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## A cross-sectional study of osteocalcin and body fat measures among obese adolescents

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### Abstract

Osteocalcin (OCN), a marker of osteoblast activity, has been implicated in the regulation of energy metabolism by the skeleton and thus may affect body fat measures. We examined the relationships of OCN to body fat measures and whether they vary according to markers of energy and vitamin D metabolism. Data was obtained from 58 obese adolescents aged 13–17.9 years (38 females, 8 black or African-American). We calculated total fat mass (FM) [dual X-ray absorptiometry (DXA)] and visceral adipose tissue (VAT) [computerized axial tomography (CT)]. Blood tests included leptin, OCN, 25-hydroxyvitamin D [25(OH)D], parathyroid hormone (PTH), thyroid function tests, and triglycerides. Markers of glucose metabolism were obtained from fasting and OGTT samples. Adolescents with 25(OH)D < 20 ng/mL were considered deficient (n=17/58); none had high PTH (PTH 65 pg/mL). OCN was associated with lower VAT ( $-84.27 \pm 33.89$  mm<sup>2</sup>) and BMI ( $-0.10 \pm 0.05$  kg/m<sup>2</sup>), not FM (p=0.597) in a core model including age, sex, race, geographic latitude, summer, height z-score, and tanner stage. Adding 25(OH)D deficiency and PTH attenuated the inverse association of OCN to VAT. We found a significant interaction of OCN and 25(OH)D deficiency on FM ( $0.37 \pm 0.18$  kg, p=0.041) and BMI ( $0.28 \pm 0.10$  kg/m<sup>2</sup>, p=0.007) in this adjusted model, which was further explained by leptin. Adding A1C to the core model modified the relationship of OCN to VAT ( $-93.08 \pm 35.05$  mm<sup>2</sup>, p=0.011), which was further explained by HOMA-IR. In summary, these findings provide evidence for a relationship between OCN and body fat measures that is dependent on energy metabolism and vitamin D status among obese adolescents.

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## Key terms

Osteocalcin; vitamin D deficiency; adolescents; obesity; fat mass; visceral fat

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## INTRODUCTION

There is increasing evidence of a cross-talk between adipose tissue and skeleton (1, 2). Osteocalcin (OCN), a marker of osteoblast activity, increases  $\beta$ -cell proliferation, insulin secretion, insulin sensitivity (fat, liver, and muscle), and energy expenditure (3). Leptin, a marker of adipocyte activity, reduces insulin secretion via several mechanisms and modulates Osteocalcin bioactivity (4).

As reviewed by Rauchenzauner (5) and Szulc (6), OCN concentrations may vary with age, gender, height, growth velocity, puberty, and time at blood draw. In vitro and in-vivo studies have also shown that insulin-like growth factor-I (IGF-I), calcium-dependent hormones such as parathyroid hormone (PTH), and metabolites of vitamin D may also affect OCN concentrations (7, 8). Higher OCN levels have been observed with exercise during puberty in (9) and in some (10) but not all adult studies (11). Lower OCN levels have been described among obese children and adolescents who were deficient in vitamin D (12, 13). In addition, there is some evidence from *in vivo* studies that vitamin D may be involved in energy metabolism (14). There is initial evidence that testosterone may affect OCN in children and adolescents, which is especially relevant during the period of rapid skeletal growth (15), and that inflammatory markers are associated with OCN in middle school aged children (16). There is also increasing evidence from *in vivo* studies that OCN may affect insulin sensitivity in both adults and children (16–20). Finally, OCN concentration has been associated with markers of the metabolic syndrome (21–22) and correlated with body mass index (BMI) (and total body fat measured by dual X-ray absorptiometry (DXA) in elderly individuals (21, 22) and more recently with markers of energy metabolism and BMI in a real life pediatric obesity clinic (20).

Given the significance of bone mass accretion during adolescence and the prevalence of obesity and 25(OH)D deficiency, the objective of this cross-sectional study was to assess the relationships of OCN with body fat measures and to determine whether these relationships varied according to markers of energy metabolism and vitamin D status. We hypothesize that the association of OCN and body fat measures may be explained at least in part by markers of energy and vitamin D metabolism among obese adolescents.

## METHODS AND PROCEDURES

### Study subjects and design

We used cross-sectional baseline data from adolescents enrolled in a Glaser Pediatric Research Network (GPRN) study of metformin, which is described elsewhere (13, 23, 24). In brief, the sample consisted of 58 obese adolescents aged 13.0 to 17.9 y. with a BMI  $\geq$  95th percentile and a weight  $<$ 136 kg (limit for the DXA-scan). Cross-sectional data included: 1) Demographic data (age, gender, race, ethnicity, medication, smoking, illicit drug use,

alcohol consumption, month of the year); 2) findings on physical examination (Tanner stage, anthropometric measurements); and, 3) laboratory assessments (blood tests, computerized axial tomography (CT), and whole-body DXA. None of the adolescents included in this study sample had diabetes or other known underlying disorders associated with obesity, had participated in a medical or surgical weight loss program, or used medications or substances that might affect growth and development, dietary intake, or physical activity.

Vitamin D status and bone metabolism were evaluated using non-fasting plasma samples obtained at each of the GCRC at the same time of the day (mid-morning) and stored frozen at  $-70^{\circ}\text{C}$  until analyzed at the *Vitamin D, Skin, and Bone Research Laboratory, Boston MA* (MFH, TC). Intact OCN was measured using an EIA from Quidel Corporation, CA, USA (Intra-assay and inter-assay CVs of 3.4–4.6% and 4.9–5.0%). Other laboratory assays were performed at *Esoterix Clinical Trials Services, Calabasas Hills, CA*; which also provided the reference range data used for the analyses. The CT scans were GE, Siemens, or Philips equipment, depending on the center (22). The DXA scans were performed using Hologic 4500 and Delphi-A scanners. Standard phantoms were circulated among four of the five GPRN sites for cross-calibration (25). All DXA scans were analyzed at the University of California, San Francisco using a standard software program (Hologic Delphi Manual software, Bedford, MA) and all CT scans were analyzed at Harvard Medical School using a standard software program (Photoshop CS2, Adobe Systems, San José, CA).

IRB approvals were obtained from the five GPRN sites (see appendix) and from the Boston University School of Medicine. Signed informed consent from a parent or other legal guardian of each subject, and age-appropriate assent was obtained prior to study.

## Analyses

Data are presented as mean $\pm$  standard deviation (SD) unless stated otherwise. We used correlation coefficients to compare sample characteristics with the independent variable of interest for the overall sample and by gender. Statistical significant is defined as  $p < 0.05$ . The outcomes for this cross-sectional data analysis included a variety of body fat measures such as total body fat mass (FM, kg), visceral adipose tissue (VAT,  $\text{mm}^2$ ), and BMI ( $\text{kg}/\text{m}^2$ ). Waist circumference, measured at the hip (24), was included as a second marker of central body fat. The independent variable of interest was OCN ( $\text{ng}/\text{mL}$ ).

A number of markers of energy and vitamin D status were selected to evaluate possible effect modification on the relationship of OCN to body fat measures. The concentration of 25(OH)D is currently considered the best indicator of vitamin D status (26, 27). 25(OH)D deficiency is presently best defined as concentrations of 25(OH)D  $< 20$   $\text{ng}/\text{mL}$  (26). As none of the adolescents had a PTH level above 65  $\text{pg}/\text{mL}$  (range upper limit) (13), we used PTH as a continuous variable in these analyses.

A number of indices were calculated based on fasting and 2 hour oral glucose tolerance test (OGTT) samples to estimate insulin resistance and glucose tolerance in relation to body fat measures (23, 24). The Homeostasis Model Assessment (HOMA) was calculated as  $[\text{glucose (mM)} \times \text{insulin } (\mu\text{U}/\text{mL})] / 22.5$  (28) to estimate fasting insulin resistance. Area-under-the-curve (AUC) for insulin and glucose were calculated from the OGTT data using the

trapezoidal rule. None of the adolescents had T2DM, as defined by fasting plasma glucose  $\geq 126$  mg/dL (7 mmol/L) or 2-hr OGTT glucose  $>200$  mg/dL (11.1 mmol/L) (29).

Pearson correlation coefficients were used to assess the associations between continuous variables, and point bi-serial coefficients (mathematically equivalent to Pearson correlation coefficient) were used to assess the association between continuous and dichotomous variables. Spearman correlation coefficients were used to corroborate the Pearson correlations and a few minor discrepancies in inference were noted, all cases of borderline  $p$ -value just above or below the conventional  $p=0.05$  cut-off point. No variables were so severely skewed in distribution as to require transformation for these analyses. We based our judgment that transformation was not required on 1) the close agreement of Pearson and Spearman correlations, and 2) the largely symmetric distribution of the dependent variables in regression analysis; the median lay within 0.1% of the mean for FM, 2.6% for VAT, 1.6% for waist circumference, and 2.5% for BMI.

We used multiple linear regression analysis to model continuous outcomes with Osteocalcin after adjusting for potential confounding variables. Covariates included variables suggested in the literature and those found to be significant in univariate analysis. We examined whether markers of energy glucose metabolism modified the relationship of OCN to VAT. We also examined whether PTH and 25(OH)D deficiency modified the relationships of OCN to VAT.

We used the multivariate models to test for interactions between relevant variables. We created an interaction term to evaluate the effect of increasing levels of OCN among vitamin D deficient adolescents on body fat measures. Interaction terms between OCN and gender or race were not found to be significant ( $p$  for interaction  $>0.05$ ) and thus were not included in the results section. As an example, the association between OCN and body fat measures did not vary significantly by gender ( $p$  for interaction = 0.736 for FM,  $p$  for interaction = 0.341 for VAT,  $p$  for interaction = 0.957 for WC, and  $p$  for interaction = 0.802 for BMI). Given that the interactions were not significant, further stratification of the data to identify whether the associations between OCN and body fat measures varied according to gender were not performed.

Among the outcome variables, three CT-scan measures and one waist circumference measure were missing. A physical activity measure based on accelerometer was only available for 24 of the 58 adolescents.

Based on a two-sided test at 0.05, a sample of 58 subjects provided 80% power for detecting a correlation coefficient of 0.35 between two variables, or for detecting an  $R^2$  of 0.12, 0.20, and 0.26 using multiple linear regression analyses with one, six and eleven variables. Analyses were performed using STATA (version 8). Given that the sample size for male adolescents ( $n=20$ ) and for female adolescents ( $n=38$ ) were much smaller, magnitude, not strength of associations between sample characteristics and OCN were presented.

## RESULTS

The overall sample OCN concentration was  $44.7 \pm 21.9$  ng/mL while OCN concentrations were  $59.6 \pm 22.3$  ng/mL for the boys and  $36.9 \pm 17.3$  ng/mL for the girls. The overall sample VAT was  $7436.5 \pm 3632.7$  mm<sup>2</sup> and FM  $39.9 \pm 9.8$  kg, which corresponded to a VAT of  $12.4 \pm 4.3\%$  and FM of  $40.0 \pm 5.5\%$ . Waist circumference was  $105.7 \pm 12.1$  cm. BMI was  $36 \pm 5$  kg/m<sup>2</sup> and 29 of the 58 subjects (50%) had a BMI between the 99.0 and 99.9th percentile. A total of 17 subjects (29%) had a 25(OH)D level below 20 ng/mL (25(OH)D deficiency). As previously reported (13), none of the subjects had an abnormal PTH concentration, as the highest value was 48 pg/mL. The HOMA-IR index was  $4.5 \pm 3.2$  and the HbA<sub>1C</sub> was  $5.4 \pm 0.3\%$ , with one subject at 6.5%, but all had normal OGTTs.

In the overall sample, we found a strong correlation between gender and OCN ( $r=0.50$ ). OCN was directly correlated with Tanner stage and geographic latitude ( $P<0.05$ ) and inversely correlated with age ( $p<0.05$ , Table 1). There were no significant correlations between OCN and black or African American race, summer, or height z-score ( $p>0.05$ ). Metabolic factors directly associated with OCN included TSH ( $r=0.28$ ), IGF1 ( $r=0.31$ ), and IGFBP-1 ( $r=0.48$ )  $p<0.05$ , while 25(OH)D ( $r=-0.28$ ) and T4 ( $r=-0.30$ ) were negatively associated with OCN ( $p<0.05$ ), but none of the other metabolic factors examined were significantly associated with OCN ( $p>0.05$ ). Overall, the correlations coefficients between sample characteristics and OCN for male and female adolescents were similar except for leptin and the markers of carbohydrate and lipid metabolism.

### Relationship of Osteocalcin to body fat measures

In simple regression analysis (Table 3), each increment of 1ng/mL OCN was inversely associated with FM ( $-0.12 \pm 0.06$  kg,  $p=0.047$ ) and BMI ( $-0.11 \pm 0.03$  kg/m<sup>2</sup>,  $p=0.001$ ) but not with VAT ( $-33.31 \pm 21.95$  mm<sup>2</sup>,  $p=0.135$ ). After adjusting for age, gender, black or African-American race, geographic latitude, summer season, height z-score, and Tanner stage 2–3, OCN was significantly and inversely associated with VAT ( $-84.27 \pm 33.89$  mm<sup>2</sup>,  $p=0.017$ ) and BMI ( $-0.10 \pm 0.05$  kg/m<sup>2</sup>,  $p=0.037$ ), but not with FM ( $-0.04 \pm 0.08$  kg,  $p=0.597$ ). The association of OCN and waist circumference was similar to those of OCN and VAT in multivariate analysis.

### Effect modification of vitamin D status

The addition of 25(OH)D deficiency and PTH to the core model did not affect the relationship of OCN to BMI ( $-0.11 \pm 0.05$  kg/m<sup>2</sup>,  $p=0.020$ ) but attenuated the inverse relationship of OCN to VAT ( $-74.40 \pm 37.39$  mm<sup>2</sup>,  $p=0.053$ ). However, vitamin D deficient adolescents with higher OCN levels were more likely to have higher FM ( $0.37 \pm 0.18$  kg,  $p=0.041$ ) and BMI ( $0.28 \pm 0.10$  kg/m<sup>2</sup>,  $p=0.007$ ) but not VAT ( $102.95 \pm 83.34$  mm<sup>2</sup>,  $p=0.223$ ) compared to those who were not deficient with higher OCN levels.

As some investigators have suggested that leptin modulates OCN activity, we further evaluated the effect of leptin on the relationship of OCN and body fat measures as well as the interaction term between OCN and 25(OH)D deficiency on body fat measures. The addition of leptin to the core model with PTH, 25(OH)D deficiency, and the interaction term

between 25(OH)D deficiency and OCN did not attenuate the relationship of OCN to body fat measures but explained the relationship of the interaction term to FM and BMI.

### Effect modification of markers of glucose metabolism

Markers of insulin resistance such as fasting insulin, HOMA-IR, and the area-under-the-curve for insulin attenuated the relationships of OCN to VAT and BMI. As an example, the relationship of OCN to VAT was modified from  $-84.27 \pm 33.89$  mm<sup>2</sup> ( $p=0.017$ ) to  $-62.56 \pm 35.75$  mm<sup>2</sup> ( $p=0.087$ ; Table 4) by HOMA-IR.

The relationship of OCN to VAT also varied with markers of glucose tolerance such as the area-under-the-curve glucose, fasting glucose, and Hb<sub>A1C</sub>. The direction of the relationship between OCN and VAT varied with the markers. The area-under-the-curve glucose explained the association between OCN and VAT ( $-56.20 \pm 36.38$ ,  $p=0.130$ ; data not shown), no magnitude change was observed between OCN and VAT ( $-86.14 \pm 33.92$ ,  $p=0.015$ ; data not shown) for fasting glucose, but there was a stronger inverse association between OCN and VAT ( $-93.08 \pm 35.04$ ,  $p=0.011$ ; Table 4) for Hb<sub>A1C</sub>. However, the addition of HOMA-IR to the core model with A1C explained the relationship of OCN to VAT ( $-69.98 \pm 38.00$ ,  $p=0.073$ ). In addition, the relationship of OCN to VAT was attenuated by markers of lipid metabolism, growth, and the thyroid axis (Table 4).

## DISCUSSION

Overall, the study findings indicate that VAT and BMI are inversely associated with OCN. To our knowledge, this is the first study to suggest that the relationship of OCN to body fat measures in obese adolescents is dependent on markers of energy and vitamin D status.

Data indicating a reciprocal relationship between bone and energy metabolism comes primarily from animal studies (2), with limited data in humans. A recently published study in elderly individuals demonstrated an association between OCN and several plasma markers of energy metabolism and body fat measures (21). This cross sectional study of 380 obese adults 65 years old and older showed an inverse relationship between OCN and body fat measures (including DXA) before and after adjusting for age, gender, physical activity, smoking, and education. Similarly, we used a multivariate model to assess the relationship between OCN and body fat measures. Covariates included age, gender, black or African-American race, geographic latitude (North of Atlanta), summer, height z-score, and Tanner stage 2–3 vs. 4–5. After adjusting for these covariates, we found a similar inverse association between OCN and body fat measures (Table 3).

In our study population, 21% were in an early to mid pubertal stage (Tanner stage II-III), when the velocity of linear growth is expected to be at its peak, and 29% of the individuals were considered to be Vitamin D deficient. In Reinehr's study 57% of the children were pre-pubertal (Tanner stage 1) but data on 25(OH)D deficiency was not available. Previous pediatric studies have shown greater levels of OCN during linear growth but lower levels among adolescents with 25(OH)D deficiency and among obese pre-pubertal African American children (12). Using a sample of obese adolescents of mixed race/ethnicity, we

confirmed that individuals at Tanner stage II-III were more likely to have higher OCN levels and those with 25(OH)D deficiency were more likely to have lower OCN.

In our study sample, PTH levels were found to be within the normal range but were higher among obese adolescents Tanner stage II-III and among those with 25(OH)D deficiency (13). There is some evidence that PTH may activate the transcription of the Osteocalcin promoter (8) and thus affect OCN in the plasma, however the effect of PTH on the osteoblast is still controversial (31). Based on current available data from studies of 25(OH)D deficiency and PTH levels, we cannot exclude the possible opposite relationship of PTH and 25(OH)D deficiency to body fat measures. In our study, the inverse association of Osteocalcin to VAT was attenuated by the addition of 25(OH)D deficiency and PTH to the model (Table 3).

Menendez (32) found that 25(OH)D may inhibit *in vitro* leptin secretion by adipose tissue but Tarcin *et al.* (14) found that leptin levels were positively correlated with 25(OH)D *in vivo*. Although the relationship between leptin and 25(OH)D remains unclear, it has been suggested that leptin may be involved in the regulation of OCN activity via several mechanisms (3). A previous pediatric obesity study showed that leptin was inversely correlated with OCN in a sample of younger and mostly pre-pubertal obese children (20). We found an inverse correlation of leptin to OCN but these correlation coefficients were smaller and non-significant. In our study, the interaction between OCN and 25(OH)D deficiency on FM and BMI was significant, but this effect was further explained by leptin levels. After adjusting for all covariates, OCN was strongly and inversely associated with VAT, WC, and BMI, but not with FM.

There is evidence from animal models (3) and adult studies (16–22, 33–34) affect glucose metabolism, and thus body fat measures. In our study sample, we found that A1C affected the relationship of OCN with body fat measures (VAT, WC, and BMI), which was further explained by HOMA-IRI. In our study, levels of HOMA\_IR index ( $5.45 \pm 3.19$ , Table 2) were higher and showed more variation than those of Reinehr ( $3.5 \pm 0.3$ ). As opposed to our study, Reinehr (20) found a significant inverse association between OCN and HOMA-IR in univariate analysis, but data on A1C and relationships of OCN to other body fat measures were not available.

OCN has not only been shown to raise insulin secretion and improve insulin sensitivity (3, 34) but there is also increasing evidence that OCN may be related to improved glycemic control in adults with T2DM (32, 35).

We are further intrigued by the effect of thyroid function tests on the relationship of OCN and body fat measures. We observed an attenuation of the relationship between OCN and VAT but not between OCN and other body fat measures when TSH and T4 were included in the core model (Table 4). Although, there is limited evidence of an association between TSH and OCN, there is evidence of an association between TSH and body fat measures. In fact, central fat accumulation has previously been associated with elevated TSH in adults (37, 38). To the best of our knowledge, this is the first study to suggest that measures of thyroid



function may modify the relationship between OCN and body fat measures in obese adolescents.

The limitations of this study include a cross-sectional design, which does not provide information on causality. As opposed to Boucher-berry, we did not have information on undercarboxylated and caboxylated OCN (16), but rather on OCN which is highly correlated with under-carboxylated and possibly a better marker for insulin sensitivity. Like Reinehr (20) and Boucher-Berry (16), we did not have data on sex hormones but we had data on Tanner stage. Therefore, we were able to adjust for the pubertal stage at the peak of skeletal growth when testosterone changes are most relevant. Our results are consistent with prior causal relationships identified in previous animal and human studies. Although our findings cannot be generalized to all populations, they may indicate directions for future investigation. Despite a limited sample size, we were able to provide initial evidence for a relationship between OCN and body fat measures, which may vary according to metabolic factors.

In conclusion, our study findings provide initial insight into the relationships of and body fat measures among obese adolescents during puberty and may serve as an important first step for future research into metabolic factors influencing adolescent obesity.

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## Study Group

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(clinical sites are listed in the order of the number of subjects enrolled into this study)

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Table 1

General sample characteristics associated with osteocalcin

Characteristics	Overall sample			Boys (n=20)		Girls (n=38)	
	Mean±SD or n(%)	Correlation coefficient <sup>1</sup>	P value	Mean±SD or n(%)	Correlation coefficient <sup>1</sup>	Mean±SD or n(%)	Correlation coefficient <sup>1</sup>
Age, y	14.9±1.4	<b>-0.35</b> **	0.008	15.21±1.54	-0.50	14.7±1.3	-0.50
Male Gender	20 (34)	<b>0.50</b> ***	<0.001	-	-	-	-
Black Race	8(14)	-0.12	0.373	3(15)	-0.06	5(13)	-0.28
Summer Season	12(21)	0.22	0.095	3(15)	0.14	9(24)	0.42
North of Atlanta	36(62)	<b>0.30</b> *	0.024	16(80)	0.05	20(53)	0.28
T5 (II-II vs. IV-V) <sup>2</sup>	12(21)	<b>0.53</b> ***	<0.001	8(40)	0.61	4(11)	0.26
Height z-score, s.d.	0.34±0.98	0.16	0.225	0.29±0.84	0.08	0.36±1.05	0.28

<sup>1</sup> Pearson correlations were used between continuous variables and point biserial correlation coefficients were used between continuous and dichotomous variables (mathematically equivalent to Pearson correlations)

<sup>2</sup> T5 (II-III vs. IV-V) corresponds to the variable Tanner stages two to three compared to stages four to five for pubic hair

\* p<0.05,

\*\* p<0.01,

\*\*\* p<0.001, testing for non-zero correlation. Testing not performed for subsamples of boys and girls.

Table 2

Biochemical characteristics associated with osteocalcin by gender

Characteristics	All subjects (n=58)			Boys (n=20)		Girls (n=38)	
	Mean±SD or n(%)	Correlation coefficient <sup>†</sup>	p value	Mean±SD or n(%)	Correlation coefficient <sup>†</sup>	Mean±SD or n(%)	Correlation coefficient <sup>†</sup>
[25(OH)D] <20 ng/mL	17(29)	<b>-0.28</b> *	0.036	4(20)	-0.20	13(34)	-0.26
PTH, pg/mL	15.9±9.8	<b>0.37</b> **	0.004	18.5±10.1	0.30	14.51±9.5	0.34
Leptin, ng/mL	36±18.5	-0.24	0.065	23.7±15.4	0.15	42.4±16.8	-0.09
HOMA-IR index	4.5±3.2	-0.06	0.642	4.9±4.1	-0.24	4.2±2.6	-0.02
A1C, %	5.4±0.3	0.04	0.776	5.5±0.4	-0.15	5.3±0.3	-0.04
Triglycerides, mg/dL	126.6±104.2	0.11	0.425	164.1±158.7	-0.12	106.9±51.3	0.15
Thyroxine, mcg/dL	7.6±1.6	<b>-0.30</b> *	0.024	7.1±1.4	0.15	7.9±1.7	-0.42
TSH, mcU/mL	1.6±1.0	<b>0.28</b> *	0.031	1.7±1.0	0.36	1.5±1.0	0.24
IGF1, ng/mL	339.6±94.0	<b>0.31</b> *	0.018	366.7±87.0	0.10	325.3±95.5	0.33
IGBP-1, ng/mL	2.4±4.7	<b>0.48</b> ***	<0.001	3.7±7.6	0.58	1.8±1.7	0.33

<sup>†</sup> Pearson correlation coefficient was used between continuous variables and point biserial correlation coefficient was used between continuous and dichotomous variables (mathematically equivalent to Pearson correlations); and

\* p<0.05,

\*\*

p<0.01,

\*\*\*

p<0.001, testing for non-zero correlation

Table 3

Relationship of osteocalcin to body fat measures after adjusting for covariates and markers of vitamin D status

Models	FM, $l$ kg ( $\beta \pm$ s.e.)	VAT, $l$ mm <sup>2</sup> ( $\beta \pm$ s.e.)	WC, $l$ mm <sup>2</sup> ( $\beta \pm$ s.e.)	BMI, $l$ kg/m <sup>2</sup> ( $\beta \pm$ s.e.)
<b>Univariate<sup>2</sup></b>				
Osteocalcin (OC)	<b>-0.12±0.06*</b>	-33.31±21.95	<b>-0.16±0.07*</b>	<b>-0.11±0.03**</b>
<b>Multivariate (MV)<sup>3</sup></b>				
OC	-0.04±0.08	<b>-84.27±33.89*</b>	<b>-0.23±0.10*</b>	<b>-0.10±0.05*</b>
<b>MV + PTH + Vitamin D deficiency (VDD)<sup>4</sup></b>				
OC	-0.05±0.08	-74.40±37.39	<b>-0.23±0.11*</b>	<b>-0.11±0.05*</b>
<b>MV + PTH + VDD + Interaction term<sup>5</sup></b>				
OC	-0.08±0.08	<b>-86.53±38.44*</b>	<b>-0.26±0.11**</b>	<b>-0.14±0.05**</b>
Interaction term	<b>0.37±0.18*</b>	102.95±83.34	0.34±0.25	<b>0.28±0.10**</b>
<b>MV + PTH + VDD + Interaction term + leptin<sup>6</sup></b>				
OC	-0.10±0.06	<b>-87.91±35.77*</b>	<b>-0.28±0.10**</b>	<b>-0.15±0.04***</b>
Interaction term	0.10±0.15	25.51±82.42	0.02±0.24	0.13±0.08

<sup>1</sup>Total body fat (FM, n=58), visceral adipose tissue (VAT, n=55), waist circumference (WC, n=57) and Body mass Index (BMI, n=58) were used as dependent variable

<sup>2</sup>Osteocalcin (OC) is used as the independent variable

<sup>3</sup>Covariates in the multivariate (MV) model of OC to body fat measures include age, sex, black or African-American race, latitude, height z-score, and Tanner stage 2-3 vs. Tanner stage 4-5

<sup>4</sup>The MV+ PTH + vitamin D deficiency (VDD) includes covariates in the multivariate model plus parathyroid hormone and vitamin D deficiency

<sup>5</sup>The MV + PTH + VDD + interaction term includes covariates in the multivariate model plus parathyroid hormone, vitamin D deficiency, and an interaction term between vitamin D deficiency and increasing osteocalcin concentrations

<sup>6</sup>The MV + PTH + VDD + interaction term + leptin includes covariates in the multivariate model plus parathyroid hormone, vitamin D deficiency, an interaction term between vitamin D deficiency and increasing osteocalcin concentrations, as well as leptin concentrations



Relationships of osteocalcin to body fat measures after adjusting for covariates and markers of energy metabolism

**Table 4**

Models	FM, $I$ kg ( $\beta \pm$ s.e.)	VAT, $I$ mm <sup>2</sup> ( $\beta \pm$ s.e.)	WC, $I$ mm <sup>2</sup> ( $\beta \pm$ s.e.)	BMI, $I$ kg/m <sup>2</sup> ( $\beta \pm$ s.e.)
<b>Multivariate (MV)<sup>2</sup></b>				
OC	-0.04±0.08	<b>-84.27±33.89*</b>	<b>-0.23±0.10*</b>	<b>-0.10±0.05*</b>
<b>MV + HOMA-IR<sup>3</sup></b>				
OC	0.03±0.08	-62.56±35.75	-0.17±0.11	-0.06±0.05
<b>MV + HbA<sub>1c</sub><sup>4</sup></b>				
OC	-0.07±0.08	<b>-93.08±35.05**</b>	<b>-0.28±0.10**</b>	<b>-0.12±0.05*</b>
<b>MV + IGF1 + IGFBP-1<sup>5</sup></b>				
OC	-0.03±0.09	-48.24±35.33	-0.14±0.11	-0.09±0.05
<b>MV + Trig<sup>6</sup></b>				
OC	-0.03±0.08	<b>-74.79±35.82*</b>	-21.08±0.11	-0.09±0.05
<b>MV + T4 + TSH<sup>7</sup></b>				
OC	-0.04±0.08	-67.96±34.64	<b>-0.22±0.11*</b>	<b>-0.10±0.05*</b>

<sup>1</sup>Total body fat (FM, n=58), visceral adipose tissue (VAT, n=55), waist circumference (WC, n=57) and Body mass Index (BMI, n=58) were used as dependent variable

<sup>2</sup>Covariates in the multivariate (MV) model of OC to body fat measures include age, sex, black or African-American race, latitude, summer, height z-score, and Tanner stage 2-3 vs. Tanner stage 4-5

<sup>3</sup>The MV + HOMA-IR includes covariates in the multivariate model plus the Homeostasis Model Assessment – Insulin resistance

<sup>4</sup>The multivariate + A1C includes covariates in the multivariate model plus hemoglobin A1C

<sup>5</sup>The multivariate + IGF1 + IGFBP1 includes covariates in the multivariate model plus the insulin growth factor one and the insulin growth factor binding protein one

<sup>6</sup>The MV + Trig includes covariates in the multivariate model plus triglycerides

<sup>7</sup>The MV + T4 + TSH includes covariates in the multivariate model plus the thyroid hormone and thyroid stimulating hormone