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High Plasma Leptin Is Not Associated with Higher Bone Mineral Density in Insulin-Resistant Premenopausal Obese Women

Rania Abou Samra, Nahla Hwalla Baba, Naji Torbay, Lea Dib, and Ghada El-Hajj Fuleihan

Department of Nutrition and Food Science (R.A.S., N.H.B., N.T.), Calcium Metabolism and Osteoporosis Program (L.D., G.E.-H.F.), Department of Internal Medicine, American University of Beirut, Beirut, Lebanon 113-6044

Obesity's protective effect on bone density may be mediated through increased muscle mass, fat mass, increased estrogen, and possibly insulin and leptin levels.

To determine the impact of leptin and insulin on bone metabolism, we studied 48 obese normally cycling premenopausal women (age, 31 ± 10 yr; body mass index, 35.7 ± 5 kg/m^2): 28 insulin resistant (IR) and 20 insulin sensitive (IS) by McAuley index. Anthropometric, body composition, and bone mineral density (BMD) measurements were made, and serum leptin, insulin, free testosterone, IGF-I, bone remodeling markers, and calciotropic hormones were measured.

Anthropometric, lifestyle, and biochemical markers were similar in the two groups. Despite higher circulating insulin and leptin levels, IR subjects had similar mean values of serum osteocalcin but higher C-telopeptide (P = 0.052). They had similar BMD at all skeletal sites compared with IS subjects. In the IR group, fat mass but not lean mass, serum leptin, insulin, testosterone, and IGF-I levels correlated positively with hip and/or total-body bone density with R varying between 0.38 and 0.65; no correlations were observed at the spine. Conversely, in the IS group, lean mass, but not fat mass, and only IGF-I correlated with hip BMD/total-body bone mineral content.

In conclusion, there is a dichotomy in the impact of body composition parameters and insulin and leptin levels on bone parameters in obese individuals. The interaction between the fat-related endocrine system and bone seems to be complex and may be modulated by local resistance to the putative protective effect of insulin and leptin on bone. (*J Clin Endocrinol Metab* 90: 2588–2594, 2005)

IN CONTRAST TO THE increasing burden of obesity on health including morbidity from cardiovascular disorders, obesity is associated with higher bone mineral density (BMD) and decreased risk of fracture (1–3). The mechanisms responsible for the higher BMD in obese subjects include muscle-mediated mechanical effects of increased weight bearing (4), increased aromatization of androgen to estrogen in adipose tissue (5), decreased sex-hormone-binding globulin with increased free sex steroids (6), hyperinsulinemia and insulin resistance (7), and possibly leptin (8). Obese subjects have high leptin levels and are more likely to have high insulin levels and to be insulin resistant. Insulin and IGF-I through the insulin receptor have a positive effect on BMD (9–12), whereas leptin's effect on BMD is complex and likely to be mediated through direct and indirect mechanisms (13–15). Indeed, a study by Ducy *et al.* (16) on mice identified leptin as a potent inhibitor of bone formation acting through the central nervous system. However, *in vitro* studies by Thomas *et al.* (17) and Holloway *et al.* (18) demonstrated that leptin has a protective effect on bone metabolism enhancing osteoblast formation and inhibiting osteoclast generation. Furthermore, recent data suggest a direct role for the peroxisome proliferator-activated receptor-γ (PPARγ) pathway in osteoblast formation (19). The PPARγ pathway has been found to be impaired in insulin resistance (20) and could explain the relationship between insulin resistance and BMD. The relative contribution of insulin and leptin to high BMD in obesity remains unclear. The purpose of this study is to dissect the effect of obesity *vs.* the leptin/insulin axis on bone metabolism in two groups of young normally cycling premenopausal women: insulin resistant (IR) and insulin sensitive (IS).

We anticipated that IR obese females have higher plasma leptin and insulin levels and therefore higher BMD compared with IS obese females.

Subjects and Methods

Study group

The study group consisted of 48 obese females recruited from the obesity clinics and the Nutrition and Food Science Department at the American University of Beirut, who met all the screening criteria. Inclusion criteria were the following: premenopausal females 18–48 yr, adult-onset obesity, normal fasting plasma glucose, body mass index (BMI) greater than 30 kg/m^2, normally cycling (≥10 cycles/yr), stable body weight for at least 2 months before study, and fasting glucose 76–110 mg/dl. Exclusion criteria included current or previous (within 6 months) use of oral contraceptive pills, cortisone, anti-epileptic drugs, cholesterol-lowering drugs, or binders; history of renal, gastrointestinal, or liver disease; alcohol intake, ethanol more than two drinks per day; smoking; current or anticipated pregnancy; obesity secondary to endocrine disease; and participation in any strenuous physical exercise. Twenty-eight females were classified as IR and 20 as IS according to the McAuley insulin sensitivity index (ISI) corrected for fat-free mass (Mffm/I) according to the following formula: ISI Mffm/I = exp(2.63 − 0.28 ln I − 0.31 ln TAG), where I is insulin in mU/liter and TAG is

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Abbreviations: BAP, Bone alkaline phosphatase; BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; CV, coefficient of variation; IR, insulin-resistant; IS, insulin-sensitive; ISI, insulin sensitivity index; PPARγ, peroxisome proliferator-activated receptor-γ.

triacylglycerol in mmol/liter. The cutoff point for insulin resistance was less than 5.8 M/mU·liter (where M = glucose disposal rate in mg/kg·min) (21). Although homeostasis model assessment (22) and quantitative insulin sensitivity check index (23) are accepted methods to assess insulin resistance, individuals in our study group were assessed using the McAuley index (24) because this method carries a sensitivity of 75% and a specificity of 91% and is suggested as an optimal method for classification of insulin resistance in obese subjects (21).

The study protocol was approved by the Institutional Review Board at the American University of Beirut, and informed consent was obtained from the subjects.

Data collection

A questionnaire was administered to collect data regarding menstrual history, dietary habits including caffeine, alcohol, and calcium consumption, and exercise habits.

Anthropometric measures included weight, height, and BMI. Subjects were weighed to the nearest 0.1 kg in light clothes, and height was measured to the nearest 0.5 cm with the person barefoot.

Fasting blood was drawn in the morning, and the following biochemical tests were obtained: fasting plasma glucose, calcium (Ca), phosphorus (P), sodium (Na), fasting insulin, leptin, IGF-I, osteocalcin, bone alkaline phosphatase (BAP), free testosterone, and cross links of collagen (CTX). All samples were stored at −70 C until analyzed.

Twenty-four-hour urine was collected. Creatinine, calcium, and phosphorus were measured using a 912 Automatic Analyzer (Hitachi and Roche Molecular Biochemicals, Indianapolis, IN).

Assays

Leptin was analyzed by RIA with intra- and interassay coefficients of variation (CV) less than 7% for values in the range 2.8–74 ng/ml (Diagnostic System Laboratories Inc., Webster, TX). Insulin was determined by antibody RIA with intra- and interassay CV less than 9% for values between 18 and 91 μU/ml (CIS Bio International, Gif-Sur-Yvette, France). Free testosterone was determined by antibody RIA, with intra- and interassay CV less than 10% for values between 1.87 and 34 pg/ml (Diagnostic System Laboratories). IGF-I was analyzed by immunoradiometric assay with intra- and interassay CV between 16 and 9.3% at values between 60 and 600 ng/ml (Nichols Institute Diagnostics, San Clemente, CA). Serum osteocalcin was analyzed by immunoradiometric sandwich assay RIA, with intra- and interassay CV less than 5% for values between 21.9 and 183.9 ng/ml (ELSA-osteo kit, CIS Bio International). Serum C-telopeptide was determined by an ELISA with intra- and interassay CV less than 6% for values between 1700 and 3500 pmol/liter (Osteometer Biotech, Herlev, Denmark). BAP was determined by an enzyme immunoassay, with inter- and intraassay CV less than 8% for values between 12 and 100 U/liter (Alkphase-B, Metra Biosystems, Mountain View, CA). Serum 25-OH vitamin D was measured by a competitive protein-binding assay with inter- and intraassay CV less than 13% for the value of 47 ng/ml (Diasorin Incstar, Diasorin, Saluggia, Italy). Serum PTH was measured with ELSA-PTH immunoradiometric assay with inter- and intraassay CV less than 7% for values between 6 and 95 pg/ml (CIS Bio International).

Dual-energy x-ray absorptiometry

Dual-energy x-ray absorptiometry measurements were used to measure body fat, lean body mass, total body bone mineral content (BMC), and lumbar spine, total hip, femoral neck, and trochanter BMD using a Hologic 4500A densitometer (Hologic, Waltham, MA). *In vivo* quality control was derived from same-day duplicate measurements performed on 168 patients during the study period. The CV percent mean ± SD for the spine duplicates was 0.89 ± 0.8%; for the total hip, 0.82 ± 0.69%; for the femoral neck, 1.4 ± 1.2%; and for the trochanter, 1.1 ± 0.85%. For the total-body measurements, the CV percent mean ± SD performed on 30 duplicates were as follows: 0.91 ± 0.69% for total-body BMD, 0.9 ± 0.77% for total body BMC, 0.54 ± 0.36% for lean mass, 1.13 ± 0.79% for fat mass, and 0.96 ± 0.76% for percentage fat mass. These values fell within the values others and we have reported (25).

Statistical analysis

For normally distributed variables, data are expressed as means ± SD, whereas nonnormally distributed variables are expressed as median (range). Comparison of outcome variables between various subgroups of subjects, *i.e.* IR and IS, was performed using a two-tailed *t* test for normal variables and K independent nonparametric test for nonnormal variables. The relationship between total BMD at three different sites and continuous variables such as leptin, insulin, IGF-I, free testosterone, lean mass, and fat mass was evaluated using a Pearson correlation coefficient for normal variables and Spearman correlation coefficient for nonnormal variables. Analysis of covariance (ANCOVA) was used to adjust for height and fat mass. The analyses were performed using SPSS software version 10.0 (SPSS, Chicago, IL). Statistical significance was at $P < 0.05$.

Results

Clinical characteristics of the study subjects

The study subjects were obese with a mean BMI in the mid thirties, young with mean age of 31 yr, and most importantly with normal menstrual function, the latter an important determinant of bone metabolism. Height, weight, lean mass, and fat mass were comparable in the two groups. Both groups had low vitamin D concentrations and similar dietary and exercise habits. Waist circumference was significantly higher in the IR group compared with the IS group. By definition, the IR group had higher insulin levels and lower ISIs. They had similar glucose, IGF-I, and free testosterone levels compared with the control IS group. Mean plasma leptin level was significantly higher in the IR group compared with the IS group (Table 1).

Bone and mineral metabolism

Mean levels for serum calcium, phosphate, vitamin D, PTH, and osteocalcin were comparable between the IR and the IS subjects. Similarly, bone density at the total body, spine, and hip were also comparable between the two groups (Table 2). The BMDs in both groups were significantly higher both at the spine and total hip compared with age-, gender-, and ethnic-matched controls (data not shown) (26).

Z-scores in the study group were derived through the densitometer software using a Western database as reference. Comparing these Z-scores against zero demonstrated that lumbar spine bone density in both groups and total hip and trochanter bone densities in the IR group were higher than that of age- and gender-matched Western women ($P < 0.01$).

Twenty-four-hour urinary calcium and calcium-to-creatinine ratio were significantly higher, and serum CTX was almost significantly higher in the IR group compared with the IS group (Table 2).

Anthropometric and biochemical correlates of BMD and turnover

Correlation analyses were implemented to study the relationship between bone parameters and anthropometric as well as metabolic parameters known to affect bone turnover and BMD. These included age, lean mass, fat mass, insulin, ISI, leptin, free testosterone, and IGF-I levels. In the overall study group, there was an inverse correlation between age and BMD and a significant positive correlation between height, lean mass, fat mass, IGF-I, and free testosterone at

TABLE 1. Anthropometric, body composition parameters, and biochemical parameters of insulin resistance between IS and IR subjects

Parameter	IR subjects (n = 28)	IS subjects (n = 20)	*P*
Age (yr)[a]	32 ± 10.9	29.2 ± 8.7	NS
Cycles/yr[a]	11.8 ± 0.5	12 ± 0.0	NS
Calcium intake (mg/d)[a]	577 ± 361	691 ± 458	NS
Height (m)[a]	159 ± 56	161 ± 79	NS
Weight (kg)[b]	89.4 (72.9–133.7)	87.6 (78–111.6)	NS
BMI (kg/m^2)[b]	36 (30.3–50.9)	33.3 (30–45.3)	NS
Waist circumference (cm)[b]	98.7 (84–131)	96 (85–108)	0.04
Lean body mass (kg)[b]	44.5 (32.9–58.7)	42.4 (37.4–61.8)	NS
Fat body mass (kg)[b]	38.8 (28–66.1)	38.8 (28.8–57.2)	NS
Insulin, μU/ml (pmol/liter)[a]	30.9 ± 9.3	18.6 ± 3.5	0.000
Glucose, mg/dl (mmol/liter)[a]	90.9 ± 7.9	87.5 ± 8.5	NS
ISI Mffm/I[a,c]	4.9 ± 0.7	6.6 ± 0.5	0.000
Leptin (ng/ml)[a]	69.2 ± 25.6	54.6 ± 21	0.04
IGF-I, ng/ml (μg/liter)[a]	210 ± 92.9	201 ± 75.1	NS
Free testosterone, pg/ml (pmol/liter)[b]	2.6 (0.7–14.8)	3.5 (0.8–16.9)	NS

The biochemical assays are reported in metric units [Systeme International (SI) units in parentheses]. To change from metric to SI, multiply insulin by 7.175, glucose by 0.0555, IGF-I by 1.0, and free testosterone by 3.47. NS, not significant; $P > 0.05$.

[a] Independent *t* test for normal variables; data are expressed as mean ± SD.

[b] K independent nonparametric tests for nonnormal variables; data are expressed as median (range).

[c] ISI Mffm/I = exp(2.63 − 0.28 ln I − 0.31 ln TAG), where I is insulin in mU/liter and TAG is triacylglycerol in mmol/liter; cutoff, <5.8 indicates insulin resistance.

multiple skeletal sites (Table 3). Whereas there was no apparent relation between insulin, insulin index, PTH, BAP, osteocalcin, CTX, vitamin D, and bone density at any site, leptin correlated significantly only with femoral neck BMD (Table 3). There were no significant associations between PTH, BAP, osteocalcin, CTX, vitamin D, and leptin in the overall study group. Also no significant associations were found between vitamin D and fat mass or lean mass.

Similar analyses were implemented in the two subgroups of obese subjects divided by insulin resistance status. In the IS group, there was a highly significant correlation between height and lean mass with BMD at the lumbar spine, hip, and total body (R = 0.47–0.68; $P < 0.05$) (Table 4). IGF-I correlated with BMD at the spine, hip, and total body (R = 0.46–0.49; $P < 0.05$). The significant correlation between lean mass and BMD disappeared when adjusting for height by ANCOVA (data not shown). Conversely, in the IR group, fat mass, but not lean mass, and leptin were both significant positive correlates of bone density at the hip and total body (R = 0.39–0.65; $P < 0.05$) (Table 5). The significant correlation between leptin and bone density disappeared when adjusting for fat mass by ANCOVA (data not shown). Testosterone levels were also significant positive correlates of bone density in the IR group (Table 5). IGF-I correlated positively with femoral neck BMD in both groups (Tables 4 and 5). There were no significant correlations between BAP, 25-OH vitamin D, and bone density in either group. In the IR group, only PTH positively correlated with spine BMD (R = 0.38; $P < 0.05$). In the IS group, a negative correlation was found between osteocalcin and total hip and femoral neck BMDs (R = −0.51

TABLE 2. Indices of mineral metabolism, BMC, and BMD in IR and IS subjects

Parameter	IR subjects (n = 28)	IS subjects (n = 20)	*P*
Serum calcium, mg/dl (mmol/liter)[b]	9.6 (8.7–10.3)	9.5 (8.9–10.1)	NS
Serum phosphorus, mg/dl (mmol/liter)[a]	3.4 ± 0.47	3.6 ± 0.40	NS
Serum 25(OH)D, ng/ml (nmol/liter)[a]	13.1 ± 4.8	13.6 ± 5.1	NS
Serum PTH, pg/ml (pmol/liter)[b]	22.8 (1.7–91.8)	23.8 (9–57.7)	NS
Serum BAP (U/liter)[a]	18.4 ± 9.2	19.1 ± 9.6	NS
Serum osteocalcin, ng/ml (μg/liter)[a]	18.3 ± 5.3	19.1 ± 6.4	NS
Serum CTX (pmol/ml)[b]	2.3 (0.6–9.4)	1.4 (0.2–7.6)	0.052
Urine calcium, mg/24 h (mmol/d)[b]	177.3 (55–322.6)	73.8 (20.2–252.2)	0.001
Urine calcium, mg/g creatinine[a]	145 ± 61.6	91.3 ± 54.7	0.005
Total body BMD (g/cm^2)[a]	0.97 ± 0.06	0.97 ± 0.08	NS
Total body BMC (g)[a]	1731 ± 191	1809 ± 331	NS
Lumbar spine BMD (g/cm^2)[a]	1.07 ± 0.09	1.1 ± 0.13	NS
Lumbar spine, Z-score	0.48 ± 0.8[c]	0.7 ± 1.14[c]	NS
Total hip BMD (g/cm^2)[a]	0.98 ± 0.11	1.00 ± 0.17	NS
Total hip, Z-score	0.46 ± 0.86[c]	0.53 ± 1.32	NS
Femoral neck BMD (g/cm^2)[a]	0.87 ± 0.11	0.89 ± 0.15	NS
Femoral neck, Z-score	0.34 ± 0.92	0.51 ± 1.26	NS
Trochanter BMD (g/cm^2)[a]	0.77 ± 0.12	0.74 ± 0.15	NS
Trochanter, Z-score	0.47 ± 0.93[c]	0.51 ± 1.42	NS

The biochemical assays are reported in metric units (SI units in parentheses). To change from metric to SI, multiply calcium by 0.25, phosphorus by 0.32, 25-OH vitamin D [25(OH)D] by 2.496, PTH by 0.1061, osteocalcin by 1.0, and urine calcium by 0.025.

[a] Independent *t* test for normal variables; data are expressed as mean ± SD.

[b] K independent nonparametric tests for nonnormal variables; data are expressed as median (range).

[c] One-sample *t* test compared with zero, significant at $P < 0.05$.

TABLE 3. Correlations between anthropometric and biochemical variables and BMD and BMC for all subjects (n = 48)

Parameter	Lumbar spine BMD	Total hip BMD	Femoral neck BMD	Trochanter BMD	Total body BMD	Total body BMC
Age[a]	−0.36[c]	−0.5[d]	−0.59[d]	−0.3[c]	−0.45[d]	−0.40[d]
Waist circumference[b]	0.08	0.13	0.09	0.11	0.19	−0.03
Height[a]	0.45[d]	0.20	0.35[c]	0.14	0.48[d]	0.77[d]
LBM[b]	0.12	0.11	0.15	0.2	0.32[c]	0.35[c]
Fat mass[b]	0.17	0.44[d]	0.45[d]	0.21	0.27	0.35[c]
Leptin[a]	0.05	0.27	0.29[c]	0.19	0.16	0.00
Insulin[a]	−0.09	0.11	0.06	0.20	0.03	−0.08
ISI Mffm/I[a]	0.16	0.14	0.16	−0.12	0.08	0.18
IGF-I[a]	0.24	0.31[c]	0.43[d]	0.21	0.32[c]	0.41[d]
Free testosterone[b]	0.05	0.47[d]	0.41[d]	0.20	0.24	0.28

[a] Pearson correlation for normally distributed variables.
[b] Spearman's correlation for nonnormally distributed variables.
[c] Values show significant correlation at $P < 0.05$.
[d] Values show significant correlation at $P < 0.01$.

and R = −0.46, respectively; $P < 0.05$) and between CTX and total hip BMD (R = −0.46; $P < 0.05$). In the IS group, leptin correlated negatively with free testosterone (R = −0.43; $P = 0.06$) and IGF-I (R = −0.47; $P < 0.05$) and positively with CTX (R = 0.45; $P < 0.05$). These correlations were not significant in the IR group.

Discussion

This study evaluated the relationship between insulin, leptin, and bone metabolism in obese normally cycling premenopausal women. Despite higher circulating insulin and leptin levels, IR normally cycling obese subjects had mean values of biochemical markers of bone remodeling and BMD at all skeletal sites that were comparable to their IS counterparts.

Hyperinsulinemia has been proposed as a potential explanation for the association between obesity and BMD in women, perhaps because of the effect of insulin on osteoblasts (9, 10), IGF-I, IGF-binding protein-1 (27), androgens (28), and PTH (29). Studies have reported increased, decreased, or unchanged BMD in diabetic females compared with females with normal glucose tolerance (30). Studies on women with polycystic ovary syndrome have reported conflicting findings regarding BMD compared with healthy controls (31–35). Discrepancies between studies can be attributed to the presence of concomitant oligomenorrhea in some studies (34). In this study, insulin levels correlated positively with BMD only in the IR group, supporting the positive role of high insulin levels on bone and consistent with previous reports. Indeed, a positive relationship between insulin and bone density was reported in a group of premenopausal and postmenopausal women (35) as well as in lean women with polycystic ovary syndrome (36).

Leptin was significantly higher in IR obese females despite similar total body fat in both groups, suggesting that hyperinsulinemia leads to enhanced leptin production. Although this could simply reflect mere association, many data point to a cause and effect relationship. Indeed, studies on diabetic subjects showed that treatment with insulin leads to higher leptin compared with BMI-matched subjects treated with other therapies (37). Rentsch *et al.* (38) and Saladin *et al.* (39) have recently shown insulin to be an important regulator of leptin expression in cultured adipocytes *in vitro*; and in rodents, elevated levels of leptin mRNA and leptin protein were noted after prolonged exposure to insulin.

In this study, leptin levels strongly correlated with bone density only in the IR group. In human beings, positive, negative, or no associations between serum leptin levels and BMD have been reported (13, 14, 37, 40–44). The relationship between leptin and bone is a complex one, with a diverging effect depending on whether central or peripheral mechanisms are operating (13–15). Peripherally, leptin has been shown to result in a positive effect on bone by increasing the production by osteoblastic cells of the potent antiresorptive factor, osteoprotegerin (17, 45). Leptin also enhances osteoblast formation and inhibits osteoclast generation (18, 46).

TABLE 4. Correlations between anthropometric and biochemical variables and BMD and BMC in IS subjects (n = 20)

Parameter	Lumbar spine BMD	Total hip BMD	Femoral neck BMD	Trochanter BMD	Total body BMD	Total body BMC
Age[a]	−0.42	−0.46[c]	−0.54[c]	−0.47[c]	−0.51[c]	−0.27
Waist circumference[b]	−0.05	0.16	0.08	0.2	0.07	0.03
Height[a]	0.55[c]	0.24	0.52[c]	0.31	0.60[d]	−0.78[d]
LBM[b]	0.34	0.31	0.47[c]	0.35[c]	0.56[d]	0.68[d]
Fat mass[b]	0.20	0.38	0.26	0.38	0.05	0.11
Leptin[a]	−0.07	−0.04	−0.02	0.01	−0.20	−0.36
Insulin[a]	0.01	−0.05	0.05	−0.09	−0.04	0.04
ISI Mffm/I[a]	−0.04	0.10	−0.07	0.04	−0.04	−0.18
IGF-I[a]	0.46[c]	0.32	0.49[c]	0.35	0.46[c]	0.46[c]
Free testosterone[b]	0.06	0.44	0.41	0.28	0.23	0.22

[a] Pearson correlation for normally distributed variables.
[b] Spearman's correlation for nonnormally distributed variables.
[c] Values show significant correlation at $P < 0.05$.
[d] Values show significant correlation at $P < 0.01$.

TABLE 5. Correlations between anthropometric and biochemical variables and BMD and BMC in IR subjects (n = 28)

Parameter	Lumbar spine BMD	Total hip BMD	Femoral neck BMD	Trochanter BMD	Total body BMD	Total body BMC
Age[a]	−0.31	−0.58[d]	0.65[d]	−0.22	−0.45[c]	−0.56[d]
Waist circumference[b]	0.19	0.21	0.18	0.09	0.27	−0.01
Height[a]	0.29	0.13	0.10	−0.03	0.32	0.73[d]
LBM[b]	−0.01	−0.07	−0.10	0.02	0.07	0.08
Fat mass[b]	0.16	0.54[d]	0.58[d]	0.11	0.39[c]	0.51[d]
Leptin[a]	0.25	0.65[d]	0.61[d]	0.28	0.48[d]	0.46[d]
Insulin[a]	0.03	0.38[c]	0.21	0.29	0.09	0.03
ISI Mffm/I[a]	0.13	0.21	0.33	−0.15	0.24	0.37[c]
IGF-I[a]	0.12	0.35	0.41[c]	0.11	0.23	0.44[c]
Free testosterone[b]	0.03	0.57[d]	0.5[d]	0.21	0.27	0.30

[a] Pearson correlation for normally distributed variables.
[b] Spearman's correlation for nonnormally distributed variables.
[c] Values show significant correlation at $P < 0.05$.
[d] Values show significant correlation at $P < 0.01$.

Centrally, leptin has been shown to inhibit bone formation through a hypothalamic relay, an effect that is inhibited using β-blockers (8, 16). The strong relationship between leptin and bone density in the IR group was paralleled by similar correlations between fat mass and bone density. Body composition parameters are known predictors of BMD (47–50), and leptin is almost exclusively produced by fat with a very strong association between serum leptin and fat mass (51–53). The above could explain the disappearance of the significant correlation between serum leptin and BMD after adjusting for body fat mass in our study. In the literature, human cross-sectional studies provide inconclusive data. The relationship between leptin and BMD disappeared after adjusting for fat mass in some (35, 54) but not all (55, 56) studies. Discrepancies between studies may relate to differences in bone mass or body composition parameters used (50), age, menopausal status, gender (57), and BMI of subjects.

Although the relationship between bone mass and fat mass has been explained in terms of PPARγ expression, it does not explain the findings in our study. Insulin and leptin are both known to be related to PPARγ signaling, and insulin resistance is associated with PPARγ inactivation (20). Studies on cells and animals have found that PPARγ haploinsufficiency enhances osteoblastogenesis *in vitro* and increases bone mass *in vivo* (19). In the IR group of our study, the PPARγ receptor would be anticipated to be relatively inactive because of the presence of insulin resistance, resulting in higher bone mass compared with IS subjects. This, however, was not observed.

A positive correlation was observed between height, lean body mass and BMD in the IS group; whereas in the IR group, fat mass and leptin were positively associated with BMD. There is ongoing controversy as to whether fat mass or lean mass is a better predictor of BMD (47–50, 58–60). Our results establish a dichotomy in the impact of body composition parameters on BMD depending on the presence of insulin resistance. Leptin may in part mediate some of the protective effect of fat mass on the skeleton (36).

Higher plasma insulin and leptin in the IR subjects did not result in increased BMD. Although IGFs (IGF-I and -II) are available to the skeletal tissue through *de novo* synthesis by bone cells and by release of stored peptide from the bone matrix, circulating IGF is another possible source (61). Insulin resistance could be an explanation for the similar BMDs between the two groups. Indeed, plasma IGF-I was not different between the two groups, and therefore bone IGF-I from circulating sources would also be comparable between the two groups. Obesity has been associated with leptin resistance because of impaired transport to the hypothalamus (62, 63), and this resistance might be extended to the periphery and more specifically to the bone level. However, this suggestion is purely speculative. Conversely, very high circulating leptin levels may overcome the resistance to cross the blood-brain barrier and subsequently cause central inhibition of bone formation in the IR group (16, 62, 63). The similar mean serum osteocalcin levels in the two subgroups, despite higher serum CTX levels in the IR group, is consistent with the above hypothesis.

The IR group had higher urinary calcium and calcium-to-creatinine ratio compared with the IS group, possibly suggesting higher rates of bone resorption. Although urinary calcium excretion is also affected by dietary calcium intake, and renal calcium handling, we believe that the higher CTX in the IR are consistent with higher bone resorption. In a study by Suzuki *et al.* (64), non-insulin-dependent diabetic Wistar rats with hyperinsulinemia were shown to have higher tartrate-resistant acid phosphatase (sensitive marker of osteoclastic function) and lower levels of osteocalcin compared with controls. Consequently, in non-insulin-dependent diabetic hyperinsulinemic rats it is suggested that the bone formation by osteoblasts was decreased, whereas bone resorption by osteoclasts was increased. In the present study, both groups had similar osteocalcin levels, but the IR group had higher serum CTX, a bone marker for resorption, which might explain the higher calcium urinary excretion in that group. Other possibilities for higher urinary calcium in the IR group include a higher dietary sodium and/or protein intake, compared with the IS group. However, that was not investigated in the study.

Our subjects were grouped according to ISI Mffm/I formula that is based on fasting serum insulin and triacylglycerol levels. This index has been shown to have a sensitivity of 75% and a specificity of 91%, whereas fasting insulin alone has 57% sensitivity and 82% specificity (21). Our IR subjects had significantly higher waist circumference than the IS, which supports our classification, because insulin resistance

is characterized by increased abdominal fat caused by large adipocytes with increased rate of lipolysis (65).

This study has several limitations. Our study group consisted of obese Caucasian individuals falling on a spectrum of hyperinsulinemia, and reflecting their obesity were low vitamin D concentrations, which may have affected our findings. It is therefore possible that our results may not be generalized to the non-insulin-resistant obese and/or vitamin D-replete obese individuals.

The sample size was not large enough, and therefore the relative contribution of various predictors on bone mass could not be explored through regression analyses. Furthermore, the study was cross-sectional, and no direct cause and effect relationship could be established. However, the study has several strengths. Leptin levels in the obese subjects spanned a wider range than previously reported (35, 37, 43, 54), thus allowing the investigation of the association between leptin and mineral metabolism across a spectrum. In addition, whereas both study groups were obese, they differed in their plasma insulin and leptin status, allowing the investigation of the effect of plasma insulin and leptin on bone, in the absence of obesity or fat mass as confounders. Finally, all subjects were normally cycling, which excluded any confounding effect of irregular menses on bone metabolism.

In conclusion, there is a dichotomy in the impact of body composition parameters and insulin and leptin levels on bone parameters in obese individuals. The interaction between the fat-related endocrine system and bone seems to be complex and may be modulated by central and peripheral mechanisms as well as local resistance to the putative protective effect of insulin and leptin on bone.

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Address all correspondence and requests for reprints to: Ghada El-Hajj Fuleihan, M.D., M.P.H. Calcium Metabolism and Osteoporosis Program, American University of Beirut Medical Center, Bliss Street, Beirut, Lebanon, 113-6044. E-mail: gf01@aub.edu.lb.

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