CLINICAL STUDY

Adipocytokine and ghrelin levels in relation to bone mineral density in physically active older women: longitudinal associations

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Abstract

Purpose: We investigated the relationship between the decrease in bone mineral mass (BMC) and bone mineral density (BMD) values with baseline adipocytokine and ghrelin concentrations in physically active postmenopausal women.

Methods: Leptin, adiponectin, ghrelin, BMC, BMD and different body composition values were measured in 35 women (age: 69.7 ± 6.0 years) before and after a 12-month prospective study period. **Results:** Significant ($P < 0.05$) decreases in fat-free mass (FFM) (by 2.56%) and BMC (by 1.63%) and increases in adiponectin (by 14.8%) were seen in older females as a result of the study period. The independent variables that were associated with decreases in total BMC were baseline fat mass (FM) and adiponectin explaining 30.6% ($R^2 \times 100$) of the total variance. In another model, baseline FFM and leptin were the independent variables that explained 20.6% ($P < 0.05$) of the total variance in the decreases in total BMD value. The variables that were associated with decreases in femoral neck BMD were FM and leptin ($R^2 = 0.102$; $P < 0.05$), while the independent variables were baseline trunk fat:leg fat ratio and adiponectin in the model with decreases in lumbar spine BMD as the dependent variable, and accounted for 13.1% ($P < 0.05$) of the decreases in BMD variance.

Conclusions: Initial adiponectin concentration together with specific body composition characteristics predicted loss in BMC and lumbar spine BMD values, while initial leptin concentration together with specific body composition parameters determined the loss in total and femoral neck BMD values in physically active older 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) (1–3), and is inversely associated with postmenopausal bone loss and bone turnover (4, 5). It is also well established that appetite and food intake decline with ageing, resulting in body mass loss (6). Furthermore, ageing is characterised with changes in body composition, including a decrease in fat-free mass (FFM) and an increase in fat mass (FM) (6). A number of cross-sectional investigations have established a strong relationship between FM and BMD in women (7–9), especially after menopause (10, 11). The age-related loss of FFM, including BMC, is caused by a reduction in anabolic hormones, physical activity and appetite (6).

The protective effect of FM on bone may be mediated by the peripheral signals of appetite regulation and energy homeostasis (3, 6–8). Among the numerous adipose-modulated biochemical signals that may

explain some of the association between FM and BMD in the elderly are leptin, adiponectin and the gut hormone ghrelin (11). Leptin, the product of *LEP* gene, regulates body mass by suppressing appetite and stimulating energy expenditure (12). Adiponectin is a polypeptide hormone expressed specifically and abundantly in adipose tissue and produced in visceral, s.c. and bone marrow fat depots (13), while ghrelin is secreted primarily by cells in the fundus of the stomach and plays a key role in the stimulation of the hypothalamic appetite centres (14) and in the coordination of feeding behaviour and energy metabolism (15). Accordingly, ghrelin has been reported to increase FM by stimulating appetite and reducing fat use (15). Circulating adiponectin (3, 16) and ghrelin (17) have been shown to increase, while no changes in leptin (3) have been observed with menopause in women.

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 (11). Furthermore, to our knowledge, no

investigations have been conducted to longitudinally examine the possible role of adiponectin and ghrelin with BMC and BMD loss in healthy postmenopausal women. Therefore, we conducted a 12-month prospective study to investigate the relationship between loss in BMC and BMD values with baseline leptin, adiponectin and ghrelin concentrations. In addition, we evaluated different body composition values that are known to affect bone metabolism.

Materials and methods

Subjects

Thirty-five postmenopausal women aged between 60 and 81 years participated in this study. They were taking part in the 60-min gymnastics lessons twice a week and had been doing so for at least the last 5 years. All subjects signed an informed consent that was approved by the Medical Ethics Committee of the University of Tartu, Tartu, Estonia. Prior to study enrolment, volunteers completed medical and physical activity questionnaires. They were excluded from the study, if they reported present or previous conditions that might have interfered with bone metabolism (such as heart disease, long-term corticosteroid use, smoking and alcoholism). At the time of the study period, no participants were receiving treatments such as calcium, vitamin D, calcitonin, bisphosphonates and diuretics, which could influence bone mineral values (2, 7, 18). If the participants had received hormone replacement therapy, they were also excluded from the study.

Experimental design

This study was a 12-month prospective study. All participants were tested twice, at the beginning of the study and 12 months later. At both times, all women were asked to come for two visits to complete the testing. On the first visit, participants had anthropometric parameters measured and a venous blood sample was taken in the morning after a 10-hour 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 depending on the participant's schedule and DXA availability.

Anthropometric, body composition and bone mineral measurements

Height was measured using a Martin metal anthropometer to the nearest 0.1 cm with a standard technique. Body mass was measured with minimal clothing to the nearest 0.05 kg using a medical electronic scale (A&D Instruments, Oxfordshire, UK) and body mass index (BMI) was calculated as body mass

(in kg) divided by height (in m²). Whole body fat, lean and BMC were measured by DXA using the 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 standard participant positioning was used for total body measurements and analysed using the extended analysis option. The standard manufacturer's skeletal landmarks were used to define trunk and leg fat. Body fat distribution was calculated as the ratio of trunk fat (in kg) to leg fat (in kg) (8, 19). BMD was determined as the total body BMD and at the skeletal sites of posterior-anterior spine (L2–L4) and femoral neck (2, 8). Coefficients of variation (CV) for measured FM, FFM, BMC and BMD parameters were less than 2%.

Blood sampling and analysis

A 10 ml blood sample was obtained from the antecubital vein in the morning (0700–0800 h) 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 RIA kit (Linco Research, St Charles, MO, USA). The sensitivity of this kit was 93 pg/ml, and the intra- and inter-assay CV values were <10% and 14.7% respectively. Total adiponectin concentration was assessed in duplicate using a commercially available RIA kit (cat. no. HADP-61 HK; Linco Research, USA). The intra- and inter-assay CV values were <7%. Leptin concentration was also determined in duplicate by RIA (Mediagnost GmbH, Reutlingen, Germany). This assay has the intra- and inter-assay CV values of less than 5%.

Statistical analysis

Statistical analysis was performed with SPSS 13.0 for Windows (Chicago, IL, USA), and the means ($\pm$ S.D.) were determined. Paired *t*-tests were performed to determine the changes in measured variables over the 12-month study period. The least significant change (LSC) for measured BMD variables was also calculated (20) resulting in an LSC of 3% at the measured sites. Pearson correlation coefficients were computed to explore the relationship between changes in bone mineral values during a 12-month study period with baseline body composition and blood biochemical variables. Backward regression elimination procedures were also used to evaluate potential associations of a decrease in bone mineral values with several baseline-independent variables. Significance was set at $P < 0.05$.

Results

The mean ($\pm$ S.D.) of measured characteristics for studied older females (age: 69.7 ± 6.0 years) before

Table 1 Mean ($\pm$ s.d.) of subject characteristics before and after a 12-month study period ($n=35$).

Variables	Before	After	P value
Height (cm)	160.0 $\pm$ 5.6	159.8 $\pm$ 5.7	0.081
Body mass (kg)	67.6 $\pm$ 9.0	67.2 $\pm$ 8.9	0.623
BMI (kg/m ²)	26.3 $\pm$ 3.7	26.0 $\pm$ 3.7	0.133
Percentage of FM	35.9 $\pm$ 7.0	35.9 $\pm$ 6.1	0.971
FM (kg)	24.1 $\pm$ 7.1	24.3 $\pm$ 6.6	0.625
Trunk fat (kg)	11.2 $\pm$ 3.2	11.3 $\pm$ 3.1	0.170
Trunk fat:leg fat ratio	1.61 $\pm$ 0.28	1.62 $\pm$ 0.27	0.146
FFM (kg)	43.0 $\pm$ 3.3	41.9 $\pm$ 3.2	0.001
Total body BMC (kg)	2.5 $\pm$ 0.4	2.4 $\pm$ 0.4	0.001
Total body BMD (g/cm ²)	1.075 $\pm$ 0.087	1.067 $\pm$ 0.089	0.312
Femoral neck BMD (g/cm ²)	1.094 $\pm$ 0.096	1.088 $\pm$ 0.096	0.326
Lumbar spine BMD (g/cm ²)	1.001 $\pm$ 0.150	0.992 $\pm$ 0.151	0.337
Leptin (ng/ml)	11.55 $\pm$ 6.00	10.69 $\pm$ 4.66	0.297
Adiponectin (μ g/ml)	16.17 $\pm$ 6.01	18.56 $\pm$ 7.17	0.034
Ghrelin (pg/ml)	1035.4 $\pm$ 426.4	1028.0 $\pm$ 377.7	0.890

BMI, body mass index; FM, fat mass; FFM, fat-free mass; BMC, bone mineral content and BMD, bone mineral density.

and after the 12-month study period are presented in Table 1. Significant ($P<0.05$) decreases in FFM (by 2.56%) and BMC (by 1.63%) and increases in adiponectin (by 14.8%) were seen as a result of the 12-month study period. In addition, a non-significant ($P>0.05$) decrease in total BMD (by 0.93%), femoral neck BMD (by 0.45%) and lumbar spine BMD (by 1.01%) occurred during the 1-year study period. The decreases in measured BMD values were smaller with respect to the calculated LSC of 3%.

Table 2 Pearson correlation coefficients of change (Δ scores) in bone mineral values during a 12-month study period with baseline body composition and blood biochemical variables ($n=35$).

Variables	Δ Total body BMC	Δ Total body BMD	Δ Femoral neck BMD	Δ Lumbar spine BMD
Height (cm)	-0.144	-0.171	-0.189	-0.152
Body mass (kg)	-0.231	-0.117	-0.150	-0.225
BMI (kg/m ²)	-0.195	-0.081	-0.137	-0.150
FM (kg)	-0.359*	-0.213	-0.337*	-0.207
Trunk fat (kg)	-0.375*	-0.119	-0.143	-0.213
Trunk fat: leg fat ratio	-0.259	-0.159	-0.207	-0.337*
FFM (kg)	-0.166	-0.324	-0.141	-0.208
Leptin (ng/ml)	-0.244	-0.342*	-0.316	-0.154
Adiponectin (μ g/ml)	0.348*	0.095	0.140	0.391*
Ghrelin (pg/ml)	0.243	0.186	0.147	0.163

BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; FM, fat mass and FFM, fat-free mass.

*Statistically significant; $P<0.05$.

Table 3 Predictive models with baseline variables explaining the variance in changes in bone mineral measures (Δ scores) over a 12-month study period.

Variables	B coefficient $\pm$ S.E.M.	P value
Δ Total BMC ($R^2=0.306$)		
FM	0.004 $\pm$ 0.001	0.015
Adiponectin	-0.003 $\pm$ 0.002	0.034
Δ Total BMD ($R^2=0.206$)		
FFM	0.004 $\pm$ 0.001	0.011
Leptin	0.001 $\pm$ 0.001	0.045
Δ Femoral neck BMD ($R^2=0.102$)		
FM	0.001 $\pm$ 0.002	0.043
Leptin	0.001 $\pm$ 0.001	0.049
Δ Lumbar spine BMD ($R^2=0.131$)		
Trunk fat: leg fat ratio	0.029 $\pm$ 0.013	0.037
Adiponectin	-0.002 $\pm$ 0.001	0.044

BMC, bone mineral content; FM, fat mass; BMD, bone mineral density and FFM, fat-free mass.

Changes in total BMC were significantly related to baseline FM, trunk fat and adiponectin values, while changes in total BMD correlated negatively with baseline leptin value (Table 2). Changes in femoral neck BMD were correlated with baseline FM, while changes in lumbar spine BMD were related to baseline trunk fat:leg fat ratio and adiponectin parameters.

Backward multiple linear regression analysis revealed that the independent variables that were significantly associated with decreases in total BMC in the multivariate analysis were baseline FM and adiponectin explaining 30.6% ($R^2 \times 100$) of the total variance (Table 3). In another model, baseline FFM and leptin were the independent variables that explained 20.6% ($P<0.05$) of the total variance in the decreases in total BMD value. The variables in the multiple regression model, which were associated with decreases in femoral neck BMD, were FM and leptin ($R^2=0.102$; $P<0.05$), while the independent variables were baseline trunk fat:leg fat ratio and adiponectin in the model with decreases in lumbar spine BMD as the dependent variable, and accounted for 13.1% ($P<0.05$) of the decreases in lumbar spine BMD variance.

Discussion

Different body composition and hormonal factors may predict a decrease in bone mineral values in healthy physically active older women. Our 12-month prospective study demonstrated significant decreases in FFM and BMC values and increases in adiponectin concentrations. However, decreases in total and areal BMD values were not significant and were smaller with respect to the calculated LSC (i.e. 20). It appeared that initial adiponectin concentration together with specific body composition characteristics was a significant predictor of a decrease in BMC and lumbar spine BMD values as a result of the 12-month observation period.

While initial leptin concentration together with specific body composition parameters determined a decrease in total and femoral neck BMD values.

The results of the present study confirm the well-established influence of leptin on bone tissue reported in postmenopausal women (3, 21, 22). The decrease in total BMD as a result of the 1-year study period was related to basal leptin concentration ($r = -0.342$; $P < 0.05$). Moreover, leptin together with FFM characterised 20.6% of the total variance in the loss of total BMD value, while leptin together with FM characterised 10.2% of the total variance in the loss of femoral neck BMD value in elderly women. Therefore, a low amount of FFM can be assumed to be associated with increased BMD loss and the development of osteoporosis in postmenopausal women (23). Similarly, FFM was also an independent variable to characterise leptin and BMD relationship in our previous study with healthy older women (11). These results together indicate that leptin exerts a positive effect on the protection of BMD loss in healthy physically active older women. However, it appears that there is no influence of leptin on BMD without specific body composition values in postmenopausal women (11, 21, 24).

To our knowledge, this is the first study which has reported that a decrease in total BMC (by 1.63%; $P < 0.05$) and lumbar spine BMD (by 1.01%; $P > 0.05$) was associated with baseline adiponectin concentration in healthy older women during a 12-month prospective study period. In addition, adiponectin and FM together characterised 30.6% of the total variance of the loss in total BMC value, while adiponectin together with trunk fat:leg fat ratio characterised 13.1% of the total variance of the loss in lumbar spine BMD value. These results demonstrate that negative changes in bone mineral parameters that occur during healthy ageing in older postmenopausal women are determined, at least in part, by initial adiponectin concentration. In support of our findings, the association between adiponectin concentration and measured bone mineral values has been reported in women (3, 11, 25). However, the relationship between adiponectin and measured bone mineral values was controlled by the amount of FFM in healthy older women (11), while an independent effect of plasma adiponectin levels on BMD has been reported in middle-aged premenopausal women (8). Adiponectin and its receptors are expressed in human osteoblast-like cells (26) and single-nucleotide T45G polymorphism in exon 2 of the adiponectin gene has been found to be associated with lumbar spine BMD in women (27). In addition, a recent study demonstrated that adiponectin was related to bone alkaline phosphatase in elderly men (28). These results together support the hypothesis that adiponectin plays an important role in the maintenance of total BMC and regional BMD in healthy physically active older women.

Similar to the results of the previous studies with older adults (29), circulating ghrelin concentration was

not associated with changes in any measured bone mineral values as a result of the 1-year study period in healthy physically active postmenopausal women. This suggests that ghrelin concentration in blood is not an important parameter in predicting the loss of bone mineral values in ageing women. By contrast, previous studies have demonstrated that ghrelin may, in part, mediate some of the protective effect of the adipose tissue on the skeleton in older men (28) and women (11). However, by adjusting the data for markers of central obesity, the association between plasma ghrelin and bone mineral values was lost in older women (11). In addition, *in vitro* study by Maccarinelli *et al.* (30) showed that ghrelin administration increased osteoblast proliferation in cell cultures and increases the levels of bone formation markers. These results demonstrate that ghrelin may play some role in total BMC and regional BMD, but does not appear to be a determinant in the loss of bone mineral values during ageing in healthy older women.

The results of the present investigation demonstrated that adiponectin concentration was significantly increased in the elderly physically active healthy women after the 12-month prospective study. This is in accordance with the previous studies that have demonstrated that adiponectin concentration is positively related to age in pre- and postmenopausal women (3, 16). It has previously been suggested that the significant relationship of adiponectin with age is the result of the changes in body composition (16, 31). Indeed, significant decreases in FFM and BMC values were seen in our physically active older women during the 1-year study period.

In conclusion, the results of the present study show a complex interaction of specific body composition parameters with leptin, adiponectin and ghrelin. The decrease in total BMC and lumbar spine BMD is characterised by initial adiponectin and specific body composition parameters, while the decrease in total and femoral neck BMD is characterised by leptin and specific body composition parameters. In addition, FFM appears to be an important determinant of decrease in total BMD in healthy physically active women. However, the selection of a healthy population and a relatively small number of subjects are 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.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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