7
FFM (kg)	42.0 ± 3.4	33.7–52.3
BMC (kg)	2.6 ± 0.4	1.7–3.8
Total BMD (g/cm^2)	1.11 ± 0.09	0.87–1.32
Lumbar spine BMD (g/cm^2)	1.05 ± 0.15	0.80–1.41
Femoral neck BMD (g/cm^2)	1.11 ± 0.10	0.88–1.33
Ghrelin (pg/ml)	889.1 ± 244.1	397.0–1396.0
Adiponectin (μg/ml)	15.0 ± 5.3	7.2–29.8
Leptin (ng/ml)	14.8 ± 7.0	3.6–28.9
IGF-I (ng/ml)	108.2 ± 29.2	53.7–212.0
Insulin (μIU/ml)	7.4 ± 4.4	2.0–22.2
Glucose (mmol/l)	5.1 ± 0.4	4.1–6.2
HOMA	1.80 ± 1.34	0.36–9.12

BMI, body mass index; WHR, waist-to-hip ratio; WTR, waist-to-thigh ratio; FM, fat mass; FFM, fat-free mass; BMC, bone mineral mass; BMD, bone mineral density; IGF-1, insulin-like growth factor-1; HOMA, homeostasis model assessment

Table 2. Correlations between bone mineral values with body compositional and blood biochemical values ($n = 88$)

Parameter	Total BMC	Total BMD	Lumbar spine BMD	Femoral neck BMD
Height (cm)	0.340*	0.259*	0.285*	0.193
Body mass (kg)	0.706*	0.608*	0.428*	0.475*
BMI (kg/m^2)	0.628*	0.597*	0.381*	0.449*
WHR	0.247*	0.327*	0.191	0.288*
WTR	0.091	0.187	0.157	0.157
FM (kg)	0.676*	0.535*	0.464*	0.467*
FFM (kg)	0.538*	0.395*	0.276*	0.311*
Ghrelin (pg/ml)	–0.210*	–0.102	–0.221*	–0.175
Adiponectin (μg/ml)	–0.032	–0.038	–0.210*	–0.061
Leptin (ng/ml)	0.264*	0.211*	0.017	0.225*
IGF-I (ng/ml)	0.011	0.021	0.085	0.062
Insulin (μIU/ml)	0.172	0.216*	0.131	0.286*
Glucose (mmol/l)	0.179	0.169	0.132	0.124
HOMA	0.210*	0.233*	0.173	0.275*

*Statistically significant, $P < 0.05$

Table 3. Predictive models explaining the variance in bone mineral measures ($n = 88$)

Variables[a]	β coefficient ± SE	*P* value
Total BMC ($R^2 = 0.58$)		
FM	0.025 ± 0.004	<0.0001
FFM	0.039 ± 0.009	<0.0001
Ghrelin	–0.001 ± 0.001	0.043
Total BMD ($R^2 = 0.35$)		
FM	0.010 ± 0.002	<0.0001
FFM	0.005 ± 0.003	0.047
Leptin	0.001 ± 0.002	0.050
Lumbar spine BMD ($R^2 = 0.30$)		
FM	0.010 ± 0.002	<0.0001
Adiponectin	–0.006 ± 0.003	0.040
Ghrelin	–0.001 ± 0.001	0.045
Femoral neck BMD ($R^2 = 0.22$)		
FM	0.005 ± 0.001	0.0001
FFM	0.006 ± 0.003	0.043
Leptin	0.002 ± 0.002	0.046

[a]Variables tested in model: BMI, WHR, WTR, FM, FFM, ghrelin, adiponectin, leptin, IGF-I, insulin, HOMA

0.0001), and femoral neck BMD (β = 0.906; $P = 0.0001$) were found. These associations remained significant ($P < 0.010$) after adjusting for ghrelin, adiponectin, IGF-I, insulin, glucose, HOMA, and BMI, but not when adjusting for WHR, WTR, FM, and FFM ($P > 0.145$) values.

Table 3 presents the backward multiple linear regression models fitted to data for total BMC, total BMD, lumbar spine BMD, and femoral neck BMD. Several independent variables including BMI, WHR, WTR, FM, FFM, ghrelin, adiponectin, leptin, IGF-I, insulin, and HOMA were entered into regression models. In the model with total BMC as the dependent variable, the independent variables that were significantly associated with total BMC in the multivariate analysis were FM, FFM, and ghrelin, explaining 58% ($R^2 \times 100$) of the total variance. In another model, FM, FFM, and leptin were the independent variables that explained 35% ($P < 0.05$) of the total variance in the total BMD value. The variables in the multiple regression model that were associated with lumbar spine BMD were FM, adiponectin, and ghrelin ($R^2 = 0.30$; $P < 0.05$), while the independent variables were FM, FFM, and leptin in the model with femoral neck BMD as the dependent variable, and accounted for 22% ($P < 0.05$) of the femoral neck BMD variance.

Discussion

In a relatively homogeneous group of healthy postmenopausal women, plasma ghrelin concentration was associated with total BMC, and total and areal BMD values, and remained associated in different analyses that controlled for measured body composition, and hormonal and insulin resistance values. However, the association between ghrelin and measured bone mineral values was no longer significant when adjusted for body fat distribution values (i.e., WHR, WTR). Similarly, plasma adiponectin concentration was significantly associated with BMC, and total and areal BMDs, and remained associated in different analyses that controlled for measured body fat, hormonal, and insulin resistance values but not when controlled for FFM. In addition, plasma leptin concentration was significantly related to BMC, and total and areal BMD values independent of the influences of measured hormonal and insulin resistance values. However, the associations between leptin and measured bone mineral values were no longer significant when adjusted for specific body composition values (i.e., WHR, WTR, FM, and FFM). The findings of the present investigation suggest that the influence of ghrelin, adiponectin, and leptin on bone tissue is mediated or confounded by the specific body composition values in this group of healthy postmenopausal women.

It has been suggested that the influence of FM on bone tissue may be mediated by hormonal factors, the primary candidate being plasma leptin concentration [9,11,32,33]. In contrast, other studies have failed to find such a relationship [14,29,34,35]. In our study, plasma leptin concentration demonstrated significant associations with measured BMC and BMD values in healthy postmenopausal women. However, the associations between leptin and bone mineral values were no longer significant when adjusted for specific body composition (i.e., FM and FFM) and body fat distribution (i.e., WHR, WTR) values. In addition, leptin together with FM and FFM values characterized 35% and 22% of the total variance in total and femoral neck BMD values, respectively (see Table 3). This finding suggests that the influence of leptin on bone tissue is mediated by specific body composition values in healthy postmenopausal women. Similar results with regard to the relationship between leptin and total BMD have also been observed in other studies, when adjusted for FM and/or BMI values in postmenopausal women [9,14]. In addition, FFM was also one independent variable to characterize leptin and BMD relationship in studied healthy postmenopausal women. A low amount of FFM can be assumed to be associated with increased bone loss and the development of osteoporosis in postmenopausal women [36]. In contrast, a positive independent effect of leptin on BMD of the growing skeleton has been observed [30]. Taken together, leptin has a role in bone growth and development, but its exact role on bone metabolism in postmenopausal women remains unclear.

Few studies have examined the association between plasma adiponectin concentration and bone mineral values in middle-aged and older adults [10,11,19,20,29,37]. Some authors have found an inverse association between adiponectin and BMD [10,20,29], while others have failed to find such a relationship [11,19]. In the present study, adiponectin showed a significant association with measured BMC and BMD values. However, the relationship between adiponectin concentration and measured bone values was controlled by the FFM in healthy postmenopausal women of the present study. In addition, FM, adiponectin, and ghrelin together characterized 30% of the total variance in the lumbar spine BMD value (see Table 3). In support of our findings, recently Lee et al. [38] found that single nucleotide T45G polymorphism in exon 2 of the adiponectin gene was associated with lumbar spine BMD in Korean women. In addition, it has been reported that adiponectin and its

receptors are expressed in bone-forming cells [39–41]. Taken together, these results support the hypothesis that adiponectin may play a role in regional BMD in a specific group of healthy postmenopausal women.

To our knowledge, no study has yet reported an association between plasma ghrelin and BMD values in healthy middle-aged and older adults [19,42]. Only one study has demonstrated an independent effect of ghrelin on BMD in healthy adolescent girls [21]. In our study, total ghrelin concentration was associated with the measured BMC and BMD values in healthy postmenopausal women. However, by adjusting the data for markers of central obesity (i.e., WHR, WTR), the association between plasma ghrelin and bone mineral values was lost. In addition, ghrelin together with FM and FFM characterized 58% of the total variance in total body BMC value (see Table 3). This finding indicates that the influence of ghrelin on BMC and BMD is mediated by specific body composition values in studied women. Accordingly, the results of present study suggest that ghrelin may explain some of the protective effect of the adipose tissue on the skeleton. In support to this, a role for ghrelin in adipogenesis has been found [43], and ghrelin mRNA is expressed in cartilage [44], while in vitro studies have demonstrated that ghrelin administration increased osteoblast proliferation in cell cultures and increases the levels of bone formation markers [45,46].

In summary, the results of present investigation demonstrate a complex interaction of specific body composition parameters with leptin, adiponectin, and ghrelin concentrations in healthy postmenopausal women. It appears that fasting leptin, adiponectin, and ghrelin concentrations have no independent effect on BMC and BMD values. If there is an important relationship of measured adipocytokines and ghrelin with bone mineral values in healthy postmenopausal women, it is mediated or confounded by specific body composition parameters. However, the cross-sectional nature of our study limits determinations of temporality and causality. In addition, the selection of a healthy population is not representative of the general population of the same age. Therefore, further interventional studies are necessary to clarify the exact role of these adipocytokines and ghrelin in the regulatory specificities of bone and mineral metabolism.

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