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Serum Adiponectin and Bone Mineral Density in Women

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Context: Bone mineral density (BMD) is positively associated with body weight. This association persists even at non-load bearing sites, suggesting that a nonmechanical factor such as an adipocyte-derived hormone may modulate BMD.

Objective: The objective of the study was to evaluate the relationship between adiponectin, an adipocyte-derived hormone, and BMD.

Design, Setting, Participants: A total of 1735 nondiabetic women were recruited from a large, population-based cohort (mean age, 50.0 yr). We employed linear regression methods to estimate the relationship between adiponectin and BMD.

Main Outcome Measures: Percentage change in BMD (as measured at total hip, spine, femoral neck, and forearm) and markers of bone turnover associated with a doubling of fasting serum adiponectin levels were measured.

Results: Employing age-adjusted analysis, each doubling of serum adiponectin was associated with a mean 2.7% decrease in BMD [total hip, −3.2% (95% confidence interval, −4.1, −2.3); femoral neck, −3.1% (−4.0, −2.1); forearm, −2.0 (−2.6, −1.4); spine, −2.6 (−3.5, −1.7)]. After adjustment for potential confounding factors, including BMI, serum leptin, central fat mass, hormone replacement therapy, smoking, and exercise, this relationship persisted, although decreased in magnitude. When stratified by menopausal status, the relationship between serum adiponectin and BMD strengthened in postmenopausal women but disappeared in premenopausal women. Serum adiponectin was positively associated with serum osteocalcin but not with urine deoxypyridinoline.

Conclusions: After adjustment of measures of body fat, increasing levels of adiponectin were associated with a decrease in BMD, even at non-load bearing sites. These data suggest that adiponectin, an adipocyte-derived hormone, may play a role in bone metabolism through nonmechanical mechanisms and that this effect may be mediated by menopausal status. **(*J Clin Endocrinol Metab* 92: 1517–1523, 2007)**

OBESITY IS STRONGLY correlated with increased bone mineral density (BMD), and this association may be explained, at least partially, by the mechanical loading effects of increased body weight (1). Consistent with this, increased body weight is associated with reduced risk of fragility fractures (2, 3). However, obesity is also associated with increased BMD at non-load bearing sites (1), leading some to suggest that obesity influences BMD through alternative mechanisms, possibly adipocyte-dependent hormonal factors (4, 5).

Adiponectin is a recently described adipocyte-produced hormone that correlates negatively with obesity in general and central adiposity in particular (6, 7). Low adiponectin levels have also been associated with a increased incidence of progression to type 2 diabetes mellitus (7, 8) and a higher incidence of myocardial infarction in men (9). Adiponectin and its receptors have recently been found to be produced by human bone-forming cells, suggesting that adiponectin may be a hormone linking bone and fat metabolism (10). Furthermore, adiponectin may have deleterious effects on bone because it appears to stimulate the receptor activator of nuclear factor-κB ligand (RANKL) pathway and inhibit production of the naturally occurring decoy receptor for RANKL, osteoprotegerin (11).

The relationship between BMD and body weight is likely to be complex and may be mediated by several factors independent of mechanical stimulation. Increased serum insulin levels may promote bone formation (12), and increased adiposity permits increased peripheral aromatization of androgens to estrogens, which in turn may increase BMD (13). Menopause may influence the relationship of adiponectin to estrogen-dependent malignancies (14, 15). Furthermore, the role in bone metabolism of another adipocyte-dependent hormone, leptin, is unclear because leptin has been shown to be both negatively and positively correlated with BMD in separate studies (16–18).

To our knowledge, the relationship between adiponectin and BMD at multiple sites has not been described in nondiabetic women. The aim of the current study was to describe the relationship between adiponectin and BMD in a large, population-based cohort of nondiabetic women across a wide age spectrum.

Subjects and Methods

Study population

The TwinsUK adult twin registry is an ongoing study investigating a wide range of age-related phenotypes including osteoporosis, obesity, diabetes, visual and cardiovascular disease (www.twinsUK.ac.uk). Female twin pairs from the cohort were invited to participate, and all subjects underwent a clinical questionnaire during the interview. This population was sampled because BMD, fat measures, adiponectin, and various serum and urine markers have been measured as part of a Wellcome Trust-funded cohort to investigate genetic and environmental

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Abbreviations: BMD, Bone mineral density; BMI, body mass index; CI, confidence interval; DPD, deoxypyridinoline; RANKL, receptor activator of nuclear factor-κB ligand.

determinants of diseases such as osteoporosis. General medical, gynecological, and lifestyle questionnaires were completed. The study was approved by the Guy's and St. Thomas' Hospital Ethics Committee, and participants provided written informed consent.

Phenotypic variables

BMD of all subjects had been measured at lumbar spine (L1–4), total hip, femoral neck, and total forearm using dual energy x-ray absorptiometry (QDR 2000W, Hologic, Bedford, MA) as described previously (19). Total fat mass and central fat mass were also measured using dual energy x-ray absorptiometry (QDR 2000W; Hologic) and calculated using standard software (version 710). Central abdominal fat was measured by a blinded investigator and was defined as the abdominal region delineated by the second lumbar to the fourth lumbar vertebrae, and laterally to the inner aspects of the ribs (20). This measure of central fat has been shown to correlate strongly with central fat as measured by computed tomography and insulin resistance (21, 22). The test-retest variability for central fat measurement was 8% (20). Subjects having a fasting glucose greater than 7.0 mmol/liter were excluded from the study. Height was measured using a stadiometer, and weight was recorded while the subjects were wearing light street clothing and no shoes. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters. Physical activity was recorded as inactive, light, moderate, and heavy exercise during leisure time. This previously validated measure of activity correlated well with an in-depth measure of physical activity in the Dunbar Health Survey (23). Fasting serum insulin levels were measured using a chemiluminescent IMMULITE assay provided by Diagnostics Products Corporation (Los Angeles, CA). Fasting morning serum total adiponectin levels were measured with a two-site DELFIA assay using antibodies and standards from R&D Systems (Minneapolis, MN). The day-to-day coefficient of variation (CV) for adiponectin was 9.9% at a concentration of 3.2 ng/ml, 7.8% at 8.5 ng/ml, and 5.2% at 14.7 ng/ml. Serum leptin concentration was determined after an overnight fast using a RIA (Linco Research, St Louis, MO). Urine deoxypyridinoline (DPD), corrected for creatinine, was measured using reversed-phase HPLC as described elsewhere (24). Serum total osteocalcin was measured using a competitive immunoassay (NovoCalcin; Metra Biosystems, Mountain View, CA).

Statistical methods

Standard descriptive statistics were calculated. Normality of variables was assessed, and adiponectin and leptin were log transformed. The relationship between adiponectin, BMD, and markers of bone turnover was assessed using linear regression, whereas the relationship between age and log transformed adiponectin was assessed using a Pearson's correlation coefficient. The relationships between the dependent and independent variables were assessed for nonlinear trends, and fasting serum insulin was log transformed. Covariates considered for the regression analyses were age, serum leptin levels, smoking status, fasting serum insulin levels, BMI, physical activity, central and total fat, menopausal and hormone replacement therapy status. Because of the correlation between multiple measures of adiposity, we checked for evidence for multicollinearity in the regressions analyses. The variance inflation factor for each covariate was assessed, and consequently total fat mass was excluded from further analyses. After removing total fat mass from the regression equation, the variance inflation factor for all variables was less than 3.5. Forward and backward stepwise variable selection with $P < 0.10$ was used as a selection cutoff to determine the final covariates in the regression analysis. Both forward and backward stepwise regression resulted in the same variables being included in the final regression. These variables were: age, smoking status, fasting serum insulin levels, BMI, physical activity, central fat, menopausal and hormone replacement therapy status. The difference in BMD and bone markers associated with a doubling of serum adiponectin levels is expressed throughout as percent difference, derived from the regression coefficients using the formula: ($100\% \times \beta$/mean BMD or mean serum marker levels). To account for the nonindependence of twin pairs, we examined the correlation between adiponectin and dependent variables using the regression cluster option in Stata 9.2. All analyses were carried out using Stata/SE 9.2 (Stata Corp., College Station, TX).

Results

A total of 1,735 women aged 18–81 yr had sufficient data for analysis. The mean age of the study population was 50.0 yr. The Pearson's correlation coefficient between age and log-transformed adiponectin was 0.191 ($P < 0.0001$) (Fig. 1). The majority of women were nonsmokers and postmenopausal. BMI levels were similar to the United Kingdom population normals (Table 1) (25).

Adiponectin levels and BMD

Simple scatter plots, with regression lines and Pearson's correlation coefficients, are displayed demonstrating the re-

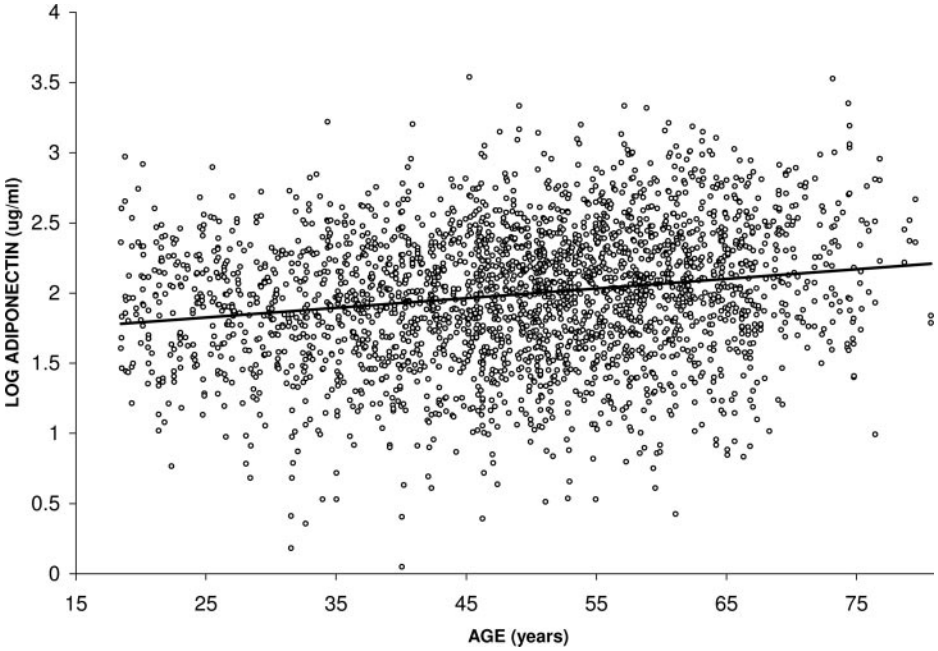

FIG. 1. Relationship between age and adiponectin levels (μg/ml) (n = 2712; Pearson's correlation coefficient = 0.191; $P < 0.0001$).

TABLE 1. Selected characteristics of study population

	n = 1,735
Age (yr)	50.0 (13.0)
Adiponectin (μg/ml)	8.3 (3.9)
Leptin (ng/ml)	17.5 (12.8)
BMI (kg/m^2)	25.5 (4.7)
Total fat mass (g)	23,045.5 (8,532.3)
Central fat mass (g)	1,300.0 (694.6)
Fasting serum insulin level (μU/ml)	7.8 (6.3)
Total hip BMD (g/cm^2)	0.94 (0.1)
Femoral neck BMD (g/cm^2)	0.81 (0.1)
Total forearm BMD (g/cm^2)	0.56 (0.1)
Spine BMD (g/cm^2)	1.00 (0.1)
Osteocalcin (μg·liter)	8.1 (3.8)
DPD (nmol/liter)	5.3 (1.8)
Smoking status	
Nonsmoker	1,304 (75.2)
Smoker	431 (24.8)
Estrogen status	
Premenopausal	699 (40.3)
Postmenopausal and never used HRT	646 (37.2)
Postmenopausal and former HRT user	207 (11.9)
Postmenopausal and current HRT user	183 (10.6)
Physical activity	
Inactive or light	869 (50.1)
Moderate or heavy	866 (49.9)

Data represent mean (SD) or number (percent). HRT, Hormone replacement therapy.

lationship between adiponectin levels and age-adjusted BMD in pre- and postmenopausal women (Fig. 2). Employing age-adjusted analysis, higher adiponectin levels were associated with a decrease in BMD at all anatomic sites in the total population [mean change in BMD associated with a doubling of serum adiponectin, −2.7%; total hip, −3.2% (95% confidence interval [CI], −4.1, −2.3); femoral neck, −3.1% (−4.0, −2.1); forearm, −2.0 (−2.6, −1.4); spine, −2.6 (−3.5, −1.7)]. When the population was stratified by menopausal status, the mean percent change in BMD associated with a doubling of serum adiponectin at all four sites was −2.0% and −2.9% in pre- and postmenopausal women, respectively (Fig. 3A). Among postmenopausal women this association persisted, despite adjustment for covariates including physical activity, serum fasting insulin levels, menopausal status, and use of hormone replacement therapy, but excluding measures of obesity. In premenopausal women the 95% CIs included zero at spine and forearm sites when these covariates were included (Fig. 3B). In addition, the inclusion of measures of obesity (BMI and central fat mass) as covariates changed the results in magnitude only minimally for postmenopausal women (mean percent change in BMD at all four sites: −1.8%) (Fig. 3C). These associations were demonstrated consistently at all sites. Of particular interest, the association was found at forearm BMD, a non-load bearing site. However, when controlling for measures of adiposity in premenopausal women, the relationship between adiponectin and BMD disappeared (Fig. 3C). Interestingly, the change in BMD associated with adiponectin appeared to be correlated with bone mineral content, rather than bone area [the change in bone mineral content at femoral neck associated with a doubling of serum adiponectin levels in postmenopausal women was −2.2% (95% CI, −0.7, −3.8), whereas similar analysis showed no appreciable relationship between adiponectin and bone area: 0.3% (95%CI, −0.4, 0.9)].

Because estrogen replacement therapy may alter the relationship between adiponectin and BMD in postmenopausal users, the regression equations were repeated for postmenopausal women while excluding those subjects who currently or previously used estrogen replacement therapy. In this separate analysis the results did not change in magnitude and remained statistically significant [mean percent change in BMD associated with a doubling of serum adiponectin levels, −2.3%; total hip, −2.4% (95% CI, −3.9, −0.9); femoral neck, −2.4% (95% CI, −4.1, −0.7); forearm, −2.2 (95% CI, −3.4, −1.0); spine, −2.1 (95% CI, −3.7, −0.5)]. To assess whether BMI altered the relationship between BMD and adiponectin, subjects were divided into normal (BMI $<$ 25 kg/m^2), overweight (BMI 25.0–29.9 kg/m^2), and obese (BMI $\geq$ 30 kg/m^2) groupings. Although the 95% CIs largely crossed the null value in this subgroup analysis, the point estimates for each site were similar to those found in the overall population (data not shown).

Adiponectin levels and bone markers

Markers of bone turnover, osteocalcin and DPD, were measured in 1418 and 1208 subjects, respectively. After adjustment for multiple potential confounding variables (age, BMI, central fat mass, insulin levels, and smoking, menopause and HRT status) increased adiponectin levels remained associated with an increase in osteocalcin, a marker of bone formation [percent change in serum osteocalcin associated with a doubling of serum adiponectin, 4.9% (95% CI, 0.9, 8.9)]. However, the association between adiponectin and DPD was not statistically significant [percent change in urinary DPD associated with a doubling of serum adiponectin, 1.2% (95% CI, −2.6, 5.1)].

Serum leptin, insulin, and BMD

Before adjustment for covariates, serum leptin levels were associated with an increase in age-adjusted BMD [mean percent change in BMD associated with a doubling of serum leptin levels, 2.3%; total hip, 2.9% (95% CI, 2.3, 3.5); femoral neck, 2.7% (95% CI, 2.1, 3.4); forearm, 1.2% (95% CI, 0.8, 1.7); spine, 2.2 (95% CI, 1.6, 2.8)]; however, after adjustment for other variables, this relationship disappeared [mean percent change in multiply-adjusted BMD associated with a doubling of serum leptin levels, −0.05%; total hip, −0.1% (95% CI, −1.0, 0.8); femoral neck, −0.2% (95% CI, −1.2, 0.8); forearm, −0.1% (95% CI, −0.8, 0.6); spine, 0.2% (95% CI, −0.9, 1.4)]. Before adjustment for covariates, there was an inconsistent relationship between fasting serum insulin levels and age-adjusted BMD [mean percent change in BMD associated with a doubling of serum leptin levels, 0.9%; total hip,1.7% (95% CI, 0.9, 2.4); femoral neck, 1.3% (95% CI, 0.5, 2.2); forearm; 0.1% (95% CI, −0.5, 0.6); spine, 0.4% (95% CI, −0.4, 1.2)]. When other covariates were included in the analysis, insulin was associated with a multiply-adjusted mean decrease in BMD by 1.2% for each doubling of serum insulin. These results were consistent across all sites: total hip: −0.8% (95% CI, −1.7, 0.0); femoral neck, −1.2% (95% CI, −2.3, −0.3); forearm, −1.1% (95% CI, −1.7, −0.5); and spine, −1.6 (95% CI, −2.5, −0.6).

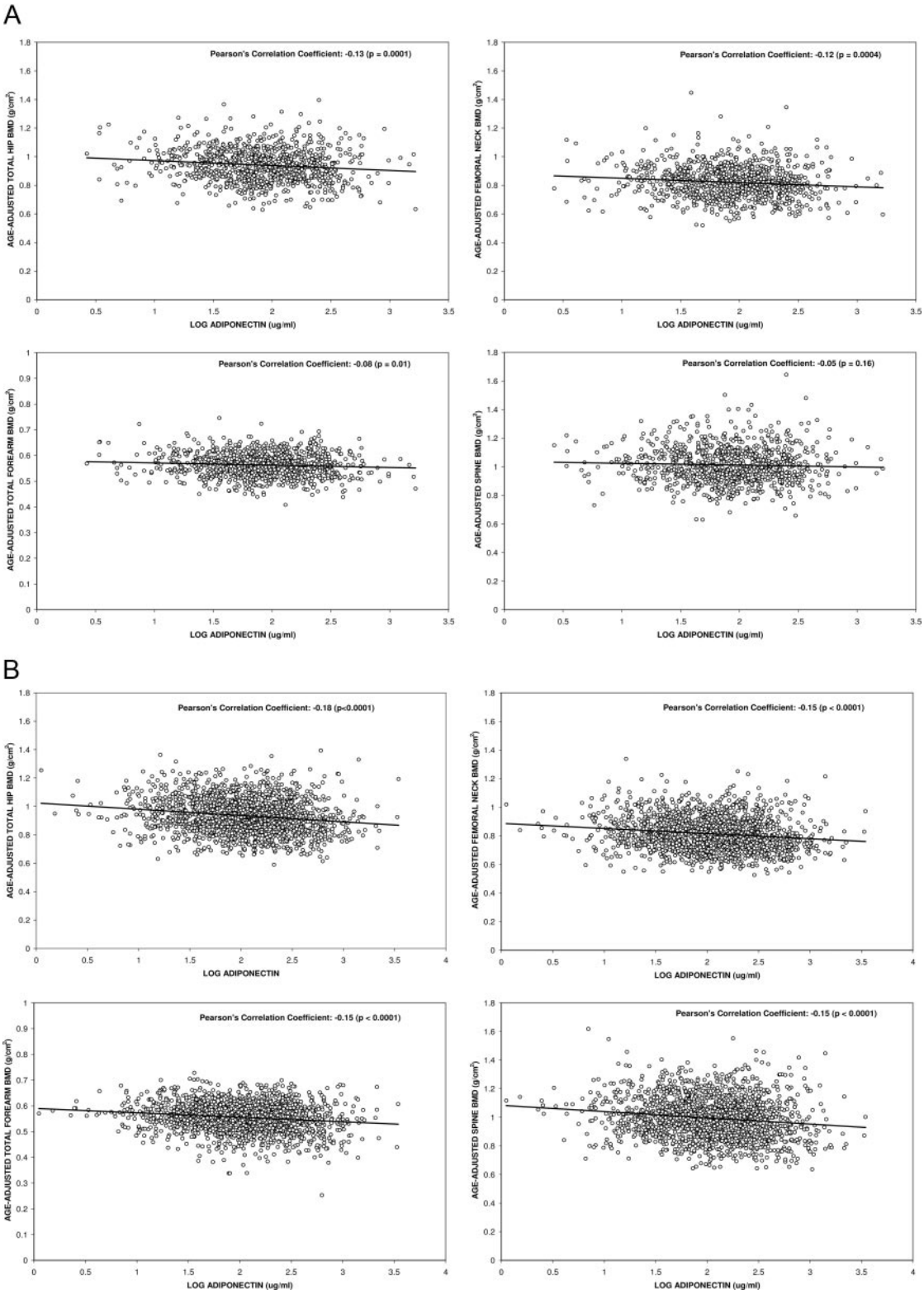

FIG. 2. Relationship between adiponectin levels (μg/ml) and age-adjusted BMD (g/cm^2) in (A) premenopausal and (B) postmenopausal women.

Discussion

In this large population of nondiabetic women, increasing adiponectin levels were associated with a potentially clinically relevant decrease in BMD. This association persisted across multiple anatomical sites, including those not stimulated by mechanical loading, despite adjustment for confounders and measures of adiposity. Taken together, these data suggest that adiponectin may play a role in the observed

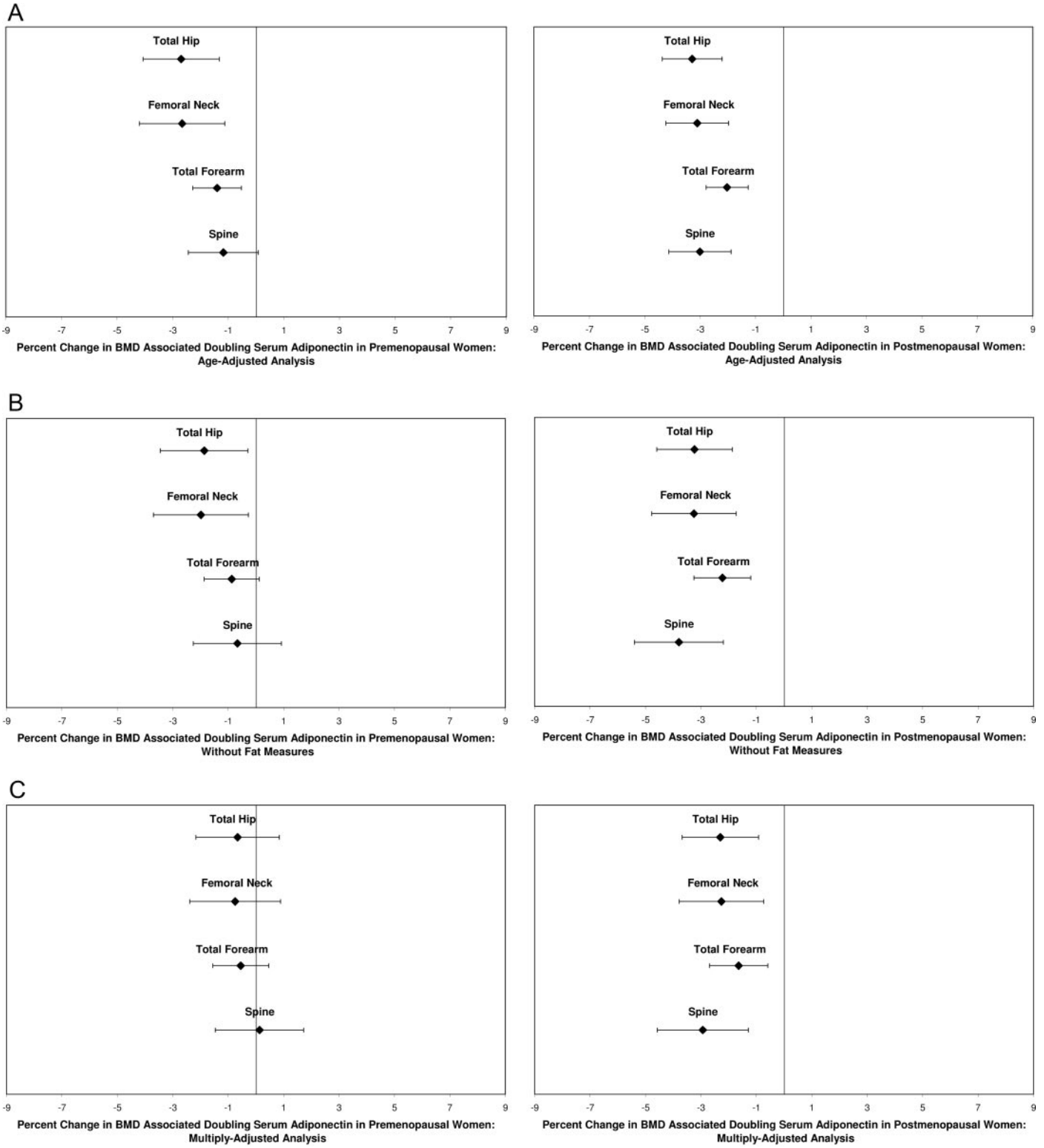

FIG. 3. Percent change in BMD associated with a doubling in serum adiponectin levels (μg/ml) in premenopausal and postmenopausal women. A, Age-adjusted analysis; B, inclusion of all covariates except measures of obesity; C, all covariates and measures of obesity. (*Horizontal error bars* represent 95% CIs.) Fat measures included BMI and central fat mass. Other covariates included age, smoking status, physical activity, hormone replacement therapy status, and serum fasting insulin levels.

relationship between body mass and BMD. This association was clearly found in postmenopausal women rather than premenopausal women, which suggests that sex hormones may influence the relationship. Finally, increased adiponectin levels were associated with an increase in makers of bone formation. These results indicate that any future pharmaco-

logical manipulation of the adiponectin pathway designed to raise adiponectin levels in the treatment of obesity related diseases may have important skeletal effects.

Recent studies have demonstrated the presence of adiponectin receptors and transcription, translation, and secretion of the adiponectin protein in human bone-forming osteoblasts (10). Furthermore, the introduction of recombinant adiponectin to human osteoblasts has been demonstrated to induce osteoblast formation, as well as stimulate the osteoclast RANKL pathway while inhibiting its decoy receptor, osteoprotegerin (11). RANKL is a potent stimulus for bone resorption, and osteoprotegerin has been shown to prevent RANKL-induced bone loss (27). Thus, adiponectin could be exerting its effect on bone metabolism through promotion of the bone-resorbing RANKL pathway.

Our study demonstrated no discernible relationship between adiponectin and BMD in premenopausal women, once measures of adiposity were controlled for, but a strong and persistent relationship was demonstrated in postmenopausal women. Similarly, adiponectin has been demonstrated to have divergent effects on estrogen-dependent cancer incidence in pre- and postmenopausal women. In a cross-sectional study of the association between hormone replacement therapy and adiponectin levels, postmenopausal women using estrogen replacement therapy were found to have lower adiponectin levels than nonusers (28), and estradiol levels have been shown to be negatively correlated with adiponectin levels (29). In postmenopausal women, adipose tissue becomes the main source of estrogen (30), and consequently, women with a low BMI would have both low estrogen and high adiponectin levels. It is of particular interest that the deleterious association between adiponectin and BMD was independent of all measures of adiposity, suggesting that adiponectin may indeed have bone-specific effects, independent of estrogen concentration. Thus, although we were unable to measure the estrogen levels in these subjects, the mechanism through which estrogens mediate the action of adiponectin remains obscure. Our data indicate that this relationship may be of particular importance in bone metabolism.

A recent study examined the relationship between adiponectin and BMD in a sample of 80 predominantly diabetic men and women and found that adiponectin was also negatively associated with BMD (31). This study was somewhat limited by the lack of control group with comparable physical activity or insulin levels, despite the fact that most subjects were obese diabetics and 36% of the study population was treated with insulin therapy. Consistent with our results, a study of 38 perimenopausal women found a negative association between adiponectin levels and total BMD, as well as BMD at lumbar spine, although forearm was not measured (32).

In our population, there was no clear relationship between leptin and BMD after inclusion of relevant confounders. There is controversy in the literature regarding the relationship between leptin and BMD, with studies reporting both positive (16, 33) and negative (5, 34, 35) correlations, whereas still others have found no evidence for a relationship (17, 36). In addition, we found that increased fasting serum insulin levels were associated with a decrease in BMD at all sites. There also exists controversy in the literature regarding the relationship between BMD and insulin in humans (reviewed in Ref. 37), and it is possible that serum insulin levels do not accurately reflect tissue-level effect (38). Previous studies found no relationship between BMD and insulin in men and women, after adjustment for potential confounders (16, 39); however a large population-based study demonstrated a positive relationship between BMD and nonfasting insulin levels (40).

There are several potential strengths and weaknesses inherent in our study. We were unable to measure estradiol levels directly in all of our subjects. However, we have controlled for menopausal status, use of hormone replacement therapy, and obesity which are the main determinants of estradiol levels in women (41). Our study population consisted of twins, which have been shown to be comparable to age-matched population singletons (26), but twins are related, and therefore observations are not strictly independent. We have adjusted for this nonindependence by employing regression techniques which control for familial clustering. We must emphasize the cross-sectional nature of our study, and therefore no inferences of causality can be made. On the other hand, strengths of this study include our large sample size and the ability to report findings at many anatomical sites, stratified by menopausal status while adjusting for many potential confounders including a wide range of adipose-related phenotypes.

In conclusion, in this large population of nondiabetic women, increasing levels of serum adiponectin were associated with a decreased BMD in postmenopausal women, despite controlling for multiple possible confounders, including measures of obesity. Our findings provide evidence that adiponectin may be a hormone linking adiposity to bone metabolism because these results were also found at non-load bearing sites. Finally, given this relationship, it will be incumbent upon researchers attempting to manipulate adiponectin pharmacologically in the treatment of obesity to study carefully the resultant effects on bone metabolism.

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