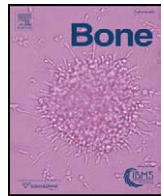




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Determinants of bone mineral density in obese premenopausal women[☆]

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ABSTRACT

Despite being a risk factor for cardiovascular disease and diabetes mellitus, obesity has been thought to protect against osteoporosis. However, recent studies have demonstrated a differential impact of specific fat compartments on bone mineral density (BMD) with visceral adipose tissue (VAT) having potential detrimental effects on BMD. Visceral obesity is also associated with dysregulation of the GH/IGF-1 axis, an important regulator of bone homeostasis. The purpose of our study was to evaluate the differential effects of abdominal fat depots and muscle, vitamin D, and hormonal determinants, including insulin-like growth factor-1 (IGF-1), testosterone, and estradiol, on trabecular BMD of the lumbar spine. We studied 68 healthy obese premenopausal women (mean BMI, 36.7 ± 4.2 kg/m²). Quantitative computed tomography (QCT) was used to assess body composition and lumbar trabecular BMD. There was an inverse association between BMD and VAT, independent of age and BMI ($p = 0.003$). IGF-1 correlated positively with BMD and negatively with VAT and, in stepwise multivariate regression modeling, was the strongest predictor of BMD and procollagen type 1 amino-terminal propeptide (P1NP). Thigh muscle cross sectional area (CSA) and thigh muscle density were also associated with BMD ($p < 0.05$), but 25-hydroxyvitamin D [25(OH)D], testosterone, free testosterone, and estradiol levels were not. 25(OH)D was associated inversely with BMI, total, and subcutaneous abdominal adipose tissue ($p < 0.05$). These findings support the hypothesis that VAT exerts detrimental effects, whereas muscle mass exerts positive effects on BMD in premenopausal obese women. Moreover, our findings suggest that IGF-1 may be a mediator of the deleterious effects of VAT on bone health through effects on bone formation.

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Introduction

Obesity is a risk factor for cardiovascular and metabolic disease. However, it has been thought that obesity protects against bone loss via mechanical loading and effects of hormones secreted or regulated by adipocytes [1–3]. Recent studies have suggested an inverse association between visceral adipose tissue (VAT) and bone mineral density (BMD) [4–6], whereas another study has demonstrated a potential protective effect of VAT on vertebral fractures in patients with diabetes mellitus [7]. Several studies have suggested that lean mass is the strongest predictor of BMD in premenopausal women [8–10], stronger than estrogen, testosterone, or progesterone [9]. Therefore, further studies are needed to determine the effects of body composition on

BMD in obese women and to investigate potential mechanisms of these effects.

The growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis is a major determinant of BMD [11–14] and is dysregulated in women in proportion to their degree of visceral adiposity [15,16]. In addition, obese women have been found to have lower rates of bone formation, as measured by type I collagen, suggesting that increased body fat suppresses new collagen formation [17]. We studied the association between BMD and IGF-1 and procollagen type 1 amino-terminal propeptide (P1NP) in obese premenopausal women to investigate the hypothesis that IGF-1 may be a mediator of effects of VAT on bone formation and bone density.

The purpose of our study was to investigate hormonal and body composition determinants of BMD in healthy obese premenopausal women. Our goal was to evaluate the effects of different abdominal fat depots, thigh muscle area and density, using quantitative computed tomography (QCT), and hormonal determinants, such as IGF-1, P1NP, 25-hydroxyvitamin D [25(OH)D], testosterone, and estradiol, on trabecular BMD of the lumbar spine using QCT. We specifically hypothesized that higher degrees of VAT would be associated with lower bone density in association with lower P1NP and IGF-1 levels in

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obese women. Furthermore, we hypothesized that thigh muscle mass and quality would be positively associated with BMD and P1NP in this population.

Materials and methods

The study was approved by Partners Healthcare Institutional Review Board and complied with Health Insurance Portability and Accountability Act guidelines. Written informed consent was obtained from all subjects after the nature of the procedures had been fully explained.

Subjects

The study group was comprised of 68 healthy obese premenopausal women who were recruited from the community through advertisements. Inclusion criteria were female gender, ages 18–45 years, BMI ≥ 30 kg/m², and eumenorrhea. Exclusion criteria included hypothalamic or pituitary disorders, diabetes mellitus or other chronic illnesses, estrogen or glucocorticoid use. Each participant underwent quantitative computed tomography (QCT) for assessment of body composition and bone mineral density (BMD) as detailed below and fasting blood tests. Body composition, BMD, and clinical characteristics have been previously reported in 54 of the 68 subjects [18–21].

Endocrine testing

Subjects underwent the following fasting blood tests: insulin-like growth factor-1 (IGF-1), procollagen type 1 amino-terminal propeptide (P1NP) (ng/ml), 25(OH)D (ng/ml), sex hormone-binding globulin (SHBG) (nmol/l), free and total testosterone (ng/dl), and free and total estradiol (pg/ml).

IGF-1, 25(OH)D, and P1NP were measured by IDS-iSYS Multi-Discipline Automated Analyser based on chemiluminescence technology (Immunodiagnostic Systems, Inc., Fountain Hills, AZ). Minimum detection limits are IGF-1 4.4 ng/ml, 25(OH)D 3.6 ng/ml, and P1NP < 1.0 ng/ml. Within-run coefficients of variation (cv) are IGF-1 1.4%–2.0%, 25(OH)D 5.5%–12.1%, and P1NP 2.6%–3.0%.

Serum testosterone was measured by a Coat-a-Count analog RIA kit (Siemens Healthcare Diagnostics Inc., Deerfield, IL) with an interassay cv of 5.9%–12% and an analytical sensitivity of 4 ng/dl.

SHBG was measured using a chemiluminescent microparticle immunoassay kit from Architect (Abbot Laboratories, Abbot Park, IL) with a within-run cv of 4.78%–5.24% and an analytical sensitivity ≤ 0.1 nmol/l. Free testosterone was calculated from total testosterone and SHBG by the laws of mass action, which has been validated in comparison to free testosterone by equilibrium dialysis in women [22].

Estradiol levels were measured using a chemiluminescent microparticle immunoassay kit from Architect (Abbot Laboratories, Abbot Park, IL), with a within-run CV of 1.5%–6.4% for concentrations of 45–192 pg/ml and a functional sensitivity ≤ 14 pg/ml.

Computed tomography (CT)—bone mineral density

Trabecular bone mineral density (BMD) assessment of the fourth lumbar vertebral body (L4) was performed using quantitative computed tomography (QCT) on a LightSpeed CT scanner (General Electric, Milwaukee, WI). Scan parameters were 144 cm table height, 80 kV, 70 mA, 2 s scan time, 1 cm slice thickness, and 48 cm field of view (FOV).

Patients were lying supine on a Mindways CT calibration phantom (Mindways, Austin, TX) containing serial solutions of dipotassium hydrogen phosphate (K₂HPO₄). Images were analyzed off-line on an IMPAX workstation (AGFA Diagnostic Software, version 4; Agfa, Ridgefield Park, NJ). A region of interest within trabecular bone of

each vertebral body was placed manually, avoiding cortical bone and posterior veins. The mean density of each vertebral body and the density of the calibration solutions were used to calculate the mineral equivalent BMD using the following formula: mg/ml of sample = (CT# of sample – intercept of calibration line) / slope of calibration line.

Computed tomography (CT)—body composition

Each subject underwent cross sectional CT of the abdomen at the level of L4 and 41 subjects underwent additional cross sectional CT of the left mid-thigh as previously described [18]. In short, a single 1 cm axial image of the abdomen and mid left thigh was obtained. Scan parameters were standardized as follows: 144 cm table height, 80 kV (abdomen), 120 kV (thigh), 70 mA (abdomen), 170 mA (thigh), 2 s scan time, 48 cm FOV. Subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), and total adipose tissue (TAT) area was calculated. In the left thigh, fat and muscle cross sectional areas (CSA), as a measure of fat and muscle mass, and mean attenuation coefficient, measured in Hounsfield units (HU) of thigh muscle tissue, as a measure of fatty infiltration, were determined [23]. Analyses were performed using Alice software (version 4.3.9; PAREXEL, Waltham, MA).

Proton MR spectroscopy (¹H-MRS)

In order to determine whether the positive correlation between IGF-1 and BMD may be a reflection of decreased bone marrow fat, we used previously published data on bone marrow fat content in a subset of 27 women. Bone marrow fat content was quantified using ¹H-MRS as previously described [19].

Statistical analysis

JMP Statistical Database Software (version 5.0.1; SAS Institute, Cary, NC) was used for statistical analyses. Non-parametric Spearman rank correlation coefficients are reported. Multivariate standard least squares regression modeling was performed to control for age, BMI, and bone marrow fat. Forward stepwise regression modeling was also performed to determine predictors of BMD and P1NP. $p \leq 0.05$ was used to denote significance and $p < 0.1$ was used to denote a trend. Data are presented as mean \pm SD.

Results

Clinical characteristics of study subjects

Subject characteristics are shown in Table 1. The age of study participants ranged from 21 to 45 years, with a mean of 35.9 ± 6.7 years. Study participants ranged in BMI from 30.2 to 49.7 kg/m², with a mean BMI of 36.7 ± 4.2 kg/m².

Associations of bone mineral density with body composition and hormones

Associations of BMD and P1NP with body composition and hormones are shown in Table 2. There was an inverse association between L4 trabecular BMD and age ($r = -0.41$, $p = 0.0006$). There were inverse associations between L4 trabecular BMD and VAT ($r = -0.42$, $p = 0.0005$) (Fig. 1), which remained significant after controlling for age and BMI using standard least squares regression modeling ($p = 0.003$). There was a trend toward an inverse association between L4 trabecular BMD and TAT ($r = -0.23$, $p = 0.06$) while there were no significant associations between L4 trabecular BMD and abdominal SAT or BMI ($p = 0.1$). There were positive correlations between L4 trabecular BMD and thigh muscle

Table 1
Clinical characteristics of all study subjects (mean \pm SD).

Variable	Subjects (n = 68)
Age (years)	35.9 \pm 6.7
Weight (kg)	97.0 \pm 13.5
BMI (kg/m ²)	36.7 \pm 4.2
IGF-1 (ng/ml)	155.1 \pm 59.0
25 hydroxyvitamin D [25(OH)D] (ng/ml)	24.1 \pm 15.2
P1NP (ng/ml)	43.9 \pm 18.0
Total testosterone (ng/dl)	34.3 \pm 21.0
Free testosterone (ng/dl)	0.54 \pm 0.43
Total estradiol (pg/ml)	115.1 \pm 94.0
Free estradiol (pg/ml)	74.6 \pm 59.4
L4 trabecular BMD (mg/cm ³)	162.4 \pm 33.0
SHBG (mmol/l)	52.8 \pm 37.3
CT: abdominal TAT (cm ²)	633.0 \pm 145.3
CT: abdominal SAT (cm ²)	510.5 \pm 121.0
CT: abdominal VAT (cm ²)	122.3 \pm 50.9
CT: thigh muscle CSA (cm ²)	147.2 \pm 19.0
CT: thigh muscle density (HU)	46.8 \pm 5.3
CT: thigh SAT (cm ²)	204.6 \pm 57.4

P1NP indicates procollagen type 1 amino-terminal propeptide; SHBG, sex hormone-binding globulin; BMD, bone mineral density; TAT, total abdominal adipose tissue; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; CSA, cross sectional area; HU, Hounsfield units.

CSA ($r=0.42$, $p=0.007$) (Fig. 2), which remained significant after controlling for BMI ($p=0.008$).

Trabecular BMD of L4 correlated positively with IGF-1 ($r=0.48$, $p=0.0001$) (Fig. 3). As IGF-1 decreases with age and increasing BMI, we controlled for age and BMI using standard least squares regression modeling. After controlling for age and BMI, the association between L4 trabecular BMD and IGF-1 remained significant ($p=0.04$). We previously demonstrated an inverse association between IGF-1 and bone marrow fat [19]. In order to determine whether the positive correlation between IGF-1 and BMD was a reflection of less bone marrow fat, we controlled for bone marrow fat in the 27 subjects for whom these data were available using standard least squares modeling. After controlling for bone marrow fat, the correlation between IGF-1 and trabecular BMD remained significant ($p=0.03$).

Fourteen subjects were vitamin D deficient [25(OH)D <20 ng/ml] [24] and 21 subjects were vitamin D insufficient [25(OH)D <30 ng/ml] [24]. There were no associations between L4 trabecular BMD and 25 (OH)D ($p=0.7$).

There were no significant associations between L4 trabecular BMD and estradiol, testosterone, or SHBG ($p=0.3$ – 0.8).

In order to determine the strongest predictors of trabecular BMD, we performed forward stepwise regression modeling. When L4 trabecular BMD was entered as a dependent variable, and VAT, IGF-1, P1NP, and thigh muscle CSA as independent variables, IGF-1 was the only predictor of BMD and explained 24% of the variability of BMD ($r^2=0.24$, $p=0.001$).

Associations of P1NP with bone mineral density and body composition

There was a positive correlation between L4 trabecular density and P1NP ($r=0.25$, $p=0.05$) (Fig. 4). P1NP demonstrated an inverse association with age ($r=-0.38$, $p=0.002$), a positive correlation with SAT ($r=0.31$, $p=0.02$), and a trend toward an inverse association with VAT ($r=-0.21$, $p=0.09$), whereas no significant associations with BMI, TAT, and thigh SAT were found ($p=0.1$ – 0.5). P1NP correlated positively with thigh muscle CSA (0.35 , $p=0.03$), thigh muscle density ($r=0.31$, $p=0.05$), and IGF-1 ($r=0.49$, $p=0.0001$) (Fig. 5).

Forward stepwise regression analysis was performed to determine the strongest predictors of P1NP. When P1NP was entered as a

dependent variable and IGF-1, and thigh muscle CSA, BMD, and SAT as independent variables, IGF-1 was the only predictor of P1NP and explained 35% of the variability of BMD ($r^2=0.35$, $p<0.0001$).

Associations of body composition with hormones

There were inverse associations between 25(OH)D and BMI ($r=-0.29$, $p=0.02$), TAT ($r=-0.27$, $p=0.04$), SAT ($r=-0.25$, $p=0.05$), but not with VAT ($p=0.1$). There was no significant association between 25(OH)D and thigh muscle CSA ($p=0.8$) or thigh muscle density ($p=0.1$). There was a positive correlation between 25 (OH)D and IGF-1 ($r=0.24$, $p=0.06$), while there was no association between 25(OH)D and P1NP ($p=0.2$).

There was an inverse association between IGF-1 and BMI ($r=0.21$, $p=0.01$), TAT ($r=0.25$, $p=0.01$), and VAT ($r=-0.35$, $p=0.0002$), while there were no significant associations with SAT and thigh fat ($p=0.9$ and $p=0.7$, respectively). IGF-1 correlated positively with thigh muscle CSA ($r=0.46$, $p=0.0027$) and muscle density ($r=0.47$, $p=0.003$).

There were no significant associations between free testosterone, free estradiol, and SHBG with BMI, abdominal and thigh fat depots, thigh muscle CSA, or thigh muscle density ($p=0.1$ – 0.9).

Discussion

We demonstrate in premenopausal obese women an inverse association between VAT and L4 trabecular BMD. Moreover, we show that IGF-1, P1NP, and thigh muscle are positive predictors of BMD. These findings support the hypothesis that VAT exerts detrimental effects, whereas the increase muscle mass seen in obesity exerts positive effects on BMD in premenopausal obese women. The GH/IGF-1 axis is dysregulated in obesity, with VAT being a strong inverse determinant of endogenous GH secretion. Our findings suggest that IGF-1 may be a mediator of the deleterious effects of VAT on bone health through effects on bone formation. Further studies are needed to investigate other potential mediators.

Despite being a risk factor for cardiovascular disease, hypertension, and diabetes mellitus, obesity has been thought to protect against osteoporosis and fat mass has been found to be a positive predictor of BMD in many studies [1,3,25–27]. However, recent studies have demonstrated a differential impact of specific fat compartments on BMD with VAT having potential detrimental effects on BMD [6,28]. Visceral obesity is also associated with dysregulation of the GH/IGF-1 axis, an important regulator of bone homeostasis [12,29,30]. In addition, obese women have been found to have lower rates of bone formation, as measured by type I collagen, suggesting that increased body fat suppresses new collagen

Table 2

Associations of trabecular BMD with body composition and hormonal determinates of bone turnover.

	L4 trabecular BMD		P1NP	
	r	p	r	p
Age	-0.41	0.0006	-0.38	0.002
Weight	-0.20	0.1	0.17	0.2
BMI	-0.20	0.1	0.16	0.2
Abdominal TAT	-0.23	0.06	0.19	0.1
Abdominal SAT	-0.21	0.1	0.31	0.02
Abdominal VAT	-0.42	0.0005	-0.21	0.09
Thigh muscle CSA	0.42	0.007	0.35	0.03
Thigh muscle density	0.36	0.02	0.31	0.05
Thigh SAT	0.26	0.1	0.11	0.5
IGF-1	0.48	0.0001	0.49	0.0001
25(OH)D	0.04	0.7	-0.15	0.2
P1NP	0.25	0.05		

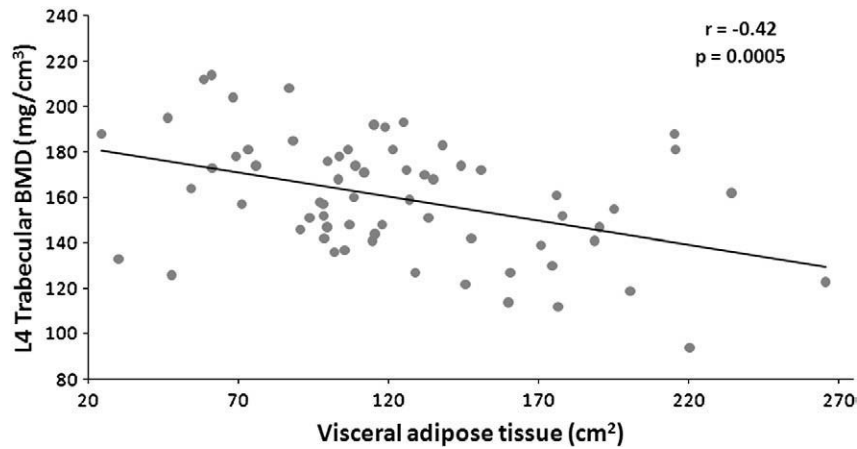


Fig. 1. Regression analysis of L4 trabecular BMD on visceral adipose tissue. There is an inverse association between bone mineral density and visceral adipose tissue.

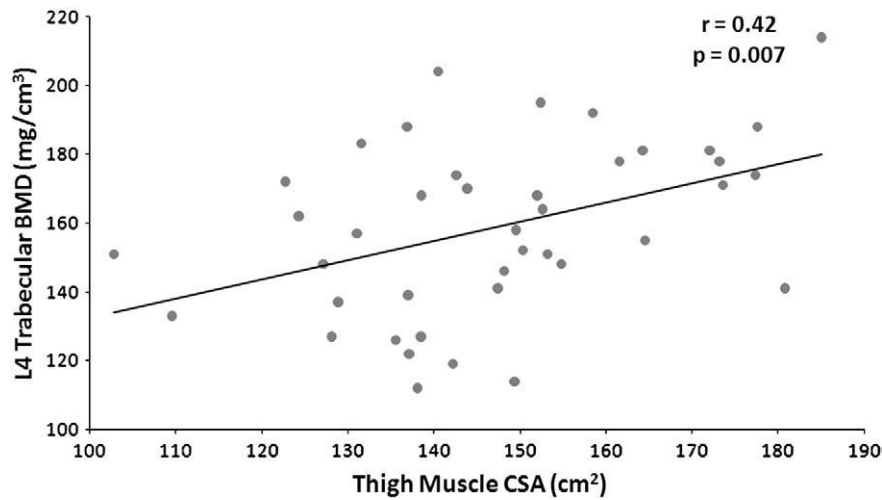


Fig. 2. Regression analysis of L4 trabecular BMD on thigh muscle cross sectional area. There is a positive correlation between bone mineral density and thigh muscle cross sectional area.

formation [17]. Vitamin D, a regulator of bone metabolism, is inversely associated with obesity and fat mass, and vitamin D deficiency is emerging as a risk factor for the metabolic syndrome

[31]. Therefore, we evaluated the effect of different abdominal fat depots, bone turnover markers, and hormones on BMD in premenopausal obese women.

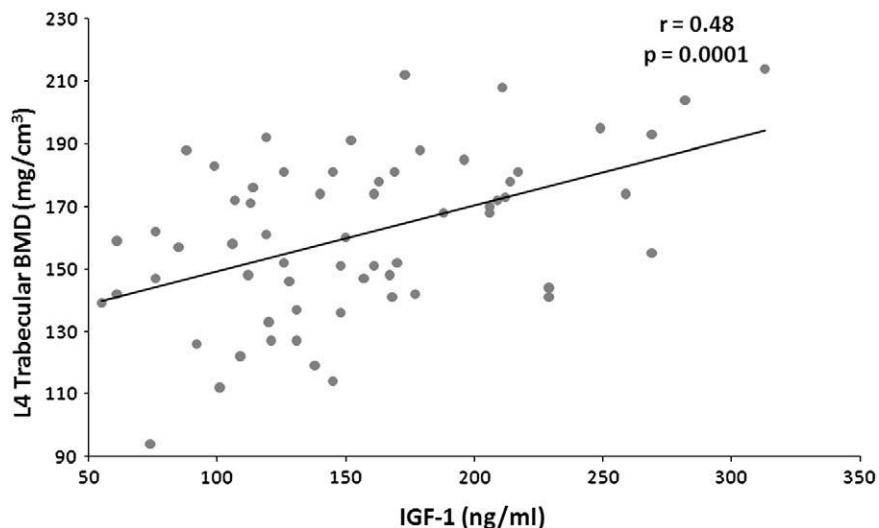


Fig. 3. Regression analysis of L4 trabecular BMD on insulin-type growth factor-1 (IGF-1). There is a positive correlation between bone mineral density and IGF-1.

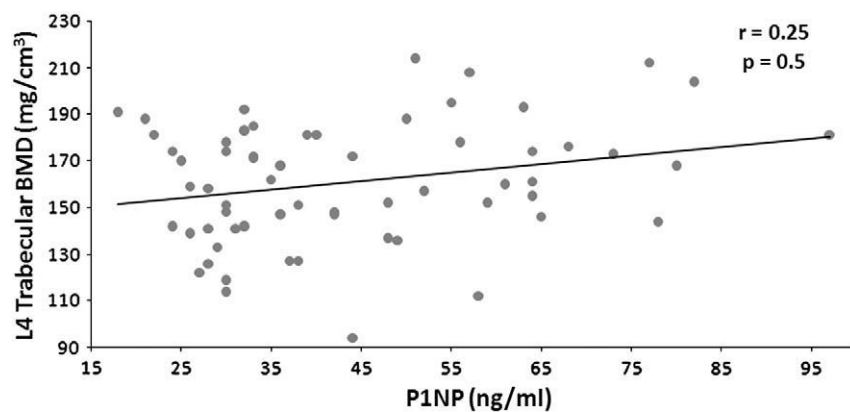


Fig. 4. Regression analysis of L4 trabecular BMD on N-terminal propeptide of Type 1 procollagen (P1NP). There is a positive correlation between bone mineral density and P1NP.

Studies in children and young adults of various BMI have shown an inverse association of VAT with BMD [6,28] and a positive correlation of SAT with BMD [6]. Other studies using DXA to determine body composition have found inverse associations of total body fat with BMD [5,32,33] and cortical CSA [5,34]. Potential explanations raised for these findings include the release of proinflammatory cytokines secreted by adipocytes, such as IL-6, TNF- α , and adipokines, such as E-selectin and adiponectin, stimulating osteoclast activity [6,35–37]. Our data suggest that IGF-1 may be an important mediator of the effects of VAT on the skeleton. VAT is a strong inverse determinant of endogenous GH secretion in women of reproductive age with obesity, and IGF-1 is also decreased in such women [15,16]. Our data demonstrate that IGF-1 correlates positively with BMD, independent of age and BMI. IGF-1 also correlated positively with P1NP, suggesting that IGF-1 increases collagen synthesis which is important to maintain appropriate levels of bone matrix. These findings are concordant with prior studies showing a strong positive correlation between IGF-1 and BMD [11–14] and between IGF-1 and P1NP [11,14]. GH and IGF-1 are key regulators of bone homeostasis and fat mass, with increased visceral fat being associated with decreased GH/IGF-1 levels [12,15]. IGF-1 regulates osteoblast activity by decreasing collagen degradation and increasing osteoblast recruitment [12], and circulating IGF-1 is necessary for bone growth [38]. We have previously demonstrated an inverse association between vertebral bone marrow fat and IGF-1 in premenopausal women ranging from normal weight to obese, supporting the role of IGF-1 as an important regulator of the fat and bone lineage [19]. A limitation of the use of single-energy QCT for the

assessment of BMD is the potential influence of marrow fat on BMD measurements. We therefore used data on vertebral bone marrow fat of 27 women. After controlling for bone marrow fat, the association between IGF-1 and BMD remained significant. This suggests that our results are not an artifact of our measurement technique. Our data from the current study therefore support a role for IGF-1 in the mediation of the negative effects of VAT on bone density.

Our data also suggest that muscle mass and quality play important roles in the maintenance of skeletal health in obese premenopausal women. Previously published data using DXA have suggested that lean body mass is an important determinant of bone density in premenopausal women. Lu et al [9] found that lean body mass, determined by DXA, was the strongest predictor of BMD of the spine and hip, whereas fat mass correlated positively with BMD of the hip in premenopausal women over a wide range of BMIs. Similarly, Wang et al [10] reported positive correlations between both lean and fat mass, determined by DXA, and BMD in young women, with lean mass having a greater positive effect on BMD than fat mass. Our data are consistent with these previously published data and are the first to examine this relationship using QCT to measure muscle area and quality, the previous studies all using surrogate measurements for muscle mass (“fat-free” or “lean body” mass), which do not directly measure muscle. Our study is also the first to focus exclusively on obese premenopausal women.

25(OH)D correlated positively with BMI and TAT, consistent with previous studies showing an inverse association between vitamin D levels and increased fat mass [39–42]. Half of our subjects had abnormal vitamin D levels, with 20% being vitamin D deficient and

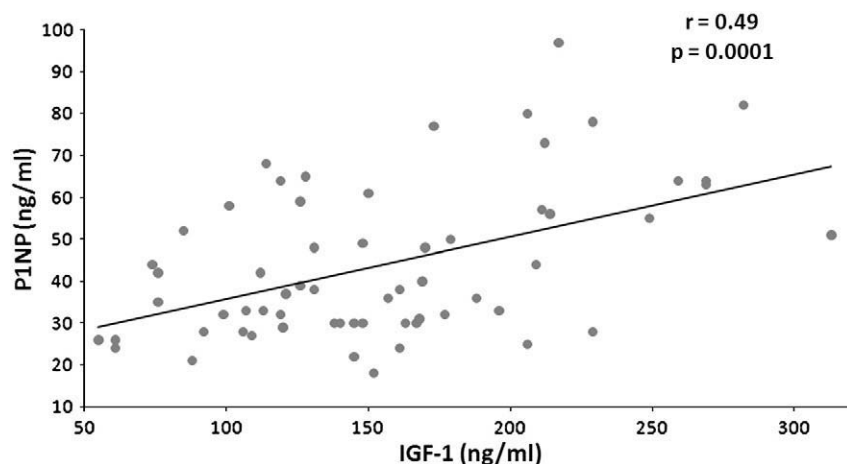


Fig. 5. Regression analysis of N-terminal propeptide of Type 1 procollagen (P1NP) on insulin-like growth factor-1 (IGF-1). There is a positive correlation between P1NP and IGF-1.

30% being vitamin D insufficient. We did not find a significant association between 25(OH)D and BMD, consistent with previous reports in adolescents and young adults [40,43]. A possible explanation for these findings might be that the detrimental effects of vitamin D deficiency on bone may not yet be present in premenopausal women. Alternatively, the effects of vitamin D levels on bone density in this population may not be strong enough to be detected in a small number of study subjects.

We failed to show a significant association between BMD and estradiol, SHBG, or testosterone. Some prior studies have shown associations between gonadal steroid levels and BMD in premenopausal women [44,45] where others, in which luteal phase [9] and follicular phase [46] serum estradiol and testosterone were measured, also failed to show significant correlations between BMD and gonadal steroids.

A limitation of our study is the cross sectional design, which limits our ability to prove causality. Second, our study was focused on obese premenopausal women, and our findings cannot be extrapolated to post-menopausal women or men. We also did not control sampling of estradiol and testosterone to the phase of the menstrual cycle. Strengths of our study were the large number of obese premenopausal women, detailed evaluation of body composition and trabecular bone using QCT, and inclusion of hormonal determinants such as IGF-1, P1NP, and 25(OH)D.

Conclusion

In conclusion, in obese premenopausal women, VAT is a negative, and thigh muscle CSA and density, IGF-1, P1NP are positive predictors of trabecular BMD. Our study supports the hypothesis that VAT exerts detrimental, and muscle positive effects on BMD. Moreover, IGF-1 was the strongest predictor of BMD and P1NP and was inversely determined by VAT in this population, suggesting that the effects of VAT on BMD may be at least partially mediated by IGF-1. Further studies are needed to investigate this hypothesis and other potential mediators of the effects of VAT on BMD.

Disclosures

The authors have no conflict of interest to declare.

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