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Association of Osteocalcin With Obesity, Insulin Resistance, and Cardiovascular Risk Factors in Young Adults

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Abstract

Low levels of osteocalcin (OCN), an osteoblast-specific hormone, have recently been associated with insulin resistance (homeostasis model assessment-insulin resistance (HOMA-IR)) and obesity, particularly in older adults. The aim of this study was to determine whether low levels of OCN would be associated with insulin resistance, obesity, and greater cardiovascular (CV) risk in young adults just emerging from adolescence. Undercarboxylated OCN and carboxylated OCN levels were measured on stored serum samples (total OCN = undercarboxylated OCN + carboxylated OCN) on 137 participants (67 males) at mean age 18.6 years (range 17–22 years). Insulin resistance was measured by hyperinsulinemic–euglycemic clamp (M_{Ibm}). Multivariable regression analyses with $\ln(OCN)$ as the independent variable were adjusted for age, sex, ethnicity, and BMI as indicated. Total OCN was inversely related to BMI, waist circumference, systolic blood pressure (SBP), interleukin (IL)-6, and directly related to M_{Ibm} ; only SBP remained significant (with M_{Ibm} $P = 0.0560$) after further adjustment for BMI. Carboxylated OCN was inversely related to BMI, waist circumference, SBP, low-density lipoprotein cholesterol (LDL-C), and directly related to adiponectin; SBP and adiponectin remained significant after further adjustment for BMI. There were no significant associations with undercarboxylated OCN. In summary, most associations with OCN were mediated via BMI. However, the significant associations of OCN with SBP, obesity, and adiponectin and borderline with M_{Ibm} , suggest a potential role for OCN in the development of insulin resistance and CV risk that becomes more apparent with aging into older adulthood.

INTRODUCTION

Recent studies have reported potential associations between the bone-specific hormone osteocalcin (OCN) and both adiposity and glucose metabolism. OCN levels positively correlate with osteoblast activity in the process of new bone formation. OCN undergoes post-translational modification whereby glutamic acid residues are carboxylated producing Gla-OCN (1); this process is, in part, regulated by leptin and sympathetic tone (2). The acidic environment of activated osteoclasts is thought to result in the decarboxylation of OCN (3) to produce undercarboxylated OCN, or Glu-OCN. The roles of total, carboxylated, and undercarboxylated OCN relative to adiposity and its related metabolic effects are currently not well defined.

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DISCLOSURE

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Data on the association between OCN and adiposity-related factors (insulin resistance and other cardiovascular (CV) risk factors) are available from some animal and human studies. Mouse studies have shown an inverse association of OCN with hepatic and peripheral insulin resistance and a direct association with insulin secretion and pancreatic β -cell mass (4,5). In addition, OCN infusion in mice decreases adiposity and is protective against the development of diabetes and obesity (5).

The majority of published reports in humans are in older adults (6–15), showing that higher total OCN is associated with greater insulin sensitivity (measured by indirect methods, e.g., homeostatic model assessment-insulin resistance (HOMA-IR) or fasting insulin), lower BMI, and lower levels of C-reactive protein (CRP), triglycerides, and visceral fat mass (8,10,13). Overweight and obese adults have been reported to have lower undercarboxylated OCN than normal weight adults (12). Middle-aged adults with OCN levels in the upper tertiles had lower fasting glucose and higher insulin sensitivity (9), and adults with type 2 diabetes mellitus had lower osteocalcin levels compared to those with normal glucose tolerance (15).

Very few studies have been published on the association between OCN and adiposity-related factors in children. Total OCN was lower in obese children compared to normal weight children and was negatively related to levels of leptin and insulin resistance measured by HOMA-IR after adjusting for age, gender, and pubertal status (16). Both undercarboxylated and carboxylated OCN were associated with insulin sensitivity by oral glucose tolerance testing in overweight children with normal glucose tolerance and prediabetes (17). Both of these studies used surrogate measures of insulin resistance.

Adult CV disease has its antecedents before adulthood (18), with gender- and BMI-related adverse changes in CV risk factors and insulin resistance emerging during the second decade (19). The present study is the first to evaluate associations between OCN and insulin resistance as determined by the “gold standard” euglycemic–hyperinsulinemic clamp, and expands on previous studies by describing the associations of OCN with adiposity, insulin resistance and related variables, and CV risk factors, in a cohort of early young adults with a broad range of BMI and insulin sensitivity.

METHODS AND PROCEDURES

Participants

The cohort for this study was derived from participants who were randomly selected after a school screening at mean age 13 years for a longitudinal study of obesity and insulin resistance (20). From an initial enrollment of 357 participants, 224 had a follow-up study visit at mean age 18.6 years (range 17–22 years) and 137 of these had stored serum samples available for measurement of OCN. This group was not significantly different from the 87 without OCN data for any of the variables listed in Tables 1 and 2.

The study was approved by the University of Minnesota Institutional Review Board. Written informed consent was obtained from the children and their parents/guardians (participant <18 years) or from adult participants.

Measures of OCN and CV risk

Undercarboxylated and carboxylated OCN were measured using EIA kits from Takara Bio USA (Madison, WI) in the Advanced Research and Diagnostic Laboratory, University of Minnesota. The undercarboxylated EIA kit uses a set of monoclonal antibodies reactive to the decarboxylated OCN and less reactive to carboxylated OCN at amino acid positions 17, 21, and 24. The carboxylated EIA kit uses a set of monoclonal antibodies reactive to

carboxylated OCN and less reactive to decarboxylated OCN at amino acid position 17. Total OCN was calculated by the sum of carboxylated and undercarboxylated OCN. All blood samples used in this study for OCN underwent one previous freeze-thaw cycle. Stability testing performed on samples from normal volunteers showed that two freeze-thaw cycles resulted in a 7% decrease in carboxylated OCN and an 11% decrease in undercarboxylated OCN levels compared to samples with only one freeze-thaw cycle. Intra-assay variability was 5.7% for carboxylated OCN and 17.3% for undercarboxylated OCN.

BP was measured twice on the right arm of seated participants with a Random Zero sphygmomanometer, and the average of the two systolic BP (SBP) was analyzed. Insulin resistance was measured by the euglycemic-hyperinsulinemic clamp and normalized to lean body mass (lbm) and expressed as M_{lbm} as described previously (20). A lower value of M_{lbm} indicates greater insulin resistance. HOMA-IR was calculated as fasting insulin concentration ($\mu\text{U/ml}$) $\times$ fasting glucose concentration (mmol/l)/22.5. Lipids were measured in the University of Minnesota-Fairview laboratory. Cholesterol was determined by a standard enzymatic-cholesterol oxidase-based method; high-density lipoprotein-cholesterol (HDL-C) was determined after precipitation of non-HDL lipoproteins with magnesium/dextran precipitating reagent. Triglycerides were determined with a standard glycerol blanked, enzymatic triglyceride method. Low-density lipoprotein-cholesterol (LDL-C) was calculated by the Friedewald equation. Adipocytokines were measured in the University of Minnesota cytokine laboratory. Interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and adiponectin were measured by enzyme-linked immunosorbent assay, and CRP was measured by an ultrasensitive colorimetric competitive enzyme-linked immunosorbent assay (21). The “CV risk factor cluster score” was used as a measurement of clustering of components of CV risk factors (based on average z -score for fasting insulin, SBP, triglycerides, and HDL-C) (21). Bone mineral content (BMC) was measured by dual-energy X-ray absorptiometry (G.E. Lunar Prodigy scanner; GE Healthcare, Madison, WI). Because we did not have a marker of bone formation besides OCN, we used total BMC as a surrogate measure of overall bone formation with the assumption that higher BMC has higher osteoblast activity. This is important because BMI is significantly correlated with BMC in our cohort ($r = 0.39$, $P < 0.0001$) and it is, therefore, difficult to separate out the influence of overall osteoblast activity on the association between OCN and BMI.

Statistical analysis

Partial Pearson’s correlation analysis was performed on undercarboxylated OCN, carboxylated OCN, total OCN, BMI, and total body BMC adjusting for age, ethnicity, and sex. Analyses with the ratio of undercarboxylated to carboxylated OCN did not provide additional information and are not presented. Separate multivariable regression analyses were performed to determine associations of $\ln(\text{OCN})$ as the independent variable (separate regressions for carboxylated, undercarboxylated, and total OCN) with each of the dependent variables (BMI, percent body fat, waist circumference, SBP, insulin sensitivity (M_{lbm}), fasting glucose, fasting insulin, HOMA-IR, total cholesterol, HDL-C, LDL-C, triglycerides, adiponectin, IL-6, TNF- α , CRP, and the CV risk factor cluster score). To study goodness of fit and to further expose the statistical role of gender for several of the interesting relationships, the mean of each dependent variable was computed within sex-specific quartile of the OCN variable, and plotted on the natural OCN scale together with the sex-adjusted line. All analyses were adjusted for age, ethnicity, and sex; all analyses except BMI were adjusted for age, ethnicity, sex, and BMI.

All variables with a maximum value more than ten times the minimum value were natural log transformed for analysis (undercarboxylated OCN, carboxylated OCN, total OCN, percent body fat, HOMA-IR, triglycerides, adiponectin, IL-6, TNF- α , and CRP) and back-transformed when appropriate for presentation of results. Statistical significance for all

analyses was set at $P < 0.05$. Statistical analyses were done using SAS version 9.2 (SAS, Cary, NC). Mean and SE are presented in descriptive statistics.

RESULTS

Participant characteristics

The mean age of participants was 18.6 ± 0.1 years and mean BMI was 25.5 ± 0.5 kg/m², with 16% obese, 21% overweight, and 63% normal weight. Based on fasting plasma glucose, only one participant had diabetes, and 7% had impaired fasting glucose. No participant had SBP >140 mm Hg, 7% had cholesterol >200 mg/dl, 13% had triglycerides >150 mg/dl, and 1% had LDL-C >160 mg/dl. None were receiving antihypertensive or lipid-lowering medications at the time of these studies.

Measures of OCN

For the entire cohort, mean carboxylated OCN was 23.7 ± 1.0 ng/ml (median 20.5 ng/ml), mean undercarboxylated OCN was 12.7 ± 0.6 ng/ml (median 11.1 ng/ml), and mean total OCN was 36.4 ± 1.3 ng/ml (median 34.2 ng/ml). Total OCN was significantly correlated with undercarboxylated OCN ($r = 0.66$, $P < 0.0001$) and carboxylated OCN ($r = 0.85$, $P < 0.0001$), as expected, since total OCN was determined by the sum of undercarboxylated and carboxylated OCN. Undercarboxylated OCN and carboxylated OCN were significantly, but not strongly, correlated ($r = 0.23$, $P = 0.008$). BMC was significantly correlated with total OCN ($r = -0.18$, $P = 0.04$) and carboxylated OCN ($r = -0.18$, $P = 0.04$), but not undercarboxylated OCN ($r = -0.04$, $P = 0.68$).

Differences in OCN distribution by gender are presented in Table 3. Women had significantly lower total and carboxylated OCN compared to men ($P < 0.0001$ and $P < 0.0001$, respectively) in association with a decreasing percent of women by increasing quartile of OCN; there was no significant difference in undercarboxylated OCN distribution by quartiles between men and women, and the percentage of women was similar in each quartile. Although females had higher percent body fat compared to males ($37.8 \pm 1.3\%$ vs. $19.5 \pm 1.2\%$, $P < 0.0001$), lower lbm compared to males (41.6 ± 0.8 kg vs. 60.8 ± 0.9 kg, $P < 0.0001$), and lower BMC compared to males (2.68 ± 0.05 kg vs. 3.31 ± 0.06 kg, $P < 0.0001$), women continued to have significantly lower total and carboxylated OCN after adjustment for percent body fat ($P = 0.004$ and $P = 0.002$ respectively), lbm ($P = 0.0001$ and $P < 0.0001$ respectively), or BMC (both $P < 0.0001$).

Relation of OCN to obesity

Total OCN was inversely related to BMI and waist circumference (both $P = 0.013$). Carboxylated OCN was inversely related to BMI and waist circumference ($P = 0.005$ and $P = 0.011$, respectively), with a trend toward an association with percent body fat ($P = 0.065$). Adjustment for BMC attenuated the relations with both total and carboxylated OCN, with only the relation between carboxylated OCN and BMI remaining statistically significant ($P = 0.038$) (Figure 1). After back transformation of total and carboxylated OCN, the sex-specific fourth quartile (Q4) OCN median minus the first quartile (Q1) OCN median was approximately equal to the regression slope, meaning that the prediction from the slope matches the observed means of the extreme cells (Tables 1 and 2). Undercarboxylated OCN was not significantly associated with BMI.

Relation of OCN to insulin resistance, CV risk factors, and adipocytokines

BMI was significantly associated with most of the dependent variables (all P values < 0.01); therefore, analyses were run with and without BMI included as an independent variable (Tables 1 and 2). Total OCN was inversely associated with waist circumference ($P = 0.013$),

SBP ($P = 0.020$), IL-6 ($P = 0.020$) and the CV risk factor cluster score ($P = 0.050$), and negatively associated with insulin resistance ($P = 0.029$). Carboxylated OCN was inversely associated with waist circumference ($P = 0.011$), SBP ($P = 0.002$), LDL-C ($P = 0.015$), and the CV risk factor cluster score ($P = 0.021$), and positively associated with adiponectin ($P = 0.004$). When BMI was added to the total and carboxylated OCN models, BMI remained significantly associated with percent body fat, waist circumference, fasting insulin, HOMA-IR, cholesterol, HDL-C, LDL-C, triglycerides, adiponectin, IL-6, and the CV risk factor cluster score in both OCN models. However, the associations between the dependent variables and OCN were significantly attenuated, except for the association of total OCN with SBP and carboxylated OCN with SBP and adiponectin (Figure 1). There were no significant associations between undercarboxylated OCN and any of the dependent variables.

DISCUSSION

This study provides new information on associations of OCN, particularly carboxylated OCN, with BMI, insulin resistance, and CV risk factors in early young adulthood (age range 17–22). Although levels among total, carboxylated, and undercarboxylated OCN were significantly correlated, there were substantial differences in their relations with measures of obesity, insulin sensitivity and the CV risk factors, and significant differences between males and females. Individuals with the lowest total or carboxylated OCN had the highest mean BMI. Most of the associations for total and carboxylated OCN with insulin sensitivity and the CV risk factors were significant but were attenuated after adjustment for BMI.

By using a direct measure of insulin resistance, i.e., the euglycemic–hyperinsulinemic clamp, not previously used in studies of OCN, this study demonstrated a significant inverse relation between total OCN and insulin resistance that is present as early as the end of adolescence in apparently healthy individuals. This relation was significantly attenuated by BMI, becoming of borderline significance ($P = 0.056$), suggesting that it is mediated to a major degree via BMI at this age. Previous studies in adults have shown an inverse relation between both total OCN and undercarboxylated OCN and insulin resistance, as measured by HOMA-IR (7–9,16,22–24) and HOMA- β -cell function (9,11,25), and adult patients with type 2 diabetes mellitus have lower OCN levels (15).

Studies in children have not been entirely consistent with each other. Results in 5–9-year-olds showed that a higher ratio of undercarboxylated to carboxylated OCN was associated with higher HOMA- β (not with HOMA-IR) (25); a second study (children mean age 11) showed a significant inverse relation between total OCN and HOMA-IR (16); and a third study in prepubertal children with normal glucose tolerance and with prediabetes by oral glucose tolerance test showed a significant inverse relation between carboxylated OCN and insulin resistance (17).

The reason for finding only a borderline significant relation between OCN and insulin resistance in the current study compared to the significant associations in previously published studies is not clear but may be related to age or selection of the cohorts, to methodological differences, or, in particular, to the fact that fasting insulin and HOMA-IR, both surrogate measures of whole body insulin resistance, are only weakly correlated with the direct clamp measurement in both children (26) and adults (27) and, therefore, less reliable in the study of insulin resistance (28). Nevertheless, because of the reported strong relation in older adults, it seems reasonable to suggest that the relation between OCN and the clamp measure of insulin resistance may become stronger as this cohort ages.

Decreased levels of OCN have been reported to be associated with increased CV risk. Studies in adults have shown (i) total OCN negatively correlated with brachial ankle pulse wave velocity and intima media thickness (both parameters of atherosclerosis) in adults with type 2 diabetes mellitus (10); (ii) using the Adult Treatment Panel III criteria for metabolic syndrome, adults in the highest quartile for total OCN had the lowest risk of metabolic syndrome (22); and (iii) total OCN was lower in men older than 70 years with metabolic syndrome compared to those without metabolic syndrome (23). Development of metabolic syndrome and CV disease are closely related to obesity, and obese individuals tend to have higher BP, lipid levels, inflammatory markers and lower adiponectin. In multiple regression analyses in the present study, with only sex, race, and age as co-variables, both total and carboxylated OCN were significantly related to a CV risk factor cluster score and to certain of the individual CV risk factors. Because of the significant relation between BMI and OCN, BMI was added as a covariate, and the relations were attenuated. However, the relation remained significant with SBP and adiponectin, both known to be significantly related to CV risk.

Intravenous infusions and daily injections of OCN to mice were shown to decrease adiposity and protect against insulin resistance (5,29). Exercise, known to improve insulin resistance (30), stimulates osteoblasts to increase bone formation (31) and has been shown in mouse and human studies to increase OCN (32–34). A 1-year exercise intervention program in obese children resulted in an increase in OCN coincident with a decrease in BMI (16). While it seems clear that levels of OCN and BMI are related, it has not yet been determined exactly how they are related or which of the two is the initial response to exercise.

There are some potential limitations to this study, including the assay used to quantify undercarboxylated OCN. Undercarboxylated OCN has been reported to be the metabolically active form of OCN in animal studies but was surprisingly not associated with any of the variables in the present study. In addition to detecting the intact completely undercarboxylated OCN molecule, this assay also detects undercarboxylated OCN fragments (35); these fragments may be metabolically inactive, thus diluting out any associations of the intact undercarboxylated OCN molecule with the variables under consideration. However, this possibility does not detract from the findings for data involving total and carboxylated OCN. A second potential limitation is the sample size which may have limited the power to detect a significant association with insulin resistance. Finally, we have no data on gender-specific hormone measurements which may have been helpful in understanding the gender differences we observed. Unlike previous investigations reporting an association between OCN and insulin resistance, one of the primary strengths of our study is that the measurement of insulin resistance was directly measured by the euglycemic-hyperinsulinemic clamp.

In summary, total and carboxylated OCN are significantly related to BMI and to SBP and adiponectin independent of BMI, with a borderline significant relation to insulin resistance. In contrast to prior reports from studies in older adults and younger children, significant associations were not found between OCN and other CV risk factors after adjustment for BMI. While these results show that the relations between OCN and the factors associated with CV risk are relatively weak in early young adulthood, the strength of the relation with BMI, the relation to SBP and adiponectin, the borderline relation to insulin resistance, and the studies suggesting a stronger relation in older adults suggest the possibility that OCN may have an impact on CV risk that begins in early adulthood and will become more apparent as this cohort ages.

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Figure 1.

Multiple regression of (a) BMI on carboxylated osteocalcin (OCN) adjusted for age, gender, and ethnicity; before (left) and after (right) adjustment for bone mineral content (BMC). Multiple regression of (b) systolic blood pressure (SBP) and (c) adiponectin on carboxylated OCN adjusted for age, gender, and ethnicity; before (left) and after (right) adjustment for BMI. Gender-specific mean OCN quartiles are included for goodness of fit assessment.

Table 1

Relation of total osteocalcin (OCN) to obesity, SBP, insulin sensitivity, adipocytokines, and CV risk factors by OCN quartile and multiple regression analysis

Dependent variable	N	Total OCN (ng/ml)				Multiple regression			
		Q1 Mean	Q2 Mean	Q3 Mean	Q4 Mean	Slope ^a P value	Slope ^a P value	Slope ^b P value	Slope ^b P value
Age, years	137	18.7	18.8	18.6	18.5	—	—	—	—
Ethnic, non-white, %	137	32	12	11	17	—	—	—	—
BMI, kg/m ²	137	28.6	24.4	25.1	23.8	-3.58	0.013		
Percent body fat, % ^c	120	29.2	30.9	25.3	17.0	-0.16	0.108	0.0	0.641
Waist, cm	137	88.4	78.1	82.7	80.2	-8.16	0.013	-0.6	0.623
SBP, mm Hg	137	113.7	105.3	112.1	109.3	-4.70	0.020	-4.1	0.047
Insulin sensitivity: M _{10m} , mg/kg/min ^d	120	10.3	11.9	10.6	11.3	1.82	0.029	1.6	0.056
Glucose, mg/dl	137	87.4	85.5	86.8	89.1	1.47	0.473	1.9	0.365
Insulin, mg/dl ^c	137	8.5	7.5	6.7	7.4	-0.04	0.768	0.1	0.570
HOMA-IR ^c	137	1.8	1.6	1.4	1.6	-0.03	0.860	0.1	0.510
Cholesterol, mg/dl	137	158.7	154.4	159.9	145.8	-7.65	0.267	-2.9	0.671
HDL-C, mg/dl	137	42.6	46.2	41.5	43.4	3.02	0.182	1.7	0.460
LDL-C, mg/dl	137	96.8	91.3	98.7	79.9	-9.58	0.133	-5.8	0.362
Triglycerides, mg/dl ^c	137	85.3	83.9	81.8	92.6	-0.04	0.714	0.0	0.690
Adiponectin, mg/ml ^c	137	5.95	7.39	5.65	5.46	0.18	0.094	0.1	0.319
IL-6, pg/ml ^c	122	3.12	2.18	2.11	2.38	-0.38	0.020	-0.3	0.118
TNF-α, pg/ml ^c	137	1.10	0.88	1.08	1.18	-0.01	0.946	0.0	0.969
CRP, ng/ml ^c	103	1.58	0.96	1.02	0.87	-0.49	0.129	-0.3	0.398
CV risk factor cluster score	137	0.27	-0.16	0.05	-0.10	-0.26	0.050	-0.1	0.351

P values < 0.05 are in boldface.

CRP, C-reactive protein; CV, cardiovascular; CV risk factor cluster score = average z-score for fasting insulin, SBP, triglycerides, and HDL-C; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; IL-6, interleukin-6; LDL-C, low-density lipoprotein-cholesterol; OCN, osteocalcin; SBP, systolic blood pressure; TNF-α, tumor necrosis factor-α.

^aMultiple regression slope adjusted for age, gender, and ethnicity. Dependent variable is the row variable.

^bMultiple regression slope adjusted for age, gender, ethnicity, and BMI. Dependent variable is the row variable.

c Natural log transformed for regression analysis.

d Lower Mlbm = higher insulin resistance.

Table 2

Relation of carboxylated osteocalcin (OCN) to obesity, SBP, insulin sensitivity, adipocytokines, and CV risk factors by OCN quartile and multiple regression analysis

Dependent variable	N	Carboxylated OCN (ng/ml)				Multiple regression		
		Q1 Mean	Q2 Mean	Q3 Mean	Q4 Mean	Slope ^a P value	Slope ^a P value	Slope ^b P value
Age, years	137	18.7	18.5	18.8	18.5	—	—	—
Ethnic, non-white, %	137	33	6	18	17	—	—	—
BMI, kg/m ²	137	28.1	26.1	23.9	23.9	-3.55	0.005	
Percent body fat, % ^c	120	27.2	26.4	23.2	23.1	-0.16	0.065	0.0 0.589
Waist, cm	137	86.9	83.8	80.1	78.9	-7.39	0.011	0.1 0.906
SBP, mm Hg	137	113.1	111.3	108.2	108.0	-5.55	0.002	-5.0 0.006
Insulin sensitivity: M _{10m} , mg/kg/min ^d	120	10.7	10.9	10.8	11.8	1.37	0.070	1.1 0.139
Glucose, mg/dl	137	86.3	86.9	86.8	88.7	2.32	0.203	2.8 0.132
Insulin, mg/dl ^c	137	8.5	6.4	7.6	7.7	-0.06	0.647	0.1 0.619
HOMA-IR ^c	137	1.8	1.4	1.6	1.7	-0.03	0.816	0.1 0.483
Cholesterol, mg/dl	137	162.2	158.7	150.8	147.6	-11.09	0.069	-6.6 0.276
HDL-C, mg/dl	137	41.5	44.6	42.4	45.0	2.61	0.195	1.3 0.532
LDL-C, mg/dl	137	101.8	95.1	86.0	84.4	-13.74	0.015	-10.3 0.068
Triglycerides, mg/dl ^c	137	85.3	86.3	89.6	82.2	-0.02	0.849	0.1 0.495
Adiponectin, mg/ml ^c	137	4.93	6.57	6.47	6.39	0.27	0.004	0.2 0.031
IL-6, pg/ml ^c	122	3.03	2.65	1.89	2.20	-0.25	0.082	-0.1 0.368
TNF-α, pg/ml ^c	137	1.01	1.05	1.06	1.11	0.03	0.730	0.0 0.634
CRP, ng/ml ^c	103	1.32	1.36	0.89	0.88	-0.37	0.189	-0.1 0.643
CV risk factor cluster score	137	0.19	-0.01	0.00	-0.12	-0.27	0.021	-0.1 0.238

P values < 0.05 are in boldface.

CRP, C-reactive protein; CV, cardiovascular; CV risk factor cluster score = average z-score for fasting insulin, SBP, triglycerides, and HDL-C; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; IL-6, interleukin-6; LDL-C, low-density lipoprotein-cholesterol; OCN, osteocalcin; SBP, systolic blood pressure; TNF-α, tumor necrosis factor-α.

^aMultiple regression slope adjusted for age, gender, and ethnicity. Dependent variable is the row variable.

^bMultiple regression slope adjusted for age, gender, ethnicity and BMI. Dependent variable is the row variable.

c Natural log transformed for regression analysis.
 d Lower Mlbm = higher insulin resistance

Table 3Osteocalcin quartiles for cohort ($N=137$) and by gender

Total osteocalcin (ng/ml)									
		Q1 ($n=34$)		Q2 ($n=34$)		Q3 ($n=35$)		Q4 ($n=34$)	
Female (%) ^a		71%		76%		46%		12%	
Male (%)		29%		24%		54%		88%	
	Median	Range	Median	Range	Median	Range	Median	Range	
All	21	7.6–25.5	30	26.3–33.9	38.4	34.2–43.7	54.7	44.5–100.9	
Female	18.1	7.6–23.2	26.5	23.5–29.1	32	29.8–36.2	40.6	36.3–63.5	
Male	24	11.7–32.4	36.2	33.1–40.4	45.8	40.9–52.2	61.6	53.3–100.9	
Carboxylated osteocalcin (ng/ml)									
		Q1 ($n=34$)		Q2 ($n=34$)		Q3 ($n=35$)		Q4 ($n=34$)	
Female (%) ^a		79%		65%		49%		12%	
Male (%)		21%		35%		51%		88%	
	Median	Range	Median	Range	Median	Range	Median	Range	
All	13.5	4.7–15.6	18.8	16.0–20.3	23.2	20.5–28.6	36.6	29.2–69.4	
Female	10.3	4.7–14.0	15.6	14.1–18.2	19.9	18.8–21.2	23.1	21.4–38.2	
Male	16.5	8.8–18.7	23.2	19.4–26.7	30.5	26.8–35.1	46.3	36.5–69.4	
Undercarboxylated osteocalcin (ng/ml)									
		Q1 ($n=33$)		Q2 ($n=35$)		Q3 ($n=35$)		Q4 ($n=34$)	
Female (%)		52%		60%		51%		41%	
Male (%)		48%		40%		49%		59%	
	Median	Range	Median	Range	Median	Range	Median	Range	
All	4.6	1.8–6.6	9.3	6.8–11.0	13.9	11.1–16.1	23.3	16.3–33.8	
Female	5.4	2.6–6.6	9.8	6.8–11	14.1	11.1–16.0	19.9	16.3–33.8	
Male	4.1	1.8–5.8	9.3	6.8–11.0	13.7	11.5–16.1	25.2	16.5–32.2	

^a P -value <0.001 for % female vs. % male.