Lower Uncarboxylated Osteocalcin Concentrations in Children with Prediabetes Is Associated with β-Cell Function

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Context: Although animal studies suggest that it is the uncarboxylated rather than carboxylated form of osteocalcin that affects glucose homeostasis, the human data are scant and equivocal.

Objective: This study investigated associations of uncarboxylated and carboxylated forms of osteocalcin with insulin sensitivity and β-cell function in 140 overweight prepubertal children (43% female, 46% black, 84% obese) with normal glucose levels (n = 99) and prediabetes (n = 41).

Methods: An oral glucose tolerance test was used to identify prediabetes and for measurement of insulin sensitivity (Matsuda index), β-cell function [oral glucose tolerance test derived insulinogenic index and disposition index (DI_{OGTT})] and uncarboxylated and carboxylated forms of osteocalcin. Visceral adipose tissue (VAT) was assessed using magnetic resonance imaging.

Results: After controlling for age, sex and race, lower uncarboxylated osteocalcin concentrations, Matsuda index, insulinogenic index, and DI_{OGTT} and higher VAT levels were found in the prediabetes *vs.* normal-glucose group (all $P < 0.03$). Carboxylated osteocalcin levels were not different between groups. Multiple linear regression adjusting for age, sex, race, and VAT revealed that uncarboxylated osteocalcin was associated with insulinogenic index and DI_{OGTT} ($\beta = 0.34, 0.36$, respectively, both $P < 0.04$) in the prediabetes group but not the normal-glucose group. In both the normal-glucose and prediabetes groups, carboxylated osteocalcin was associated with insulin sensitivity ($\beta = 0.26, 0.47$, respectively, both $P < 0.02$).

Conclusions: These data suggest that the lower uncarboxylated osteocalcin concentrations found in children with prediabetes may be associated with β-cell dysfunction. In addition, our findings between carboxylated osteocalcin and insulin sensitivity suggest that carboxylated osteocalcin plays a role in human glucose homeostasis. **(*J Clin Endocrinol Metab* 96: E1092–E1099, 2011)**

Obesity, insulin resistance, and type 2 diabetes are related disorders of energy metabolism for which current therapies are suboptimal. Recently a novel alternate pathway for approaching these common disorders has emerged, suggesting that bone, a tissue not previously thought to influence energy metabolism, may regulate the glucose-insulin axis (1–4). In mice lacking the gene for osteocalcin, a bone-derived protein and well-known biomarker of bone formation, Lee *et al.* (1) observed phenotypes of glucose intolerance, insulin resistance, and visceral obesity. When the researchers administered recombinant uncarboxylated osteocalcin *in vivo*, improvements in glucose tolerance and insulin secretion were observed (1). Likewise, *in vitro* experiments showed that uncarboxylated osteocal-

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Abbreviations: CV, Coefficient of variation; DI_{OGTT}, OGTT-derived insulinogenic index and disposition index; ICTP, carboxyterminal telopeptide region of type I collagen; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; 25-OHD, 25 hydroxyvitamin D; PA, physical activity; P1NP, procollagen type 1 amino propeptide; SAAT, sc abdominal adipose tissue; VAT, visceral adipose tissue.

cin enhanced insulin production by islets and increased insulin sensitivity in adipocytes (1, 3). Taken together, these data support a regulatory role of the skeleton on glucose homeostasis, which appears to be mediated by the uncarboxylated form of osteocalcin.

Osteocalcin, the most abundant noncollagenous protein of the bone extracellular matrix, undergoes γ-carboxylation in a vitamin K-dependent process before secretion (5). During the carboxylation process, a small fraction of osteocalcin is uncarboxylated and released into circulation. It is postulated from the existing animal work that only the uncarboxylated form of osteocalcin has the capacity to regulate insulin production and sensitivity, ultimately improving glucose metabolism (1–4).

Clinical data indicate that total osteocalcin concentrations, which include both carboxylated and uncarboxylated forms, may play a role in glucose homeostasis (6–10); however, our understanding of the uncarboxylated form alone is uncertain. Studies in nondiabetic children (11) and adults (12, 13) showed that uncarboxylated osteocalcin was not related to either fasting glucose or insulin resistance (*i.e.* homeostasis model assessment of insulin resistance). However, Kanazawa *et al.* (14) found in adults with type 2 diabetes that uncarboxylated osteocalcin was negatively associated with fasting glucose and hemoglobin $A1_C$, a biomarker of 1- to 3-month glucose control. Although a clear explanation for these disparate findings is unknown, they may reflect the imprecision of static-derived glucose homeostasis measurements. To date, only one study has investigated relationships between uncarboxylated osteocalcin and dynamic-derived measures of glucose homeostasis (15). Hwang *et al.* (15) performed an oral glucose tolerance test (OGTT) in Korean men who mostly had prediabetes (23%) and type 2 diabetes (73%) and found that higher uncarboxylated osteocalcin levels were associated with better glucose tolerance. Based on findings by Hwang *et al.* (15) and the aforementioned studies in participants with (14) and without (11–13) diabetes, it is plausible that the role of uncarboxylated osteocalcin in glucose homeostasis may only be relevant in individuals with abnormal glucose regulation; however, this proposition warrants further investigation.

To our knowledge, relationships between uncarboxylated osteocalcin and dynamic measurements of glucose and insulin concentrations have not been determined in children. Therefore, the purpose of this study was to investigate associations of both uncarboxylated and carboxylated forms of osteocalcin with OGTT-derived measures of insulin sensitivity and β-cell function in prepubertal overweight children with and without prediabetes. Furthermore, we explored whether any observed associations between the different forms of osteocalcin and insulin sensitivity and β-cell function were dependent on visceral adiposity.

Participants and Methods

Participants

Participants included 140 prepubertal overweight children who were part of an investigation to determine the effects of aerobic exercise on metabolism (16). Inclusion criteria for the trial were the following: white or black race, aged 7–11 yr, overweight (body mass index 85th or greater percentile for age and sex) (17), and sedentary (no regular participation in an exercise program more than 1 h/wk). Children were excluded if they had a medical condition that would affect study results or limit physical activity (PA). Only those children who were prepubertal (Tanner stage I; absence of secondary sex characteristics), as determined by a pediatrician, were included in this study to minimize the confounding effects of puberty on bone and glucose metabolism (18). Participants underwent anthropometrics, body composition scans, OGTT, and interviews that assessed PA and diet. Informed consent and assent were obtained from all parents and children, respectively. All procedures were approved by the Medical College of Georgia Human Assurance Committee (institutional review board).

Biochemical assays

All participants underwent a 2-h OGTT after an overnight fast and were administered a standard oral glucose solution (1.75 g/kg of ideal body weight up to a maximum of 75 g) at time 0. Assay procedures for glucose and insulin have been described in detail in a previous publication (19). Using additional fasting sera, concentrations of total osteocalcin and uncarboxylated osteocalcin were analyzed in duplicate using a RIA with an antibody that recognizes both carboxylated and uncarboxylated osteocalcin (20). Carboxylated osteocalcin was separated from uncarboxylated osteocalcin by adsorption on hydroxyapatite. Carboxylated osteocalcin was calculated from total osteocalcin minus uncarboxylated osteocalcin. Given that the assay's capacity to identify uncarboxylated osteocalcin depends on the concentration of total osteocalcin, it is recommended that uncarboxylated osteocalcin be expressed as a percentage of the total osteocalcin (20). Therefore, we also determined associations of insulin sensitivity and β-cell function with percentage uncarboxylated osteocalcin, which was calculated as (uncarboxylated osteocalcin/total osteocalcin) × 100. The mean intra- and interassay coefficients of variation (CV) for total osteocalcin, carboxylated osteocalcin, and uncarboxylated osteocalcin ranged from 3.7 to 8.2%. Procollagen type 1 amino propeptide (P1NP), a marker of bone formation, and carboxyterminal telopeptide region of type I collagen (ICTP), a marker of bone resorption, were both measured in serum and assayed in duplicate using RIA (Orion Diagnostica, Espoo, Finland). Intra- and interrassay CV were 2.8 and 5.6%, respectively, for P1NP and 3.4 and 5.9%, respectively, for ICTP. Serum 25 hydroxyvitamin D (25-OHD) concentrations were assayed using RIA (DiaSorin Laboratories, Stillwater, MN) and run in duplicate, with intra- and interassay CV of 5.6 and 7.1%, respectively.

Insulin sensitivity and β-cell function parameters

The Matsuda index, an insulin sensitivity index that reflects a composite estimate of hepatic and muscle insulin sensitivity, was calculated using the following formula: Matsuda index = 10,000/$\sqrt{}$(fasting glucose × fasting insulin) × (mean glucose × mean insulin), where fasting glucose and insulin data are taken from the OGTT and mean data represent the average glucose and insulin values obtained during the OGTT (*i.e.* fasting, 30, 60, 90, and 120 min) (21). The Matsuda index has been validated using the euglycemic clamp method (21).

β-Cell function parameters included OGTT-derived insulinogenic index and disposition index (DI_{OGTT}), which have been shown to correlate well with insulin secretion measured by euglycemic clamp and each predict future development of type 2 diabetes (22, 23). The insulinogenic index was calculated by dividing the increment in insulin at 30 min by the increment in glucose at 30 min of the OGTT (*i.e.* $\Delta insulin_{0-30}/\Delta glucose_{0-30}$). The DI_{OGTT} was calculated as the product of insulin sensitivity (Matsuda index) and insulin secretion (insulinogenic index).

Adiposity, PA, and dietary intake measurements

Percentage body fat was measured by dual-energy x-ray absorptiometry (QDR-4500W; Hologic Inc., Waltham, MA). Visceral adipose tissue (VAT) and sc abdominal adipose tissue (SAAT) were quantified in cubic centimeters via magnetic resonance imaging (1.5T; General Electric Medical Systems, Milwaukee, WI) from five 1-cm transverse slices around the L4-L5 disk of the lumbar spine. Assessments of VAT and SAAT are described in detail elsewhere (24).

Information on moderate and vigorous PA was collected using questions 80 and 81 from the 2001 Youth Risk Behavior Survey (25). Moderate PA (days per week) was determined by the question, "On how many of the past 7 days did you participate in PA for at least 30 min that did not make you sweat or breathe hard, such as fast walking, slow bicycling, skating, pushing a lawn mower, or mopping floors?" Vigorous PA (days per week) was determined by the question, "On how many of the past 7 days did you exercise or participate in PA for at least 20 min that made you sweat or breathe hard, such as bicycling, fast dancing, or similar aerobic activities?" These moderate and vigorous PA variables have shown modest reliability and validity in this age group (26).

To assess mean daily intakes of energy, dietary calcium, vitamin D, and vitamin K and the percentage of kilocalories per day from carbohydrates, protein, and fat, the participants completed three 24-h diet recalls with a registered dietitian or trained research assistant on the day of blood collection (software version 2006; Nutrition Data System for Research, Minneapolis, MN). During the recall, participants were able to use their diet record for assistance. Before the recall, a registered dietitian trained the child and parent in how to maintain a diet record using food models, portion booklets, and serving containers for estimating serving size.

Statistical analysis

The sample was divided into normal-glucose and prediabetes groups (27). A child was considered to have prediabetes if he or she had impaired fasting glucose (IFG; fasting plasma glucose ≥ 100 mg/dl but < 126 mg/dl) or impaired glucose tolerance (IGT; 2 h glucose ≥ 140 mg/dl but < 200 mg/dl). Normal distribution and homogeneity of variances were confirmed by Shapiro-Wilks *W* and Levene's tests, respectively. Before analyses, fasting insulin, Matsuda index, insulinogenic index, and DI_{OGTT} were log transformed so that each of these variables followed an approximate normal distribution. An *F* test was performed to test the assumption of homogeneity of regression slopes for the interactions between the independent variable (*i.e.* normal glucose and prediabetes groups) and the covariates (age, sex, and race). Because there were no interactions, analysis of covariance was used to compare the differences in adiposity and biochemical measurements between the normal-glucose and prediabetes groups after adjusting for age, sex, and race (Table 1). Further analyses were conducted to determine group differences in the osteocalcin, insulin sensitivity, and β-cell function variables after control for the same covariates plus VAT. The adjusted means of the variables in the adjusted analyses are reported as mean ± SE.

Multiple linear regression analyses were used to determine whether any measure of osteocalcin (*i.e.* carboxylated, uncarboxylated, total, or percentage uncarboxylated) was independently related to insulin sensitivity and β-cell function after adjusting for age, sex, race, and VAT. The adiposity variable VAT was chosen as the confounding adiposity variable of interest because VAT has been shown in overweight children to have a greater influence on insulin resistance than either SAAT or percentage body fat (28). Because clinically meaningful and significant differences between groups were observed on many variables of interest, linear regression analyses were conducted separately for the normal-glucose group and prediabetes group. There were no interactions with race or sex, and thus, white and black males and females were analyzed in the same linear regression model. To determine whether any observed association between osteocalcin parameters and insulin sensitivity and β-cell function outcomes were not simply an association between bone turnover and glucose metabolism, we repeated the multivariate regression analysis with ICTP and P1NP. Data were analyzed using SPSS software package (version 18.02; PASW Statistics, Chicago, IL) and statistical significance was set at $P < 0.05$.

Results

Participant characteristics

Participant characteristics for the normal-glucose and prediabetes groups are presented in Table 1. Of the 140 participants, 30% were identified with prediabetes, and the numbers of children with IFG, IGT, and IFG + IGT were 21, 14, and 16, respectively. No significant differences were observed between groups in age, body mass index percentile, percentage body fat, SAAT, 25-OHD, ICTP, PA, and dietary intake. The prediabetes group, however, was found to have 17% lower P1NP concentrations and 25% greater levels of VAT than the normal-glucose group after adjustment for age, race and sex (both $P < 0.04$).

Osteocalcin, insulin sensitivity, and β-cell function parameters between normal-glucose and prediabetes groups

After controlling for age, sex, and race, the prediabetes group *vs.* normal glucose group had significantly lower uncarboxylated and total osteocalcin levels, insulinogenic in-

TABLE 1. Participant characteristics

Variable	Normal glucose	Prediabetes	P^a
N	99	41	
Sex (M/F)[b]	51/48	29/12	0.073
Race (W/B)[b]	48/51	27/14	0.021
Age (yr)[c]	9.1 ± 0.1	9.3 ± 0.2	0.212
BMI percentile[c]	96.7 ± 0.4	97.2 ± 0.4	0.571
Percent body fat (%)	40.0 ± 0.7	40.9 ± 1.0	0.727
VAT (cm^3)	31.7 ± 1.6	40.8 ± 2.5	0.003
SAAT (cm^3)	250.1 ± 10.8	270.4 ± 16.0	0.188
25-OHD (ng/ml)	27.4 ± 1.0	26.6 ± 1.6	0.678
P1NP (μg/liter)	605.9 ± 23.2	511.8 ± 30.4	0.038
ICTP (μg/liter)	17.0 ± 0.4	16.1 ± 0.5	0.169
Osteocalcin			
Carboxylated (ng/ml)	19.2 ± 0.7	17.3 ± 1.2	0.154
Uncarboxylated (ng/ml)	7.8 ± 0.4	5.6 ± 0.7	0.011
Total (ng/ml)	26.9 ± 0.9	22.8 ± 1.5	0.026
Uncarboxylated (%)	28.2 ± 1.1	24.7 ± 1.8	0.058
Insulin sensitivity			
Fasting glucose (mg/dl)	90.2 ± 0.6	99.2 ± 1.0	<0.001
Fasting insulin (μU/ml)	17.6 ± 1.1	22.7 ± 1.8	0.018
Matsuda index	3.2 ± 0.2	2.1 ± 0.2	<0.001
β-Cell function			
Insulinogenic index	2.4 ± 0.2	1.9 ± 0.3	0.041
DI_{OGTT}	6.7 ± 0.4	3.9 ± 0.5	<0.001
PA			
Moderate (d/wk)	2.3 ± 0.3	1.9 ± 0.4	0.154
Vigorous (d/wk)	4.1 ± 0.2	4.8 ± 0.4	0.412
Dietary intake			
Energy intake (kcal/d)	1722 ± 68	1767 ± 108	0.732
Carbohydrate (%)	50.7 ± 0.8	49.8 ± 1.4	0.542
Fat (%)	35.4 ± 0.6	35.5 ± 1.1	0.983
Protein (%)	14.8 ± 0.4	15.5 ± 1.4	0.223
Calcium (mg/d)[d]	725 ± 38	682 ± 62	0.565
Vitamin D (μg/d)[d]	4.7 ± 0.6	4.8 ± 1.0	0.941
Vitamin K (μg/d)[d]	55.5 ± 7.4	44.2 ± 11.0	0.081

Values are means ± SE. BMI, Body mass index; M, male; F, female; W, white; B, black.

[a] Test of significance between groups were based on analysis of covariance (controlling for age, sex, and race).

[b] Test of significance between groups were based on Fisher's exact test.

[c] Test of significance between groups were based on independent samples *t* test (two tailed).

[d] Test of significance between groups were based on analysis of covariance (controlling for age, sex, race, and energy intake).

dex, Matsuda index and DI_{OGTT} and higher fasting glucose and insulin levels (Table 1). There was a trend toward lower percentage uncarboxylated osteocalcin levels in the prediabetes group ($P = 0.058$). Further analyses revealed that adjusting for the higher levels of VAT in the prediabetes group did not alter outcomes for uncarboxylated and total osteocalcin, fasting glucose, Matsuda index, and DI_{OGTT} between groups (all $P < 0.05$). However, there was no group difference in fasting insulin when VAT was included as a covariate ($P = 0.15$). No significant differences in carboxylated osteocalcin concentrations were observed between groups.

Associations between osteocalcin parameters and indices of insulin sensitivity and β-cell function

Normal-glucose group

After adjustment for age, sex, and race, carboxylated osteocalcin ($\beta = 0.231$), uncarboxylated osteocalcin ($\beta = 0.185$), and total osteocalcin ($\beta = 0.258$) were positively associated with Matsuda index (all $P < 0.05$). Table 2 shows that when VAT was included as a covariate, these associations remained (all $P < 0.04$). Percentage uncarboxylated osteocalcin was inversely associated with fasting glucose after control for age, sex, and race ($\beta = -0.189$, $P = 0.048$), and this relationship persisted after including VAT as a covariate ($P = 0.041$). No significant associations were observed between osteocalcin variables and fasting insulin or β-cell function (all $P > 0.05$).

Prediabetes group

Carboxylated osteocalcin ($\beta = 0.481$) and total osteocalcin ($\beta = 0.334$) were positively associated with Matsuda index after adjustment for age, sex, and race (both $P < 0.05$). The associations of Matsuda index with carboxylated osteocalcin and total osteocalcin

TABLE 2. Carboxylated osteocalcin, uncarboxylated osteocalcin, total osteocalcin, and percentage uncarboxylated osteocalcin as independent predictors of indices of insulin sensitivity and β-cell function in the normal-glucose and prediabetes groups[a]

	Carboxylated OC		Uncarboxylated OC		Total OC		Uncarboxylated OC (%)	
	β	*P*	*β*	*P*	*β*	*P*	*β*	*P*
Normal glucose (n = 99)								
Fasting glucose	0.147	0.145	−0.091	0.370	0.090	0.376	**−0.206**	**0.041**
Fasting insulin[b]	−0.100	0.278	−0.120	0.181	−0.122	0.188	−0.058	0.504
Matsuda index[b]	**0.262**	**0.005**	**0.192**	**0.038**	**0.273**	**0.004**	0.041	0.656
Insulinogenic index[b]	−0.072	0.479	−0.124	0.205	−0.122	0.228	−0.055	0.568
DI_{OGTT}[b]	−0.022	0.855	−0.051	0.651	−0.064	0.588	−0.054	0.625
Prediabetes (n = 41)								
Fasting glucose	0.204	0.243	0.050	0.785	0.192	0.277	−0.187	0.331
Fasting insulin[b]	**−0.360**	**0.045**	0.130	0.350	-0.140	0.296	0.252	0.079
Matsuda index[b]	**0.467**	**0.022**	0.034	0.822	**0.426**	**0.031**	-0.171	0.261
Insulinogenic index[b]	−0.048	0.731	**0.338**	**0.016**	0.071	0.611	**0.362**	**0.011**
DI_{OGTT}[b]	0.183	0.276	**0.359**	**0.037**	0.265	0.114	0.290	0.096

OC, Osteocalcin.

[a] Multiple regression analyses were conducted between osteocalcin parameters *vs.* indices of insulin sensitivity and β-cell function, adjusted for age, sex, race, and visceral adipose tissue. Standardized β-values and *P* values are presented. Significant associations are in *bold*.

[b] Log transformed for analyses.

persisted after including VAT as a covariate (both $P < 0.04$, Table 2). Fasting insulin was negatively associated with carboxylated osteocalcin before ($\beta = -0.417$) and after ($\beta = -0.360$) control for VAT (both $P < 0.05$). In regard to β-cell function, uncarboxylated osteocalcin and percentage uncarboxylated osteocalcin were positively associated with insulinogenic index ($\beta = 0.341$ and $\beta = 0.405$, respectively) and DI_{OGTT} ($\beta = 0.367$ and $\beta = 0.342$, respectively) after adjustment for age, sex, and race (all $P < 0.04$). Table 2 displays similar findings of β-cell function with uncarboxylated osteocalcin and percentage uncarboxylated osteocalcin after additional adjustment for VAT; however, it attenuated the significant association between percentage uncarboxylated osteocalcin and DI_{OGTT} ($P = 0.096$). Total osteocalcin and carboxylated osteocalcin were not associated with β-cell function (all $P > 0.05$).

In both the prediabetes and normal-glucose groups, there were no associations between concentrations of ICTP, P1NP, or 25-OHD with insulin sensitivity or β-cell function after adjustment for age, sex, and race (all $P > 0.05$). However, in the normal-glucose group only, P1NP was negatively associated with fasting glucose ($\beta = -0.274$, $P = 0.02$).

Discussion

Since the recent discovery that treating osteocalcin-deficient mice with uncarboxylated osteocalcin ameliorates glucose intolerance and insulin resistance (1), it is essential from a clinical (therapeutic) perspective to learn the role of uncarboxylated osteocalcin in those at risk for diabetes, particularly in children who face a lifetime of chronic illness if they get diabetes. In the present study, we found that uncarboxylated osteocalcin concentrations were lower in children with prediabetes than in children with normal glucose levels. Carboxylated osteocalcin levels were not different between groups. In both the prediabetes and normal-glucose groups, positive relationships were found between carboxylated osteocalcin concentrations and insulin sensitivity, independent of age, sex, race, and visceral adiposity. In the prediabetes group only, uncarboxylated osteocalcin concentrations were positively associated with β-cell function. Taken together, these results suggest that the lower uncarboxylated osteocalcin levels observed in the overweight children at risk for diabetes could be contributing to β-cell dysfunction. In addition, our findings linking carboxylated osteocalcin and insulin sensitivity indicate that carboxylated osteocalcin plays a role in human glucose homeostasis.

Several clinical reports have shown that higher total osteocalcin levels are associated with better glucose homeostasis (6–10, 12–14). However, few studies have investigated the role of uncarboxylated osteocalcin alone in the glucose-insulin axis, and the findings have been disparate (11–15). The discrepancies may be attributed, in part, to glucose tolerance status in study participants. For instance, in the studies linking low uncarboxylated osteocalcin levels to glucose homeostasis, the participants had either prediabetes or type 2 diabetes (14, 15). It is possible that uncarboxylated osteocalcin may be relevant only to glucose homeostasis when circulating uncarboxylated os-

teocalcin concentrations are deficient. Therefore, uncarboxylated osteocalcin may not have any additional effect on the glucose-insulin axis when circulating levels are sufficient. To date, there is no established criterion for uncarboxylated osteocalcin deficiency. Given that the prediabetes *vs.* normal-glucose group had lower uncarboxylated osteocalcin concentrations, further study is needed to determine whether a certain level of uncarboxylated osteocalcin is required for optimal glucose-insulin function and regulation.

Animal studies suggest that carboxylated osteocalcin does not have an active role in the regulation of glucose homeostasis (1–4). However, in this study and others (6–15), higher concentrations of both carboxylated osteocalcin and total osteocalcin, which reflects mostly the carboxylated form, are associated with lower insulin resistance, suggesting that the carboxylated form of osteocalcin may play a role in the glucose-insulin axis. The disparate findings may reflect a species difference as to which form of osteocalcin (carboxylated *vs.* uncarboxylated) is the active form with regard to glucose homeostasis (29). It is plausible that, in humans, both forms of osteocalcin may have significant, but different, roles in the glucose-insulin axis. Based on our findings, carboxylated osteocalcin may have more relevance to insulin sensitivity, whereas uncarboxylated osteocalcin may be more important for β-cell function. However, an effect of uncarboxylated osteocalcin on β-cell function may be observed only in individuals with abnormal glucose metabolism, given that we and others (14, 15) have observed this relationship exclusively in prediabetes and diabetes. Given the observational nature of our study and others (6–15), experimental investigations are needed to confirm the roles of both forms of osteocalcin in the glucose-insulin axis, particularly in those with diabetes and at risk for diabetes.

Although osteocalcin has long been considered a marker of bone formation, it can also be released into circulation during bone resorption (30), so the serum concentration may reflect components of both formation and resorption. Because animal work by Ferron *et al.* (31) suggests that the major source of uncarboxylated osteocalcin comes from bone resorption rather than formation, we assessed P1NP and ICTP concentrations to better understand glucose homeostasis with bone turnover status. Although ICTP levels were not different between groups, the P1NP concentrations were lower in the children with prediabetes. These findings, along with higher total osteocalcin concentrations found in the normal-glucose group, indicate that prediabetes may affect bone formation but not bone resorption.

It is thought that the link between bone formation and glucose metabolism is regulated via leptin, an adipocyte-derived hormone. It has been shown that as leptin levels increase, there is a subsequent decrease in bone formation, which in turn depresses insulin sensitivity and secretion via decreased production of uncarboxylated osteocalcin (32). It is possible that the group differences in uncarboxylated osteocalcin could have been attributed to higher leptin concentrations in the children with prediabetes, which may have suppressed osteocalcin formation. Alternatively, lower adiponectin concentrations in the prediabetes group could have been a contributing factor to the study findings because it is hypothesized that uncarboxylated osteocalcin decreases insulin resistance by inducing secretion of adiponectin (1). Although leptin and adiponectin were not assessed in this study, our analyses did take into account adiposity (because of its strong correlation to both leptin and adiponectin) as a potential surrogate for the adipocyte-derived hormones. Interestingly, the groups were not distinguishable by total or sc abdominal adiposity; however, visceral adiposity levels were, on average, 25% higher in the prediabetes group than in the normal-glucose group. When we sought to determine whether visceral adiposity could explain the findings between osteocalcin parameters and insulin sensitivity and β-cell function, our data suggest that factors other than visceral adiposity (or percentage body fat and SAAT, but data not shown) are imperative to the relationship between osteocalcin and glucose homeostasis.

Given that vitamin K is involved in the γ-carboxylation of osteocalcin, it is important to note that, although not significantly different, the normal-glucose group reported dietary vitamin K intakes of 56 μg/d compared with only 44 μg/d in the prediabetes group, which is below the recommended adequate intake of 55–75 μg/d for this age group (33). The percentage uncarboxylated osteocalcin level is considered a measure of vitamin K status of bone, which increases in vitamin K depletion and decreases in response to vitamin K supplementation (34). In this study, we did not find significant differences in percentage uncarboxylated osteocalcin levels between groups, suggesting that vitamin K status may not be an important factor in glucose homeostasis. Notwithstanding, recent data suggest that increasing dietary vitamin K intake is beneficial for reducing the progression of insulin resistance (35) and is associated with a reduced risk of type 2 diabetes (36). Vitamin K interventions are warranted to understand the role of osteocalcin in glucose homeostasis.

A major strength of this investigation is that this is the first pediatric study to use dynamic measurements of glucose and insulin concentrations for assessment of insulin sensitivity and β-cell function and their relationship to carboxylated and uncarboxylated forms of osteocalcin. Another strength was that our participants were homogenous with respect to pubertal stage (all Tanner stage I),

thus allowing us to minimize potential confounding effects of the pubertal transition on bone and glucose metabolism (18). In contrast, we acknowledge study limitations. In addition to not having biomarker information on leptin and adiponectin, our study used cross-sectional data, and thus, we cannot be certain that uncarboxylated or carboxylated osteocalcin has a direct effect on glucose homeostasis. Furthermore, some of the associations between osteocalcin variables and measures of insulin sensitivity and β-cell function failed to reach statistical significance, likely due to our relatively small sample size. Therefore, we recognize that assessing these relationships in larger cohorts is needed.

In summary, our data suggest that the lower uncarboxylated osteocalcin concentrations found in children with prediabetes may be associated with β-cell dysfunction. In addition, our findings between carboxylated osteocalcin and insulin sensitivity suggest that carboxylated osteocalcin plays a role in human glucose homeostasis.

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