Association between Serum Osteocalcin and Markers of Metabolic Phenotype

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Context: Osteocalcin has been reported to contribute to the regulation of glucose tolerance and insulin secretion and sensitivity in experimental animals.

Objective: Our objective was to examine the association between serum osteocalcin concentration and markers of dysmetabolic phenotype using data from a completed clinical trial in adults age 65 and older [n = 380, mean age 71 yr, body mass index (BMI) 26.9 kg/m^2, 5% with diabetes].

Research Design and Methods: In cross-sectional analyses (baseline data), we estimated the associations of serum osteocalcin and urine N-telopeptide with markers of metabolic phenotype including fasting plasma glucose (FPG) (primary outcome), fasting insulin, insulin sensitivity estimated by homeostasis model assessment for insulin resistance, plasma high-sensitivity C-reactive protein, IL-6, and measures of adiposity (BMI and body fat) (secondary outcomes) after multivariate adjustment for potential confounders. In prospective analysis (placebo arm), we estimated the associations of osteocalcin and N-telopeptide with change in the primary outcome, FPG, over a 3-yr period.

Results: In cross-sectional analyses, serum osteocalcin concentration was inversely associated with FPG ($P = 0.01$), fasting insulin ($P = 0.006$), homeostasis model assessment for insulin resistance ($P = 0.002$), high-sensitivity C-reactive protein ($P = 0.01$), IL-6 ($P = 0.02$), BMI ($P < 0.001$), and body fat ($P < 0.001$). When participants were divided into tertiles by serum osteocalcin, mean FPG was 97.1 *vs.* 104.8 mg/dl in the highest *vs.* lowest osteocalcin tertile, respectively ($P < 0.01$). In prospective analyses, exposure to higher osteocalcin levels during follow-up was associated with a significantly lower rise in FPG at 3 yr. Urine N-telopeptide was not associated with any marker of metabolic phenotype.

Conclusions: Serum osteocalcin concentration was inversely associated with blood markers of dysmetabolic phenotype and measures of adiposity. Our findings should be considered hypothesis generating, and they need to be replicated in human studies designed to test the hypothesis that osteocalcin affects metabolism. **(*J Clin Endocrinol Metab* 94: 827–832, 2009)**

Recent studies have revealed a new neuroendocrine circuit linking bone and energy homeostasis. Leptin, an adipocyte-derived hormone, has been previously shown to inhibit bone formation by acting on osteoblasts via central neural pathways (1–4). More recently, evidence from animal studies suggests that the skeleton may exert an endocrine regulation of glucose metabolism (5), thereby closing the feedback loop between bone and peripheral organs involved in energy homeostasis. In elegant animal experiments, Lee *et al.* (5) showed that mice lacking the gene that encodes osteocalcin (osteocalcin −/−), an osteoblast-specific secreting molecule, have an abnormal amount of visceral fat and exhibit glucose intolerance, insulin resistance, and impaired insulin secretion compared with wild-type mice. In *ex vivo* studies, when pancreatic β-islets isolated from wild-type

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2008-1422 Received July 7, 2008. Accepted December 4, 2008.
First Published Online December 16, 2008

Abbreviations: BMI, Body mass index; Cr, creatinine; CV, coefficients of variation; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment for insulin resistance; hsCRP, high-sensitivity C-reactive protein; NTX, N-telopeptide.

mice were cocultured with wild-type osteoblasts or in the presence of supernatants from cultured osteoblasts, insulin secretion increased, suggesting the presence of an osteoblast-derived circulating factor that regulates β-cell function. Furthermore, coculture of wild-type osteoblasts with adipocytes increased adiponectin expression and action (5). In mice, administration of recombinant osteocalcin significantly decreased glycemia and increased insulin secretion (5). Taken together, these data support a regulatory role of the skeleton on glucose and energy homeostasis, which appears to be mediated by osteocalcin. If osteocalcin contributes to the regulation of insulin sensitivity, it also raises the possibility that osteocalcin may contribute to other components of the dysmetabolic phenotype (systemic inflammation, adiposity), given that insulin resistance plays a central role in the pathogenesis of the phenotype.

Whether the skeleton, via release of osteocalcin, plays a role in humans in relation to the metabolic phenotype, as defined by glucose intolerance, insulin resistance, and systemic inflammation, has not been evaluated. The purpose of the present study was to examine the association between serum osteocalcin and dysmetabolic phenotype in humans.

Subjects and Methods

Study participants

Healthy ambulatory adults 65 yr of age or older were recruited for a randomized trial on vitamin D and calcium supplementation to prevent bone loss (clinicaltrials.gov ID NCT00357643) (6). Criteria for exclusion included current cancer, laboratory evidence of kidney or liver disease, dietary calcium intake exceeding 1500 mg/d, and bone-altering conditions (hyperparathyroidism, nephrolithiasis, renal disease, bilateral hip surgery, or therapy with bisphosphonate, calcitonin, estrogen, tamoxifen, or testosterone in the past 6 months or fluoride in the past 2 yr). The trial enrolled 445 (199 men and 246 women) participants. The present *post hoc* analyses are limited to participants who had stored specimens available for measurement of the outcomes of interest at baseline (cross-sectional analyses, n = 380) and those in the placebo arm with available measurements of the primary outcome, fasting plasma glucose (FPG), both at baseline and end of study at 3 yr (prospective analyses, n = 198). The study was conducted at the Human Nutrition Research Center on Aging at Tufts University with approval from the Tufts Medical Center Human Investigation Review Committee and written informed consent by all participants.

Variables

Ascertainment of exposure

Serum osteocalcin concentration was measured in the morning after an 8-h overnight fast, as described below. The assay detects both uncarboxylated and carboxylated osteocalcin.

Ascertainment of outcomes

The primary outcome was FPG. Assessment of insulin sensitivity in the basal (nonstimulated) state was estimated by the homeostasis model assessment for insulin resistance (HOMA-IR) based on fasting glucose and insulin measurements as follows: HOMA-IR = [glucose (millimoles per liter) × insulin (milliunits per liter)]/22.5 (7). High HOMA-IR scores denote low insulin sensitivity (increased insulin resistance). HOMA-IR has a high correlation with measures of insulin sensitivity obtained from the euglycemic clamp procedure (8, 9), including prediction of age-related insulin resistance in older people (10). We did not assess pancreatic β-cell function using HOMA because HOMA modeling of β-cell function is not valid in older persons in whom dynamic testing is required to estimate age-related impairment of β-cell function (10). Plasma high-sensitivity C-reactive protein (hsCRP) and IL-6 were measured as indicators of systemic inflammation (11, 12). Body fat (kilograms or percent total body mass) was estimated by dual-energy x-ray absorptiometry (model DPX; Lunar Radiation Corp., Madison, WI). The coefficient of variation (CV) for fat mass in our laboratory is 2.2% (13).

Assessment of potential confounders

Height (to ±0.1 cm) was measured at baseline using a wall-mounted stadiometer, and body weight (to ±100 g) was measured using an electronic calibrated scale (model CN-20; DETECTO-Cardinal Scale Manufacturing Co., Webb City, MO). Body mass index (BMI) was calculated as weight/height^2 (kilograms per square meter). Data on age, sex, and smoking status (yes/current, yes/formerly, never) and education (highest educational level achieved, no college *vs.* any college) were self-reported at baseline. Leisure, household, and occupational activity were assessed at baseline with use of the Physical Activity Scale for the Elderly (PASE) questionnaire (14). As a marker of bone turnover, 24-h urine N-telopeptide (NTX) corrected for urine creatinine (Cr) was measured as described below.

Blood and urine analyses

All measurements were done in the morning after an 8-h overnight fast. Serum osteocalcin was measured with two-site immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA) with intra- and interassay CV of 4.0 and 6.0%, respectively. Urine NTX was measured by ELISA (Ostex International, Seattle, WA) with CV ranging from 5.6–7%. Plasma glucose was measured by an oxygen rate method employing the Beckman Synchron LX System (Beckman Coulter, Inc., Fullerton, CA) with intra- and interassay CV of 2.0 and 3.0%, respectively. Serum insulin was measured by RIA commercial kit (DPC Coat-A-Count insulin assay; Diagnostic Products Corp., Los Angeles, CA) with intra- and interassay CV of 3.1–9.3% and 4.9–10.0%, respectively. Plasma hsCRP was measured by the Olympus autoanalyzer (Smith-Kline Beecham Laboratories, Santa Cruz, CA) using reagent from Equal Diagnostics (Exton, PA) with intra- and interassay CV of 2.0 and 3.3%, respectively, at midrange. IL-6 was measured by immunoassay commercial kit (Quantikine HS; R&D Systems, Minneapolis, MN) with intra- and interassay CV of 6.9–7.8 and 6.5–9.6%, respectively. Osteocalcin was measured in a blinded fashion as the samples were collected. Measurements of FPG, insulin, hsCRP, IL-6, and NTX/Cr were done in a blinded fashion, in duplicate and in pairs (before/after intervention) at the same time in the same analytical run and in random order to reduce systematic error and interassay variability.

Statistical analysis

All variables of interest were examined for normality. CRP and IL-6 had a skewed distribution and were transformed to their natural logarithm for analyses (normal distribution was confirmed after transformation) but are back-transformed when we present results for ease of interpretation.

Cross-sectional analyses

Separate multivariate regression analysis was performed with osteocalcin as the predictor/independent variable and FPG (primary outcome), insulin, HOMA-IR, hsCRP, IL-6, BMI, and fat mass (secondary outcomes) as the metabolic outcome variables of interest (dependent variables). All analyses were adjusted for age, sex, and variables that have been previously associated with dysmetabolic phenotype (BMI, smoking, physical activity score, and education level). The analyses on adiposity markers as outcomes (BMI and fat mass) were not adjusted for BMI. We tested for an interaction between osteocalcin (predictor) and sex on all outcomes, and it was not statistically significant; therefore, we present results for both men and women together. Evidence from animal

studies suggest that different concentrations of osteocalcin may be required to regulate β-cell and adipocyte gene expression (15) and thereby metabolic outcomes. Therefore, we divided the cohort into tertiles by osteocalcin concentration, and we used general linear models (PROC GLM procedure SAS software) adjusting for several potential confounders to compare differences in metabolic outcomes of interest across osteocalcin tertiles. To test the hypothesis that any observed association between osteocalcin and outcomes of interest does not simply reflect an association between bone turnover and metabolic outcomes, we repeated the multivariate regression analysis with urine NTX/Cr as the predictor variable.

Prospective analysis

To assess whether the change in metabolic phenotype that occurs over time is dependent on long-term exposure to osteocalcin, we regressed the 3-yr change in the primary outcome, FPG, according to osteocalcin exposure by estimating the mean osteocalcin concentration of all available osteocalcin measurements [at baseline and during follow-up (months 6, 12 and 18)]. For this analysis, we used participants in the placebo arm (n = 198), which represents the natural history of the metabolic phenotype and to avoid confounding with the intervention (vitamin D and calcium), which is known to affect both the exposure (osteocalcin) (6) and outcome (FPG) (16). We adjusted for baseline values to avoid potential bias that might result if the magnitude of the change depended on starting value for FPG and for other potential confounders (age, sex, physical activity, smoking, education, and change in BMI). We repeated the prospective analysis with baseline urine NTX/Cr as the predictor variable.

Statistical significance for all analyses was set at $P < 0.05$. Statistical analysis was done using SAS version 9.1 (SAS, Cary, NC). The regression coefficients reported are unstandardized. Values are presented as means ± SEM.

Results

Cross-sectional analyses (baseline data)

Participant characteristics

The mean age of the participants (n = 380) was 70.9 ± 0.2 yr, and BMI was 26.9 ± 0.2 kg/m^2 (Table 1). Based on FPG criteria and self-reported diagnosis of diabetes, 5% of the cohort had diabetes, 29% had impaired fasting glucose, and 66% had normal fasting glucose.

Osteocalcin and metabolic phenotype

Multiple regression coefficients for the markers of metabolic phenotype of interest (FPG, fasting insulin, HOMA-IR, hsCRP, and IL-6) as outcome variables were calculated against serum osteocalcin concentration as the independent variable, adjusting for age, sex, BMI, physical activity, smoking, and education. Osteocalcin concentration was inversely associated with blood markers of metabolic phenotype, FPG (regression coefficient $\beta = -1.159$, P for regression coefficient $\beta = 0.012$; R^2 for the model = 0.14), fasting insulin ($\beta = -0.317$; $P = 0.006$; $R^2 = 0.18$), HOMA-IR ($\beta = -0.123$; $P = 0.002$; $R^2 = 0.17$), hsCRP ($\beta = -0.065$; $P = 0.010$; $R^2 = 0.17$), and IL-6 ($\beta = -0.044$; $P = 0.020$; $R^2 = 0.09$) after multivariate adjustment. Osteocalcin concentration was also inversely associated with markers of adiposity, BMI ($\beta = -0.578$; $P < 0.001$; $R^2 = 0.10$), body fat ($\beta = -1.082$; $P < 0.001$; $R^2 = 0.15$), and percent body fat ($\beta = -0.669$; $P < 0.001$; $R^2 = 0.49$) after multivariate adjustment for age, sex, physical activity, smoking, and education.

To minimize potential confounding of the observed association by diabetes-related variables (*e.g.* duration, severity, and type of diabetes-specific therapy) or comorbidities (17, 18), we repeated the analyses after excluding 20 participants (5% of the cohort) with diabetes based on either self-reported diagnosis or FPG of 126 mg/dl (7 mmol/liter) or higher. After multivariate adjustment, the inverse association between osteocalcin concentration and FPG remained, but it was not statistically significant ($\beta = -0.281$; $P = 0.28$). After multivariate adjustment, osteocalcin concentration remained inversely associated with fasting insulin ($\beta = -0.282$; $P < 0.01$; $R^2 = 0.17$), HOMA-IR ($\beta = -0.080$; $P = 0.003$; $R^2 = 0.19$), hsCRP ($\beta = -0.072$; $P = 0.007$; $R^2 = 0.16$), IL-6 ($\beta = -0.053$; $P = 0.009$; $R^2 = 0.09$) and markers of adiposity, BMI ($\beta = -0.531$; $P < 0.001$; $R^2 = 0.09$), body fat ($\beta = -1.02$; $P < 0.001$; $R^2 = 0.15$), and percent body fat ($\beta = -0.666$; $P < 0.001$; $R^2 = 0.50$).

When we divided the cohort into tertiles by serum osteocalcin concentration, all markers of metabolic phenotype varied inversely with osteocalcin tertile (Table 2). There appeared to be a threshold of osteocalcin concentration (middle tertile) above

TABLE 1. Characteristics of study participants at baseline

Characteristic	Entire cohort	By osteocalcin tertile (ng/ml)			*P*
		<5.20	5.20–7.10	≥7.10	
No. of participants	380	123	132	125	
Serum osteocalcin (ng/ml)	6.3 ± 0.1	4.14	6.00	8.76	<0.01
Age (yr)	71.0 ± 0.2	70.2 ± 0.4	70.4 ± 0.4	72.3 ± 0.5	0.01
Sex, no. of women (%)	209 (55)	48 (39)	68 (51)	93 (74)	<0.01
Race, no. of Caucasians (%)	366 (96)	120 (98)	125 (95)	121 (97)	0.49
Physical activity score	116 ± 2.8	116 ± 5	119 ± 5	112 ± 5	0.31
Smoking, n (%)					0.03
Yes, currently	20 (5)	7 (6)	10 (8)	3 (2)	
Yes, formerly	197 (52)	71 (57)	71 (54)	55 (44)	
Never	163 (43)	45 (37)	51 (38)	67 (54)	
Education, highest grade completed, n (%)					0.16
No college	143 (38)	45 (37)	45 (34)	53 (42)	
Any college	237 (62)	78 (63)	87 (66)	72 (58)	
24-hr urine NTX/Cr (nmol/mmol)	40.6 ± 1.3	26.9 ± 0.8	36.9 ± 1.3	58.1 ± 3.0	<0.01

Data are presented as mean ± SEM; *P* values are for differences in characteristics between osteocalcin tertiles.

TABLE 2. Markers of metabolic phenotype by tertiles of serum osteocalcin (cross-sectional analyses) adjusted for covariates

	Tertile of serum osteocalcin		
	Lowest, <5.20	5.20–7.10	Highest, ≥7.10
n	123	132	125
Median osteocalcin (ng/ml)	4.2	6.0	8.3
Markers of metabolic phenotype			
Blood markers			
FPG (mg/dl)	104.8 ± 2.3	97.7 ± 0.9[b]	97.1 ± 1.1[b]
Fasting insulin (mU/liter)	7.5 ± 0.2	6.4 ± 0.3[a]	6.2 ± 0.3[a]
HOMA-IR	2.1 ± 0.2	1.6 ± 0.1[b]	1.5 ± 0.1[b]
hsCRP (mg/liter)	4.2 ± 0.7	2.8 ± 0.2	2.7 ± 0.4[a]
IL-6 (pg/ml)	4.3 ± 0.4	3.5 ± 0.3	3.5 ± 0.5[a]
Adiposity markers			
BMI (kg/m^2)	28.2 ± 0.4	27.0 ± 0.3[a]	25.5 ± 0.3[c]
Body fat (kg)	28.2 ± 0.8	25.5 ± 0.7[b]	22.5 ± 0.7[c]
Body fat, % total mass (kg)	36.3 ± 0.8	34.7 ± 0.8[a]	32.6 ± 0.7[c]

Blood markers of metabolic phenotype are expected means ± SEM by general linear model analysis after adjustment for age, sex, BMI, physical activity, smoking, and education. Adiposity markers of metabolic phenotype are expected means ± SEM by general linear model analysis after adjustment for age, sex, physical activity, smoking, and education. HOMA-IR = [glucose (mmol/liter) × insulin (mU/liter)]/22.5 (7). To change from traditional (mg/dl) units to SI (mmol/liter) for glucose, multiply by 0.0555. *P* values are for markers of metabolic phenotype (expected mean) after multivariate adjustment compared with lowest osteocalcin tertile (osteocalcin < 5.20 ng/ml).

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

which blood markers of metabolic phenotype did not decrease further. For adipose markers, the inverse association was continuous across all tertiles.

Urine NTX and metabolic phenotype

As expected, there was a positive correlation between osteocalcin and NTX/Cr (r = 0.31, $P < 0.01$). In regression analysis, there was no association between urine NTX/Cr and any of the measured markers of metabolic phenotype after multivariate adjustment (data not shown) with the exception of hsCRP (regression coefficient $\beta = -0.005$; $P = 0.029$; $R^2 = 0.16$). However, the association between NTX/Cr and hsCRP was entirely driven by two outliers (NTX/Cr concentration of 267 and 272 nmol/mmol, respectively). After these two outliers were removed from the analysis, the association became nonsignificant ($P = 0.9$).

Prospective analyses (placebo arm only)

Participant characteristics

The mean age of the participants in the placebo group (n = 198) was 71.3 ± 0.3 yr, and BMI was 26.8 ± 0.3 kg/m^2. There were no differences in baseline characteristics compared with the entire cohort (n = 380).

Osteocalcin and change in FPG

In regression analyses, mean osteocalcin concentration during follow-up predicted change in FPG at yr 3, after multivariate adjustment for baseline FPG, age, sex, physical activity, smoking, education, and change in BMI at yr 3 (regression coefficient $\beta = -0.889$; $P = 0.029$; $R^2 = 0.14$). We repeated the analyses after excluding participants (n = 23) with diabetes at baseline or during follow-up. After multivariate adjustment, the inverse association between mean osteocalcin concentration and change in FPG remained ($\beta = -0.759$; $P = 0.008$; $R^2 = 0.19$).

Urine NTX and change in FPG

In regression analysis, after excluding the two participants with very high NTX/Cr, baseline urine NTX/Cr concentration did not predict change in FPG at yr 3, after multivariate adjustment for baseline FPG, age, sex, physical activity, smoking, education, and change in BMI at yr 3 ($P = 0.22$).

Discussion

In older individuals, we found that circulating serum osteocalcin was inversely associated with blood and adiposity markers of dysmetabolic phenotype, including hyperglycemia, insulin resistance, systemic inflammation, BMI and body fatness. In prospective analysis, we found that serum osteocalcin predicts change in FPG that occurs over time in older individuals. Our findings are consistent with recent studies in animals showing that bone and energy metabolism exert reciprocal regulation (5, 19, 20).

A link between bone and energy metabolism has long been suspected, based on observations that obesity is inversely associated with osteoporosis. However, the mediators of such relationship had not been identified until recently when, leptin, an adipocyte-derived hormone, was shown to be a major regulator of bone turnover (1–4). Working on the hypothesis that bone may also exert feedback control on energy homeostasis, Lee *et al.* (5) recently showed that the skeleton via the release of osteocalcin, an osteoblast-specific secreting molecule often used in clinical studies as a marker of bone formation, has a profound effect on glucose homeostasis, insulin sensitivity, and fat metabolism (5, 19). Both in osteocalcin-deficient and wild-type mice (15), osteocalcin induces expression and secretion of insulin via a direct effect on β-cells, improves insulin sensitivity and glucose tolerance, and can significantly attenuate the deleterious effects of diet- or hyperphagia-induced obesity.

Several human studies have previously reported that osteocalcin and other markers of bone turnover are lower in patients with established diabetes (21–28, 29), which is taken to suggest a low bone turnover state in these patients with diabetes. These studies are case control, most with small numbers of participants, and the reported associations may have been confounded by the lack of adjustment for important risk factors for diabetes such as BMI, physical activity, or diabetes-specific therapy (*e.g.* thiazolidinediones, which are known to affect bone turnover) (30). Furthermore, osteocalcin itself has been reported to vary by age, sex, smoking status, and physical activity (31). In our study,

the inverse association between osteocalcin and dysmetabolic phenotype was significant even after adjusting for multiple potential confounders. Furthermore, to explore the possibility that osteocalcin may simply reflect increased bone turnover associated with or caused by diabetes, we repeated our analyses after excluding participants with diabetes. The inverse association between osteocalcin concentration and FPG remained, although not statistically significant, whereas the inverse association with other markers remained strongly statistically significant. In the prospective analyses, mean serum osteocalcin concentration predicted change in FPG over time both in a cohort that included diabetes and a cohort that excluded participants with diabetes, suggesting that the association between osteocalcin and glycemia may be causal.

The observed inverse association between osteocalcin and insulin resistance in humans, as measured by HOMA-IR, has not been previously reported. Our findings are consistent with the animal studies (15), where the inverse association appears to be partially mediated by secretion of adiponectin. We do not have measurements of adiponectin; therefore, we cannot test this hypothesis in our cohort. Nevertheless, the inverse association with insulin resistance may have important implications in relation to the metabolic syndrome, given that insulin resistance plays a central role in the pathogenesis of the syndrome.

Our findings that markers of adiposity were inversely associated with serum osteocalcin after adjustment for potential confounders are in line with studies in cell cultures and animals. In various models of obesity (diet-induced or hyperphagia), osteocalcin was protective against obesity and type 2 diabetes (15). Specifically, mice fed a high-fat diet and received osteocalcin gained significantly less weight and had significantly smaller fat pads and normal levels of triglycerides compared with mice not given osteocalcin (15). Treated mice were also more glucose tolerant and more insulin sensitive. In the animal studies, there was no difference in food intake, and the benefit appeared to be via an increase in genes involved in energy expenditure.

Because of evidence from animal studies that different but overlapping amounts of osteocalcin were required to regulate β-cell and adipocyte gene expression (15), we divided the cohort into tertiles by osteocalcin concentration and compared metabolic outcomes among tertiles. Our findings that glycemia and other blood markers of dysmetabolism were lower in the middle tertile but not progressively lower in the highest tertile confirm results from the dose-response experiments in animals, where the effect of osteocalcin on insulin expression by cultured β-cells plateaued at high concentration of osteocalcin (15). Similarly, markers of adiposity continued to improve in the highest tertile *vs.* second tertile, which is also in accord with results by Ferron *et al.* (15) where there was no apparent threshold in adiponectin expression or decreased deposition of fat by increasing doses of osteocalcin.

Because diabetes has been inversely associated with bone turnover markers in previous studies (21–27), to test the hypothesis that any observed association between osteocalcin and outcomes of interest was not simply due to an association between bone turnover and metabolic outcomes, we did a multivariate regression analysis with urine NTX/Cr as the main predictor variable. Our results showed that NTX/Cr was not associated with markers of metabolic phenotype, confirming our hypothesis that the observed association between osteocalcin and dysmetabolic phenotype is not confounded by measures of bone turnover.

Osteocalcin undergoes posttranslational modification whereby three glutamic acid residues are carboxylated to form γ-carboxyglutamic acid residues. Carboxylated osteocalcin has a higher affinity for hydroxyapatite and is thought to be involved in bone extracellular matrix mineralization, although conclusive data are lacking (32–36). In the studies by Lee *et al.* (5) and Ferron *et al.* (15), the uncarboxylated form appeared to be mediating the effects of osteocalcin on metabolic phenotype. Other studies in cell cultures have suggested that both uncarboxylated and carboxylated forms increase basal and insulin-stimulated glucose transport, although the effect of the carboxylated form was less robust (37). In our study, we measured total osteocalcin and do not have measurements of uncarboxylated osteocalcin; therefore, we cannot further this hypothesis.

The directionality of our findings from the cross-sectional analyses cannot be determined with certainty. However, the findings from the prospective analyses suggest that the association between osteocalcin and glycemia may be causal in humans similarly to the animal studies (5, 15). Because the number of nonwhite persons was very low, we cannot directly extrapolate our findings to the nonwhite population.

In conclusion, in older individuals, serum osteocalcin concentration was inversely associated with blood markers of dysmetabolic phenotype and measures of adiposity. Because of the *post hoc* nature of our analysis using data from a study designed for skeletal primary outcomes, our findings should be considered hypothesis generating, and they need to be replicated in other populations and in human studies specifically designed to test the hypothesis that osteocalcin affects metabolism. Nevertheless, our results provide support for an important role in humans of osteocalcin to regulate glucose tolerance, insulin sensitivity, and systemic inflammation, confirming recent findings from animal studies.

Acknowledgments

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This work was supported by Research Grants DK76092, DK79003, and DK78867 (to A.G.P.) from the National Institutes of Health, RR25752 (to Tufts University Clinical Translational Research Center) from the National Center for Research Resources, AG10353 from the National Institute of Aging, and the U.S. Department of Agriculture Cooperative Agreement No. 58-1950-4-401 (to B.D.-H.).

Disclosure Statement: The authors have nothing to disclose.

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