Relation between adiponectin and bone mineral density in elderly post-menopausal women: Role of body composition, leptin, insulin resistance, and dehydroepiandrosterone sulfate

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ABSTRACT. ***Introduction:*** **Adipocytokines have been proposed as new mediators of the protective effects of fat mass on the skeleton. The aim of this study was to test the relationship between adiponectin, leptin, and bone mineral density (BMD), independently of body composition, insulin resistance, and other factors known to affect bone metabolism.** ***Methods:*** **Thirty-six post-menopausal non-diabetic elderly women, with ages ranging from 66 to 77 yr took part in the study. In all subjects we evaluated body weight, height, body mass index (BMI), waist circumference, adiponectin, leptin, insulin, DHEAS, and homeostasis model assessment of insulin resistance (HOMA), as well as yr since menopause. Total body fat mass (FM) and BMD at whole body and femoral level were measured with Dual energy X-ray Absorptiometry (DXA). Volumetric BMD was defined as the ratio between total body BMD and height.** ***Results:*** **Leptin was positively and adiponectin negatively related with whole body and femoral BMD. Positive associations between insulin, HOMA, DHEAS, and BMD measures were also found. After adjusting for FM, only adiponectin maintained a significant relation with whole body and femoral BMD; the strength of this association was reduced after adjustment for insulin resistance, estimated by HOMA. In stepwise multiple linear regression analyses adiponectin explained 11.7% of total BMD variance, 17.4% of femoral neck BMD variance, and 30.7% of volumetric BMD variance, independently of BMI, FM, leptin, HOMA, and DHEAS.** ***Conclusions:*** **The present study may suggest possible involvement of adiponectin in bone metabolism, independently of FM and insulin resistance even in elderly post-menopausal women.**
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INTRODUCTION

Adipocytokines have been proposed as possible factors mediating the protective effects of fat mass on the skeleton (1, 2).

A functional role in bone homeostasis has lately been suggested for adiponectin (3-6), a recently discovered adipocytokine, specifically and highly expressed in adipose tissue and abundantly secreted into the bloodstream (7). Adiponectin receptors, adipoR1 and adipoR2 are expressed in human osteoblasts (3, 5, 6) as well as in osteoclasts (4, 6). Small amounts of adiponectin mRNA are detectable in human femur and tibia, and human osteoblasts in primary culture secrete this adipocytokine (3). Adiponectin appears to stimulate the receptor activator of nuclear factor-kB ligand (RANKL) pathway and to inhibit production of osteoprotegerin in human osteoblasts, thus indirectly increasing osteoclastogenesis (8). However, the effects of adiponectin on bone metabolism differ among experimental systems and *in vivo* or *in vitro* studies (4-6).

Lenchik et al. (9) first reported a negative relation between adiponectin levels and bone mineral density

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(BMD) measured at different sites, in a population of 80 diabetic and non-diabetic men and women with a wide range of age and body mass index (BMI), after adjustment for multiple confounders. Seemingly, a negative association of adiponectin with total BMD was found in healthy pre-menopausal (10) and peri-menopausal women, even after adjusting for body composition, leptin, and insulin (11). Recently, the role of adiponectin in bone metabolism has received further support by data from a large population-based cohort of 1735 non-diabetic women with a wide range of age and BMI (12). In this study, increasing levels of adiponectin were associated with a significant decrease in BMD, even at non-load bearing sites, after adjustment for total body fat mass (FM) (12). This association disappeared when considering the group of pre-menopausal women only (12), in accordance with other studies conducted in non-diabetic female adolescents (13), in smaller groups of peri-menopausal women (14) or in non-diabetic middle-aged men (15). Thus, it seems likely that the relationship between adiponectin and bone in humans should be analyzed, taking into account several potential confounders and, in particular, leptin and insulin resistance.

Leptin has been investigated for its metabolic effects on bone, with both a peripheral and positive action on bone metabolism as well as a central and negative effect (16-18). Leptin receptors have been identified in human osteoblasts, and leptin has been shown to stimulate osteoblastic cell differentiation and to inhibit osteoclast generation (16, 17). In contrast, leptin-deficient (ob/ob) mice present a phenotype characterized by increased bone mass, and the intracerebroventricular infusion of leptin decreased bone formation rate in ob/ob mice as well as in wild-type animals (18). Moreover, conflicting results have also been reported about the relationship between leptin levels and bone mass in humans (2, 16, 17, 19, 20).

The present study was addressed to simultaneously examine the interrelationships between adipocytokines and BMD in a sample of healthy post-menopausal elderly women after controlling for body composition, insulin resistance, and hormones known to affect bone metabolism.

MATERIALS AND METHODS

Thirty-six elderly non-diabetic women, with ages ranging from 66 to 77 yr, took part in the study. All subjects underwent a careful clinical assessment before the study, and were considered eligible if they were free from drugs or any disease known to affect bone metabolism. We excluded patients with a previous diagnosis of diabetes and osteoporosis as well as women with renal or hepatic insufficiency, congestive heart disease and lung disease. All subjects had been weight-stable in the previous 6 months; BMI ranged from 19.9 to 37.2 kg/m^2. None of the subjects engaged in regular physical exercise. Only 8.3% of patients were current smokers, whereas 77.8% and 13.9% of women were respectively never and past smokers. All women were post-menopausal (yr since menopause: 22.7±5.9, mean±SD) and were not under hormonal replacement therapy.

Characteristics of the study sample are summarized in Table 1. All participants gave their informed consent, and the experimental protocol was approved by the Ethics Committee of our University.

Anthropometry

With the subjects wearing light indoor clothes and no shoes, body weight was measured to the nearest 0.1 kg (Salus scale, Milan, Italy), and height to the nearest 0.5 cm using a stadiometer (Salus stadiometer, Milan, Italy). BMI was calculated as body weight adjusted by stature squared (kg/m^2). Waist circumference was obtained with a measuring tape as the narrowest circumference between the xyphoid process and the umbilicus.

Dual energy x-ray absorptiometry

BMD (g/cm^2) was determined at the whole body and femoral level with Dual energy x-ray absorptiometry (DXA) (Hologic QDR 2000, Waltham, USA) array beam with software version 7.2. Volumetric BMD was defined as the ratio between total body BMD and height (BMD/h). Total body FM was also determined. Characteristics and physics concepts of DXA measurement have been described elsewhere (21). Daily quality-assurance tests were performed according to the manufacturer's direction. All the scans were subsequently analyzed by a single trained investigator. Coefficient of variation (CV) for double determination in 11

Table 1 - *Characteristics of the study population (no.= 36 women).*

	Mean±SD	Range
Age (yr)	70.83±2.65	66-77
Leptin (ng/ml)	18.63±15.99	2.80-92.00
Adiponectin (μg/ml)	16.90±5.50	4.43-31.00
Insulin (μU/ml)	9.71±4.91	1.76-21.41
HOMA	2.32±1.28	0.39-5.28
DHEAS (μg/dl)	120.19±73.27	19.70-366.00
Weight (kg)	65.44±12.06	49.30-94.00
BMI (kg/m^2)	27.68±4.80	19.85-37.18
Waist circumference (cm)	85.74±10.98	69-112
FM (kg)[a]	27.84±8.62	15.39-50.21
BMD whole body (g/cm^2)[a]	0.90±0.09	0.78-1.13
BMD whole body/height [(g/cm^2)/m][a]	0.57±0.06	0.48-0.71
BMD hip (g/cm^2)[a]	0.74±0.14	0.54-1.09
BMD femoral neck (g/cm^2)[a]	0.65±0.11	0.47-0.96

HOMA: homeostasis model assessment of insulin resistance; BMI: body mass index; FM: fat mass; BMD: bone mineral density. [a]Measured by Dual energy x-ray absorptiometry.

women (with ages ranging from 68 to 75 yr) was 1% for FM, 1.2% for whole body and 1% for total hip BMD respectively.

Biochemical measures

A blood sample was obtained from each participant after an overnight fast. Plasma leptin and serum adiponectin were measured using commercially available radioimmunoassay kits (Linco Research, Inc., St. Charles, MO). Sensitivity was 0.1 ng/l for leptin and 1 ng/ml for adiponectin; intra-assay and inter-assay CV were respectively 0.7% and 7.8% for leptin and 3.9% and 8.5% for adiponectin. Plasma immuno-reactive insulin underwent duplicate measurements by double antibody radioimmunoassay using a commercial kit (Diagnostic Products Corp., Los Angeles, CA). Sensitivity was 6 pmol/l and the intra-assay CV 4.9%. Insulin resistance was estimated by the homeostasis model assessment (HOMA) method (22). DHEAS was measured as previously described (23).

Statistical analysis

Mean±SD as well as range values were provided to describe the main characteristics of the study population. Leptin and DHEAS concentrations were log-transformed in order to normalize data before analyses. Pearson and partial correlations were used to test associations between variables. Stepwise multiple regression analysis models were used to test the effects of independent variables on total body, volumetric and femoral neck BMD. Only variables with significant associations with BMD at univariate analysis were included in the models; age, smoking status, and years since menopause did not show any significant relation with measures of BMD in this group of elderly women.
A significant level of 0.05 was used throughout the study. All statistical analyses were performed using the statistical Package for the Social Science (24).

RESULTS

Table 2 shows correlation matrix for the main metabolic and body composition variables. In this group of elderly women BMI, waist circumference, and FM were all positively and strongly related to BMD. Age was not significantly associated with BMD, measured at the whole body as well at the femoral level. Leptin levels displayed positive correlations with BMD measured at the whole body and at the femoral level. A similar pattern of relation was also found for insulin and HOMA, whereas adiponectin was negatively associated with whole body and femoral BMD. Leptin and adiponectin were not significantly related each other. DHEAS was negatively correlated with adiponectin levels and positively associated with femoral BMD.
After adjustment for total body FM, leptin, insulin, HOMA, and DHEAS did not maintain a significant relation with BMD parameters (data not shown in tables). By contrast, after taking into account the amount of total body FM, adiponectin still showed significant negative correlations with BMD values (Table 3). The strength of the association between adiponectin, whole body and femoral BMD was reduced only after adjustment for insulin resistance as estimated by HOMA (Table 3).
Stepwise multiple regression analysis was used to test the effects of independent variables on total body, volumetric, and femoral neck BMD (Table 4). Adiponectin explained 11.7% of total BMD vari-

Table 2 - *Correlation matrix for metabolic and body composition variables (no.= 36 women).*

	Leptin	Adiponect.	Insul.	HOMA	DHEAS	BMI	Waist	FM	BMD tot	BMD/h	BMD hip	BMD fem neck
Leptin	1											
Adiponect.	–0.226	1										
Insul.	0.617**	–0.427**	1									
HOMA	0.669**	–0.442**	0.968**	1								
DHEAS	–0.030	–0.402*	0.221	0.250	1							
BMI	0.682**	–0.404*	0.642**	0.697**	0.117	1						
Waist	0.711**	–0.451**	0.634**	0.702**	0.033	0.906**	1					
FM	0.765**	–0.338*	0.565**	0.643**	0.068	0.917**	0.889**	1				
BMD tot	0.415*	–0.523**	0.420*	0.479**	0.235	0.459**	0.596**	0.539**	1			
BMD/h	0.422*	–0.571**	0.458**	0.506**	0.302	0.528**	0.587**	0.524**	0.939**	1		
BMD hip	0.364*	–0.462**	0.475**	0.525**	0.339*	0.484**	0.558**	0.494**	0.892**	0.878**	1	
BMD fem neck	0.242	–0.445**	0.359*	0.384**	0.324	0.369*	0.436**	0.399*	0.874**	0.869**	0.907**	1

Adiponect.: adiponectin; Insul.: insulin; HOMA: homeostasis model assessment of insulin resistance; BMI: body mass index; Waist: waist circumference; FM: fat mass; BMD tot: whole body bone mineral density; BMD/h: whole body bone mineral density height ratio; BMD fem neck: femoral neck bone mineral density. *p<0.05; **p<0.001.

Table 3 - *Adjusted correlations between adiponectin and bone mineral density (BMD) (no.= 36 women).*

	Adiponectin (adjusted for FM)	Adiponectin (adjusted for HOMA)	Adiponectin (adjusted for FM and HOMA)
BMD whole body	–0.424**	–0.380*	–0.380*
BMD whole body/height	–0.492**	–0.449**	–0.448**
BMD hip	–0.359*	–0.293	–0.286
BMD femoral neck	–0.358*	–0.328	–0.322

FM: fat mass; HOMA: homeostasis model assessment of insulin resistance. *$p<0.05$; **$p<0.001$.

ance, 17.4% of femoral neck BMD variance and 30.7% of volumetric BMD variance, independently of BMI, FM, leptin, HOMA, and DHEAS (Table 4).

DISCUSSION

Our study provides clinical evidence of possible involvement of adiponectin in bone metabolism, independently of body composition and insulin resistance, in a group of healthy elderly post-menopausal women. In stepwise multiple regression analysis models adiponectin explained a significant part of total body, volumetric, and femoral neck BMD variance.

Recently published studies seem to point to the existence of a significant negative association between adiponectin and bone metabolism (10, 12). However, it seems difficult to concordantly interpret the findings of clinical and experimental studies and include a new hormone such as adiponectin in the complicated network of factors regulating bone metabolism (25), because of its possible interrelationships with other hormonal factors, besides fat mass, involved in the system.

In a large population of non-diabetic women with a wide range of age and BMI, Richards et al. (12), reported that each doubling of serum adiponectin was associated with a mean 2.7% decrease in BMD at different sites, even after adjustment for potential confounders such as BMI, leptin, central fat mass, hormone replacement therapy, smoking, and exercise. After stratifying for menopausal status, this association between adiponectin and BMD persisted only in post-menopausal women, once FM was checked (12). Our study, investigating the effects of several covariates all together, in particular adiponectin, leptin, insulin resistance, and DHEAS on bone metabolism seem to confirm and expand these recently published results to an older population of post-menopausal non-diabetic women. In fact, to our knowledge this is the first study conducted exclusively in a geriatric population with a narrow age range, across a wide range of body composition parameters. Results obtained in pre- and peri-menopausal women are in conflict with data supporting a significant negative relation between adiponectin and bone (10, 11) as well as other data against any significant association, after adjustments (12-14, 26).

The relationship between adiponectin and bone in humans should be analyzed taking into account several potential confounders and in particular sex hormones. This may at least partly explain the discrepancy of results between studies conducted in pre- and peri-menopausal women and those including post-menopausal women. In fact, estradiol levels, which have been proposed as one of the main confounding factors on the relation between adiponectin and bone density (27), were not measured in any of the studies published on this topic (10-14, 26). Unfortunately, in this study we also did not have the opportunity to directly evaluate estradiol levels, even though we measured DHEAS, the most abundant steroid in human plasma whose biological effects are mainly related to testosterone and estradiol conversion (28, 29). Moreover, in our subjects in a stepwise regression analysis with several independent variables including DHEAS, the strongest predictors of BMD at different sites were FM and adiponectin.

Recently, a new hypothesis regarding the role of adiponectin on bone metabolism has been advanced through experimental *in vivo* and *in vitro* studies (6). Shinoda et al. (6) speculated on the existence of at least 3 distinct actions of adiponectin on bone: a positive action through an autocrine/paracrine pathway, a negative direct endocrine action on bone and finally a positive indirect endocrine action through enhancement of insulin osteogenic signaling. Thus the strength and direction of the correlations between adiponectin and BMD, in different clinical studies (10-14, 26), may be dependent on the balance of the direct and indirect actions of adiponectin which oppositely affect bone metabolism. Thus, the prevalence of diabetes and/or insulin resistance may at least partly explain the discrepancy of results among different studies (10-14, 26).

Table 4 - *Stepwise multiple linear regression analysis where total body bone mineral density (BMD), volumetric BMD or femoral neck BMD were the dependent variable and body mass index, fat mass, leptin, adiponectin, homeostasis model assessment of insulin resistance, and DHEAS the independent variables (no.= 36 women).*

Models	B coefficient±SE	*p*	R^2
1. Total body BMD:			
Fat mass	0.409±0.001	0.006	27.0%
Adiponectin	–0.385±0.001	0.010	38.7%
2. BMD/h:			
Adiponectin	–0.445±0.001	0.003	30.7%
Fat mass	0.374±0.001	0.010	41.7%
3. Femoral neck BMD:			
Adiponectin	–0.445±0.001	0.007	17.4%

BMD/h: whole body BMD-height ratio.

There are several possible mechanisms through which adiponectin may affect bone mass. Adiponectin has been shown to stimulate the RANKL pathway and to inhibit production of osteoprotegerin in human osteoblasts, increasing osteoclastogenesis (8). Moreover, peroxisome proliferator-activated receptor γ agonists, the thiazolidinediones (glitazones), increase the production of adiponectin (7) and were shown to be associated to a shift of progenitor cells from osteoblastogenic to adipogenic in different laboratory systems (30, 31), thus potentially reducing bone formation.

We previously described a small independent effect of leptin on bone metabolism, even though adjustments for confounding hormonal factors were not possible (20). In the present study, leptin appears to be mainly a simple surrogate of adiposity in the relation with BMD, since any significant association disappeared after adjustment for total body FM. However, this result should be interpreted with caution by considering the small sample size of this study and the conflicting results in several previously published papers (2, 16, 17, 19).

In this study, age was not significantly associated with BMD measures. Advancing age is considered one of the main predictors of low bone mass (32). However, the simple correlations between age and BMD evaluated at different sites were not significant in this population, even after adjusting for BMI. In our opinion, this lack of association may be due to the relatively narrow age range (66-77 yr) of this population of elderly women.

Several limitations must be considered in interpreting the results of this study. First of all, the small sample size and the impossibility to extend these observations to a group of men. Second, determinations of free sexual hormones, estradiol, free-testosterone, SHBG, as well as of bone turnover markers would have completed the characterization of the subjects participating in the study. We did, however, check the analyses for menopausal status, use of hormone replacement therapy and obesity, which are the main determinants of estradiol levels in women (33). Finally, cross-sectional design does not make it possible to establish any cause-effect relationship, but only to describe associations.

In conclusion, our data seem to suggest a possible involvement of adiponectin in bone metabolism, independently of FM and insulin resistance, even in elderly post-menopausal women.

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