Influence of Insulin-Like Growth Factor–1 and Leptin on Bone Mineral Content in Healthy Premenopausal Women

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The aim of the present investigation was to study the influence of plasma insulin-like growth factor–1 (IGF-1) and leptin levels on bone mineral mass (BMC) and bone mineral density (BMD) in premenopausal women and the relationship between IGF-1 and leptin levels. Two hundred and four healthy women participated in this study. All participants had a body mass index (BMI) <30 kg/m^2 and were matched for their level of mean daily energy expenditure. BMC and BMD were correlated with measured body composition and blood biochemical parameters. No association was observed between BMC and BMD values with measured physical performance characteristics. Leptin had a significant association with BMC (β = 0.840; P = 0.0001), total BMD (β = 0.833; P = 0.0001), femoral neck BMD (β = 0.829; P = 0.0001), and lumbar spine BMD (β = 0.833; P = 0.0001). However, these associations were no longer independent when adjusted for body fat mass (FM) and trunk fat:leg fat ratio (P > 0.385). IGF-1 was significantly related to BMC (β = 0.920; P = 0.0001), total BMD (β = 0.918; P = 0.0001), femoral neck BMD (β = 0.921; P = 0.0001), and lumbar spine BMD (β = 0.917; P = 0.0001), but did not remain significant when adjusted for fat free mass (FFM; P > 0.062). In addition, a significant association between IGF-1 and leptin was found (β = 0.801; P = 0.0001), and it remained significant after controlling for age, FM, FFM, insulin, and fasting insulin resistance index (FIRI), but not when adjusted for BMC and body mass values. In conclusion, it appears that fasting IGF-1 and leptin concentrations have no direct effect on BMC and BMD values. In addition, if there is an important relationship between IGF-1 and leptin, it is mediated or confounded by BMC in premenopausal women. Exp Biol Med 231:1673–1677, 2006

Key words: bone mineral mass; bone mineral density; IGF-1; leptin; premenopausal women

Introduction

Body mass has been reported to be one of the strongest predictors of bone mineral mass (BMC) and is associated with increased bone mineral density (BMD) and decreased risk of bone fractures in women at different ages (1, 2). Body fat mass (FM) has been proposed as a better predictor of BMD than body mass and fat free mass (FFM) in elderly women (2). Excessive FM, consistent with obesity, induces greater mechanical loading that contributes to the significant relationship between total body mass and BMD (3). In addition, the increase in body FM in obesity is also accompanied by an increase in muscle mass, which may also mediate the relationship between total body mass and BMD (4). Accordingly, in addition to mechanical loading, body composition parameters influence changes in BMC and BMD in women at different ages.

Because of the prominent role of insulin-like growth factor–1 (IGF-1) in muscle development, it is possible that increased muscle mass in obesity is a result of enhanced IGF-1 secretion and/or activity (5). In addition, the role of IGF-1 in bone metabolism is well established (6, 7). Specifically, IGF-1 is a bone-promoting polypeptide that mediates the effects of growth hormones at the bone tissue level (8). Another factor that may play a role in bone mass and BMD is leptin, the product of the *LEP* (previously denoted *OB*) gene. Leptin is a polypeptide hormone mainly secreted by adipose tissue and is strongly correlated to FM (7, 9). It has emerged as a potential candidate for explaining the protective effect of FM on bone (10). However, cross-sectional studies to assess the role of leptin in bone metabolism have not been conclusive (11), as some studies have indicated no relationship between leptin concentration and BMD (12, 13). It is also interesting to note that an inverse association between plasma IGF-1 and leptin concentration has been observed (14).

In this study, we examined a group of healthy premenopausal women to determine (i) the influence of plasma levels of IGF-1 and leptin on BMC and BMD values, and (ii) the possible relationship between IGF-1 and leptin levels. In addition, we evaluated different body

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composition and physical performance factors that are known to affect bone metabolism.

Materials and Methods

Subjects. Two hundred and four healthy premenopausal women between the ages of 18 and 49 years were recruited for this study. All participants signed an informed consent that was approved by the Medical Ethics Committee of the University of Tartu, Tartu, Estonia. Prior to study enrollment, volunteers completed medical and physical activity questionnaires and were excluded if they reported current or previous conditions that might have interfered with bone metabolism (such as heart disease, long-term corticosteroid use, smoking, alcoholism, and long-term high levels of physical activity). At the time of the study, no participants were taking oral contraceptives or receiving treatments such as calcium, vitamin D, calcitonin, bisphosphonates, and diuretics, which could influence bone mineral values (11, 15). All participants had a body mass index (BMI) of <30 kg/m^2 and were matched for their level of mean daily energy expenditure (15, 16).

On the first of two visits, participants had a venous blood sample taken in the morning after an overnight fast. Functional tests were completed 2 hrs after a light breakfast. The first measurement session was conducted during the early follicular phase of the menstrual cycle (17). The second measurement session consisted of body composition and bone mineral assessments by dual energy x-ray absorptiometry (DXA). Measurement sessions were separated by approximately 1 week dependent on the participant's schedule and DXA availability. In addition, all participants completed a 3-day energy expenditure questionnaire (18).

Body Composition, BMC, and BMD. Height was measured using a Martin metal anthropometer to the nearest 0.1 cm with a standardized technique. Body mass was measured with minimal clothing to the nearest 0.05 kg using a medical electronic scale (A&D Instruments, Abingdon, UK) and BMI was calculated as body mass (in kg) divided by height (in m^2).

Whole-body fat and lean and bone mineral mass were measured by DXA using a DPX-IQ densitometer equipped with adult, proprietary software, version 3.6 (Lunar Corporation, Madison, WI). Participants were scanned in light clothing while lying flat on their backs with arms at their sides. The fast scan mode and standard participant positioning were used for total body measurements and analyzed using the extended analysis option. 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 g) to leg fat (in g) (19). BMD values were determined as the total body BMD and at the sites of posterior-anterior spine (L2–L4) (15, 20) and femoral neck (19).

Physical Performance. Physical working capacity (PWC) was determined on a cycle ergometer (Tunturi T8, Turku, Finland) using three progressive workloads at intensities of 50, 100, and 150 W for a period of 6 mins per level (15). Heart rate at the end of each workload was measured using a Polar Vantage NV (Kempele, Finland) heart rate monitor. Individual PWC was calculated at the level of predicted maximal heart rate (205 − [age/2]) by extrapolation. Leg extensor power was assessed with a vertical counter movement jump (CMJ) using a contact platform (Newtest Oy, Oulu, Finland) and recording the maximal height of the jump. The best result from three trials was taken as the value for a participant's leg extensor power (21).

Blood Biochemistry. A 10-ml blood sample was obtained from the antecubital vein with the participant in the upright position in the morning (0700–0800 hrs) after an overnight fast. Plasma was separated and frozen at –20°C for later analysis. Leptin concentrations were measured in duplicate by a radioimmunoassay (Mediagnost GmbH, Reutlingen, Germany). This assay has intra- and interassay coefficients of variation (CVs) of $<5\%$. Insulin and IGF-1 levels were determined in duplicate on an Immunolite 2000 (DPC, Los Angeles, CA). The intra- and interassay CVs for insulin were 4.5% and 12.2%, respectively, at an insulin concentration of 6.6 μIU/ml and for IGF-1 $<7\%$. Glucose concentration was measured using the hexokinase/glucose-6-phosphate dehydrogenase method with a commercial kit (Boehringer, Mannheim, Germany) and insulin resistance was calculated using the fasting insulin resistance index (FIRI): (fasting glucose [m*M*] × fasting insulin [μIU/ml])/25 (22).

Statistical Analysis. Statistical analysis was performed with SPSS 11.0 for Windows (Chicago, IL) and means and standard deviations were determined. Associations were reported as Spearman's rank correlation coefficients. Regression analysis models were also used to evaluate potential associations of leptin, IGF-1, or measured bone mineral indices with several independent variables. Significance was set at $P < 0.05$. A sample size power calculation indicated that a minimum of 187 participants was sufficient to perform the study with a power of 80% and an alpha error of 5%.

Results

The mean (± SD) values of measured characteristics for premenopausal women are presented in Table 1. Mean daily energy expenditure averaged at 2636.3 ± 661.3 kcal. BMC and total BMD were associated with measured body composition and blood biochemical parameters, while no associations were observed between BMC and total BMD values and measured physical performance characteristics (Table 2). Femoral neck BMD was related to FFM, body mass, BMI, and physical performance characteristics. Lumbar spine BMD was also related to parameters that characterized the amount and distribution of body fat. No

Table 1. Baseline Body Composition, Physical Performance and Biochemical Markers of Premenopausal Women ($N = 204$)

Parameters[a]	Mean ± SD	Range
Age (years)	35.0 ± 7.7	18–49
Height (cm)	166.9 ± 7.8	144.9–181.1
Body mass (kg)	65.0 ± 10.3	50.8–82.6
BMI (kg/m^2)	24.1 ± 5.2	18.9–29.6
% FM	28.6 ± 9.4	19.2–36.4
FM (kg)	18.4 ± 8.4	10.4–28.9
Trunk fat (kg)	7.94 ± 4.39	3.79–12.78
Trunk fat:leg fat	1.15 ± 0.29	0.79–1.65
FFM (kg)	46.7 ± 5.2	40.1–53.4
Total BMC (kg)	2.9 ± 0.4	2.2–4.6
Total BMD (g/cm^2)	1.22 ± 0.08	1.02–1.57
Lumbar spine BMD (g/cm^2)	1.32 ± 0.13	0.92–1.72
Femoral neck BMD (g/cm^2)	1.26 ± 0.09	1.00–1.71
PWC (W)	185.9 ± 46.9	93.0–352.0
CMJ (cm)	19.6 ± 5.6	12.0–38.0
Leptin (ng/ml)	10.9 ± 7.4	1.9–28.8
IGF-1 (ng/ml)	199.8 ± 75.9	75.0–308.0
Insulin (μIU/ml)	7.4 ± 5.3	2.8–23.3
Glucose (m*M*)	5.0 ± 1.5	3.8–8.8
FIRI	15.8 ± 14.7	2.3–63.3

[a] BMI, body mass index; FM, fat mass; FFM, fat free mass; BMC, bone mineral content; BMD, bone mineral density; PWC, physical working capacity; CMJ, counter movement jump; FIRI, fasting insulin resistance index.

relationships were observed between measured areal BMD values and leptin or IGF-1.

In separate regression models, including age with each variable of interest, we found that BMC was correlated ($P \leq 0.0001$) with leptin concentration ($\beta = 0.207$), IGF-1 concentration ($\beta = 0.498$), insulin concentration ($\beta = 0.212$), FIRI ($\beta = 0.170$), FFM ($\beta = 0.940$), FM ($\beta = 0.472$), trunk fat:leg fat ratio ($\beta = 0.258$), PWC ($\beta = 0.658$), and CMJ ($\beta = 0.630$). Total BMD significantly correlated ($P \leq 0.005$) with leptin ($\beta = 0.165$), IGF-1 ($\beta = 0.477$), insulin ($\beta = 0.183$), FIRI ($\beta = 0.144$), FFM ($\beta = 0.902$), FM ($\beta = 0.374$), trunk fat:leg fat ratio ($\beta = 0.809$), PWC ($\beta = 0.645$), and CMJ ($\beta = 0.635$) after adjustment for age in separate regression models. Similar results were seen when relationships with areal BMDs were examined (results not shown).

A positive association of leptin concentration with BMC was found ($\beta = 0.840$; $P = 0.0001$), which remained significant ($P = 0.0001$) after controlling for IGF-1, insulin, FIRI, FFM, PWC, and CMJ, but not when adjusting for FM ($P = 0.605$) and trunk fat:leg fat ratio ($P = 0.085$). A similar positive association between leptin and total BMD was found ($\beta = 0.833$; $P = 0.0001$), and it remained significant ($P \leq 0.009$) after adjusting for IGF-1, insulin, FIRI, FFM, PWC, and CMJ, but not when adjusting for FM ($P = 0.545$) and trunk fat:leg fat ratio ($P = 0.502$). Similar results were seen when relationships with areal BMDs were examined, where leptin was related to femoral neck BMD ($\beta = 0.829$; $P = 0.0001$) and lumbar spine BMD ($\beta = 0.833$; $P = 0.0001$). These relationships remained significant after adjusting for IGF-1, insulin, FIRI, FFM, PWC, and CMJ, but not when adjusting for FM (femoral neck BMD: $P = 0.500$; lumbar spine BMD: $P = 0.496$) and trunk fat:leg fat ratio (femoral neck BMD: $P = 0.578$; lumbar spine BMD: $P = 0.385$).

Serum IGF-1 concentration was significantly related to BMC ($\beta = 0.920$; $P = 0.0001$), and remained significant when adjusting for leptin, insulin, FM, trunk fat:leg fat ratio, PWC, and CMJ, but not when adjusting for FFM. Similarly, IGF-1 was significantly related to total BMD ($\beta = 0.918$; $P = 0.0001$) and remained significant when adjusting for leptin, insulin, FM, trunk fat:leg fat ratio, PWC, and CMJ, but not when adjusting for FFM. Finally, IGF-1 was significantly related to femoral neck BMD ($\beta = 0.921$; $P =$

Table 2. Relationships Between Bone Mineral Values and Body Compositional, Blood Biochemical, and Physical Performance Parameters in Study Population ($N = 204$)

Parameters[a]	Total BMC	Total BMD	Femoral neck BMD	Lumbar spine BMD
Body mass (kg)	0.636**	0.340**	0.289**	0.323**
BMI (kg/m^2)	0.386**	0.335**	0.181**	0.261**
% FM	0.306**	0.264**	0.076	0.170*
FM (kg)	0.407**	0.294**	0.129	0.213**
FFM (kg)	0.583**	0.235**	0.372**	0.318**
Trunk fat (kg)	0.325**	0.264**	0.075	0.184**
Trunk fat:leg fat	0.215**	0.242**	0.080	0.168*
Leptin (ng/ml)	0.243**	0.204**	0.089	0.136
IGF-1 (ng/ml)	0.148*	0.140*	0.129	0.061
Insulin (μIU/ml)	0.188**	0.168*	0.074	0.139*
FIRI	0.240**	0.171*	0.111	0.178*
PWC (W)	0.123	0.054	0.223**	0.121
CMJ (cm)	0.067	0.027	0.153*	0.090

[a] Abbreviations used are the same as in Table 1.
* $P < 0.05$; ** $P < 0.01$.

0.0001) and lumbar spine BMD ($\beta = 0.917$; $P = 0.0001$), and remained significant when adjusting for leptin, insulin, FM, trunk fat:leg fat ratio, PWC, and CMJ, but not when adjusting for FFM (femoral neck BMD: $P = 0.062$; lumbar spine BMD: $P = 0.239$).

A significant association between IGF-1 and leptin was found ($\beta = 0.801$; $P = 0.0001$), and it remained significant after controlling for age ($P = 0.0001$), FM ($P = 0.003$), FFM ($P = 0.009$), insulin ($P = 0.0001$), and FIRI ($P = 0.000$), but not when adjusting for BMC and body mass values.

Discussion

In a relatively homogeneous group of premenopausal women, a positive association of leptin concentration with measured BMC and total and areal BMDs was observed and found to be independent of the influences that measured physical performance, insulin resistance, and FFM factors may exert on BMD. In contrast, the association between leptin and measured BMDs was no longer significant when adjusted for values of total body FM and body fat distribution. Similarly, IGF-1 concentration was significantly associated with BMC and total and areal BMD, and remained associated in different analyses that controlled for physical performance parameters, insulin resistance values, and body composition parameters. However, the association between IGF-1 and BMC was no longer significant when adjusted for FFM. The findings of the present investigation suggest that the influence of leptin and IGF-1 on bone metabolism is mediated or confounded by specific body composition values in a group of healthy premenopausal women.

Leptin, the product of the *LEP* gene, has multiple biological effects on nutritional status, metabolism, and the neuroendocrine axis (9). In addition, leptin strongly correlates with body FM, but the relationship of leptin with BMD still remains controversial (7, 9). Some authors have found positive relationships between leptin levels and BMDs (10, 19, 23, 24), while others have failed to find such a relationship (7, 12, 13). In our study, leptin was strongly related to measured BMC and BMD values. However, by adjusting the data for FM values, the correlation between leptin and bone values was lost, which indicates that there is no influence of leptin on BMC and BMD independent of adiposity. Similar results with regard to the relationship between leptin and total BMD in adults have also been obtained in other studies (25–27), whereas a positive effect of leptin on BMD of the growing skeleton has been observed (19, 28). Recently, Garnett *et al.* (19) were the first to report the fat mass–independent effect of leptin concentration on lumbar spine BMD in healthy prepubertal children. In another study, Iwamoto *et al.* (29) reported that serum leptin did not play an important role in overall bone metabolism and only influenced regional BMD in premenopausal women. Collectively, these results suggest that leptin may have a role in bone growth and development, but its role in mature bone is not clear.

IGF-1 is a growth-regulating peptide hormone with a wide array of physiological actions (5–7). In epidemiological analyses of large cohorts and *in vitro* studies of bone cells, circulating IGF-1 has been linked to the process of bone acquisition (30). For example, previous cross-sectional studies have reported that higher IGF-1 concentrations are associated with greater BMD in women (12, 31), and lower IGF-1 levels are found in women with established osteoporosis compared with healthy women (32, 33). In contrast, no relationship between serum IGF-1 and BMD of the entire skeleton was found in healthy postmenopausal women (12). Furthermore, Seck *et al.* (34) found that bone IGF-1 was not related to BMC. In the present study, IGF-1 showed a significant association with measured BMC and BMD values. However, the relationship between IGF-1 concentration and measured bone values was controlled by the FFM in healthy premenopausal women of the present study. In accordance with our results, reduced serum levels of IGF-1 have been observed in conditions characterized by reduced FFM and decreased strength (8).

Another interesting finding of the present investigation was the association between IGF-1 and leptin levels, which remained significant after adjusting for FM, FFM, insulin concentration, and FIRI but not when adjusting for body mass or BMC values. In contrast, Nyomba *et al.* (5) found no relationship between IGF-1 and leptin, while Martini *et al.* (12) observed a significant correlation between IGF-1 and leptin, which was eliminated after adjustment for BMI. The results of the present investigation and that of Martini *et al.* (12) might suggest that IGF-1 cannot regulate plasma leptin concentration independent of its effect on specific body composition parameters. IGF-1 is regulated by growth hormone and is used as an index of growth hormone secretion and action (5). Elevated plasma leptin concentrations have been reported in hypopituitary patients, and is reduced after growth hormone treatment (35). Furthermore, it has been postulated that leptin might regulate adipocyte sensitivity to growth hormone (36). This suggests the presence of a feedback loop whereby growth hormone inhibits leptin secretion and leptin stimulates growth hormone secretion or action (5). In addition, IGF-1 and leptin may be correlated because they are both regulated by insulin (5). However, this study would not likely be able to determine an effect of insulin on IGF-1 and leptin since subjects are young, lean, and healthy without evidence of insulin resistance. Taken together, these studies suggest that there could be a relationship between IGF-1 and leptin in healthy premenopausal women.

In summary, the results of the present investigation demonstrate a complex interaction between specific body composition parameters with IGF-1 and leptin concentration in a relatively homogeneous group of healthy premenopausal women. It appears that fasting IGF-1 and leptin concentrations have no direct effect on BMC and BMD

values. In addition, if there is an important relationship between IGF-1 and leptin, it is mediated or confounded by BMC. Future studies are needed to further investigate the possible relationship between IGF-1 and leptin, and how these peptides may regulate BMC and BMD values in different groups of women.

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