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Greater inflammation and adiposity are associated with lower bone mineral density in youth with type 1 diabetes

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Abstract

Aims: The objectives of this study were to investigate relationships of inflammation and adiposity with bone mineral density (BMD) in youth with type 1 diabetes followed prospectively for 18 months.

Methods: Participants were youth with type 1 diabetes (n=136, 8–16.9 years) enrolled in an 18-month behavioral nutrition intervention trial. BMD of the total body, subtotal, lumbar spine, pelvis leg, arm and rib, percent body fat and percent trunk fat (by dual energy x-ray absorptiometry) and C-reactive protein (CRP), a marker of inflammation, were assessed at baseline, 12 and 18 months. Linear mixed-effects models estimated associations of time-varying BMD with time-varying CRP, and with percent body and trunk fat.

Results: CRP was inversely associated with BMD of the total body, pelvis and leg (n=136). Percent body fat was inversely associated with BMD of the total body and pelvis; whereas percent trunk fat was related only to total body BMD.

Conclusions: Greater inflammation and adiposity were related to lower BMD in youth with type 1 diabetes. Investigating the impact of inflammation and adiposity on bone turnover markers could provide insights on mechanisms that contribute to this relationship.

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Author contributions

Tonja R. Nansel and Leah M. Lipsky obtained the data for analyses. Namrata Sanjeevi researched the data and wrote the manuscript. Tonja R. Nansel and Leah M. Lipsky reviewed and edited the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

Keywords

type 1 diabetes; inflammation; adiposity; bone mineral density

1. Introduction

Autoimmune destruction of the β -cells of the pancreas (1) resulting in insulin deficiency and disruption of glucose homeostasis (2) has been implicated in bone mineral loss in type 1 diabetes patients. Previous studies have observed low bone turnover (characterized by impaired bone formation) (3, 4) and osteopenia (5) among youth with type 1 diabetes. Moreover, poorer glycemic control has been associated with low bone mineral density (BMD) in this population (6, 7). Meta-analyses have consistently demonstrated an increased risk for fractures (8, 9) attributed to low bone mass (10) among individuals with type 1 diabetes compared to healthy controls. Thus, understanding correlates of BMD in individuals with type 1 diabetes may inform efforts to mitigate bone loss in this population.

Accumulation of bone mass in childhood is largely driven by linear growth and bone modeling involving the coordinated processes of bone deposition by osteoblasts and bone resorption by osteoclasts (11). Derangement of this process may interfere with bone accrual resulting in reduced peak bone mass in adulthood. Inflammation may interfere with bone mineral acquisition, as it has been shown to inhibit osteoblast function and promote osteoclast differentiation (12). Consistent with this hypothesis, higher serum C-reactive protein (CRP), a marker of inflammation, is associated with reduced bone mass in healthy children (13) and in those with impaired glucose tolerance (14). Given that low bone mass and high inflammation are common characteristics of type 1 diabetes (6), research is warranted to understand how these factors are interrelated in this population. However, only one study has examined the association of inflammation with bone mass in the context of type 1 diabetes, and is limited by small sample size ($n=30$) and the cross-sectional design (6).

Body composition may be another important consideration in understanding the relationship of inflammation with BMD among type 1 diabetes patients. Greater adiposity has been associated with pancreatic β -cell hypersecretion of hormones, such as insulin and amylin (15, 16) in the general population, which have an anabolic effect on bone (15–17). The increased BMD observed in individuals with obesity and type 2 diabetes has been attributed to hyperinsulinemia (17). However, this physiologic mechanism may be uncoupled in type 1 diabetes due to the destruction of pancreatic β -cells. Further, the association of increased adiposity with increased inflammation (18, 19) may lead to lower BMD. Previous studies investigating BMD predictors in type 1 diabetes have relied on BMI as an indicator of adiposity (20, 21). Thus, research is needed to examine the relationship of more direct measures of body composition with BMD in type 1 diabetes.

The primary objectives of this study were to investigate the relationship of inflammation with BMD (measured as the amount of calcified bone tissue in grams/centimeter²), and to estimate associations of total and central adiposity with BMD in youth with type 1 diabetes followed prospectively for 18 months.

2. Materials and Methods

2.1. Study Design

This is a secondary analysis of data from a randomized clinical trial (RCT) of a behavioral nutrition intervention conducted from 2010–2013 at a tertiary diabetes center in the northeast United States. Details regarding the intervention have been described previously (22). Eligibility criteria included youth age ranging from 8.0 to 16.9 years, 1 year of diagnosis with type 1 diabetes, 0.5 units per kilogram of daily insulin dose, most recent HbA1c ranging from 6.5% (48 mmol/mol) to 10.0% (86 mmol/mol), 3 injections per day or use of insulin pump, at least one clinic visit in the past year, and able to communicate in English. Youth were excluded from the study based on the following criteria: daily use of premixed insulin, transition to insulin pump therapy in the past three months, use of real-time continuous glucose monitoring in the past three months, participation in another intervention study in the past six months, use of medications that significantly interfere with glucose metabolism, or presence of gastrointestinal disease, multiple food allergies, or significant mental illness. Of 622 eligible invited youth, 148 (24%) consented to participate and 139 (22%) completed baseline assessments. Exclusion of data from one sibling each of 3 sibling pairs resulted in a sample of 136; 125 (92%) were retained through study completion.

The goal of the intervention was to increase intake of whole plant foods (whole fruits, vegetables, whole grains, legumes, nuts, and seeds). The control arm received equal frequency of contacts with research staff, but did not receive any dietary advice besides that provided as part of standard type 1 diabetes care. Study participation spanned a duration of 18 months.

All youth provided assent, and written informed consent was obtained from parents at the time of enrollment. Additionally, youth turning 18 years of age during the study provided written informed consent. Institutional review boards of the participating institutions approved the study procedures.

2.2. Measures

2.2.1. Bone mineral density (BMD)—The bone mineral content (BMC) and bone area (BA) for total body and subtotal (calculated by subtracting head region from total body measurements) were measured by dual energy X-ray absorptiometry (DXA; Hologic Inc.) at baseline, 12 and 18 months. The BMC and BA of both weight bearing (23) (lumbar spine, pelvis, and left and right legs) and non-weight bearing sites (left and right arms, and ribs) were determined as they could be differentially impacted by adiposity. BMD, calculated by dividing BMC by BA, was obtained for total body, subtotal, lumbar spine, pelvis, and the average of both legs, arms and ribs. The DXA manufacturer provided total body BMD z-scores by comparison of individual measurements with an age- and sex-matched reference population (24).

2.2.2. Adiposity—Percent body fat and percent trunk fat were assessed by dual energy x-ray absorptiometry (DXA; Hologic, Inc.) at baseline, 12 and 18 months. Total and central

adiposity were included because they reflect different distributions of body fat, and are differentially associated with health outcomes (25).

2.2.3. Serum laboratory measurements—Blood samples were drawn from youth at baseline and 6, 12 and 18 months follow-up. Samples were stored at room temperature for 20–30 minutes following collection, then centrifuged for 15 minutes at ~3000 RPM at temperature of 4 °C, aliquoted and frozen at –80 °C for later assay. A high sensitivity enzyme-linked immunosorbent assay (Architect c8000, Abbott) with a lower detection limit of 0.2 mg/l was used to quantify CRP in the serum samples. Circulating vitamin D was determined by serum concentrations of 25-hydroxyvitamin D assessed by radioimmunoassay, and the continuous variable was used for all analyses.

2.2.4. Diabetes characteristics and socio-demographics—Height, weight, age, sex, insulin regimen and dosage were extracted from medical records at baseline and at 3, 6, 9, 12 and 18 months follow-up. BMI (kg/m^2) was calculated from height and weight, and weight status was determined with respect to Centers for Disease Control and Prevention age- and sex-adjusted cut-offs (26). The date of diagnosis of diabetes, as indicated in the medical records, was used to calculate baseline diabetes duration. A laboratory assay standardized to the Diabetes Control and Complications Trial (reference range, 4–6%, [20–42mmol/mol]) was utilized to measure glycated hemoglobin (HbA1c) at each visit. Initial HbA1c measurements were conducted using Tosoh (Tosoh Medics, South San Francisco, CA, USA) followed by the Roche Cobas Integra (Indianapolis, IN). Laboratory analysis of results from the Tosoh and Roche instruments on identical samples indicated no clinically significant bias between the two methods. Tanner stage was abstracted from medical records to assess pubertal status. The previous-visit Tanner stage was carried forward for missing visit-specific data.

Parents reported youth race/ethnicity at baseline. Moderate and vigorous physical activity (hours/week) was calculated from responses to items from the Behavioral Risk Factor Surveillance System at baseline, 6, 12 and 18 months (27).

2.2.5. Calcium intake—Calcium intake was determined from three-day food records completed by families at baseline and at 3, 6, 9, 12 and 18 months follow-up. Families were provided a sample dietary record along with instructions on accurately measuring and reporting food and beverage intake. Scales, measuring cups and spoons were used to enable portion-size estimation. Participants also were requested to provide detailed information of each food item consumed, such as brand or restaurant names, and food labeling (for example, fat-free, 1% milk). Research staff reviewed the dietary records for completeness and contacted participants to request information that was found missing on the records. If a family did not complete records, a registered dietitian obtained 2 non-consecutive 24-hour dietary recalls (1.7% of assessments). Calcium intake was estimated from the records using the Nutrition Data System for Research (NDSR; Nutrition Coordinating Center, University of Minnesota). The mean calcium intake from the food records was divided by the age-based recommended dietary allowance for calcium to indicate the percentage of recommendation that was met by intake.

2.3. Statistical analysis

Differences in baseline characteristics between treatment groups were tested using independent samples t-tests for continuous variables and Pearson's chi-square tests for categorical variables. BMD classification was determined for participants using BMD z-scores, where scores of -2.0 , between -2.0 and -1.0 , and -1.0 indicated low, low normal, and normal BMD, respectively (28). Serum CRP concentration was classified as normal (<1.0 mg/l), borderline ($1-3$ mg/l) and high risk (>3 mg/l) for cardiovascular disease (CVD) based on the American Heart Association and Centers for Disease Control and Prevention (29). Continuous serum CRP concentration was log-transformed to improve normality. BMD, log-transformed CRP and adiposity measures obtained at baseline, 12 and 18 months were used for the main analyses. Linear mixed effects models estimated associations of time-varying continuous BMD with time-varying continuous CRP and adiposity indicators (percent body fat and percent trunk fat). Models included a random intercept representing the subject-specific baseline variation in the outcome. Covariates included baseline sex, treatment assignment, and diabetes duration, and time-varying age, height, weight, Tanner stage, glycemic control, insulin dose, insulin regimen, serum vitamin D, physical activity, and ratio of calcium intake to age-based recommendations. Weight was included as a covariate to adjust for the positive effect of mechanical stress on BMD (30). We additionally adjusted for height to account for the impact of vertical size on weight and BMD. Linear mixed effects models also estimated the association of percent body fat and percent trunk fat with BMD measurements. This analysis included similar covariates, but weight was not included due to high collinearity with percent body fat and trunk fat. The linear mixed effects models were then additionally adjusted for log-transformed CRP. Due to the exploratory nature of the study, analyses were not adjusted for multiple testing (31, 32). SPSS version 21 were used for all analyses.

3. Results

3.1. Baseline participant characteristics

As shown in Table 1, baseline percent body fat was higher in the control compared to the intervention group. No other differences were observed between the two groups.

3.2. Classification of participants based on BMD z score and CRP levels

The proportion of participants classified as having a low total body BMD (z score ≤ -2.0) was 3.3%, whereas 15.4% of the youth had a z-score in the low range of normality ($-1.0 < z$ score < -2.0). Fifteen percent and 10.4% of the recruited sample had CRP levels in the range of $1-3$ mg/l and >3 mg/l, respectively.

3.3. Association of BMD with CRP

There was no intervention effect on BMD (data not shown). CRP was negatively associated with BMD of the total body ($p=0.02$), such that a one-unit increase in log-transformed CRP was related to a BMD decrease of 0.02 g/cm². CRP also was inversely associated with BMD of subtotal body ($p=0.02$), lumbar spine ($p=0.005$), and leg ($p=0.002$) (Tables 2 & 3).

Inverse associations of CRP with pelvis ($p=0.31$), arm ($p=0.11$) and rib ($p=0.14$) BMD were not statistically significant.

3.4. Association of adiposity indicators with bone outcomes

Percent body fat ($p=0.006$) and trunk fat ($p=0.01$) were inversely related to total body BMD (Model 1, Tables 2 & 3). A one percent increase in total body fat and trunk fat was associated with a BMD decrease of 0.002 and 0.001 g/cm², respectively. Given that percent total body fat at baseline varied from 11.5 to 47.5% in this sample, this difference of 35% would be associated with a difference in BMD of 0.07 g/cm², which is approximately equivalent to the difference between the 50th and 25th percentile in children (33). In addition, percent body fat was negatively associated with BMD of the pelvis ($p=0.01$). These associations remained significant after additionally adjusting for log-transformed CRP (Model 2, Tables 2 & 3). The relationships of percent body fat and trunk fat with subtotal, lumbar spine, leg and arm BMD were not statistically significant. Percent trunk fat was significantly associated with rib BMD, after adjusting for log-transformed CRP ($p=0.03$).

4. Discussion

In this study of youth with type 1 diabetes, CRP was inversely related to BMD of the total body, subtotal body, lumbar spine, and leg; associations with pelvis, arm and rib BMD did not reach statistical significance. Adiposity, measured as percent body fat and percent trunk fat, was negatively associated with total body BMD. Further, percent body fat was inversely associated with BMD of the pelvis, but associations with other weight bearing sites (lumbar spine and leg) were not statistically significant. These findings are clinically relevant given the elevated risk of hip fractures among type 1 diabetes patients (34, 35). In contrast, non-weight bearing sites may not be sensitive to the effects of adiposity, thereby explaining lack of significant inverse associations of adiposity measures with arm and rib BMD. These findings collectively suggest an unfavorable role of inflammation and adiposity in reducing total body BMD among youth with type 1 diabetes.

Disruption of bone metabolism by inflammation (12, 36) could explain the inverse relationship between CRP and BMD observed in this study. This finding is consistent with previous research showing that CRP negatively impacts bone outcomes in a general sample of children (13), as well as in overweight children with impaired glucose tolerance (14). In contrast, a previous cross-sectional study in 30 youth with type 1 diabetes found no correlation between CRP and BMD parameters (6). This discrepancy could be attributed to the longitudinal nature and greater sample size of the current study. Previous research has shown positive (37–39) and negative (40–43) association of body fat with bone outcomes in healthy children. To the best of our knowledge, this is the first study to indicate an inverse relationship between adiposity and bone density in youth with type 1 diabetes, who lack endogenous insulin production. Previous evidence in this sample of youth with type 1 diabetes indicates that adiposity is positively associated with CRP (19). However, the relationship of adiposity with total body BMD remained significant after adjusting for CRP. Collectively, the study results imply that the association of increased adiposity with lower BMD may be attributable to mechanisms in addition to inflammation. Total body BMD was

at least 1 standard deviation lower than the reference population for nearly one-fifth of the study sample, suggesting impaired bone acquisition and achievement of suboptimal peak bone mass. However, BMD z-scores were calculated based on comparison to an age- and sex-matched reference population (24), without any consideration of the pubertal stage. Since puberty represents an important period for bone mass accrual (44) and delayed pubertal onset occurs in type 1 diabetes (45), differences in pubertal stage between type 1 diabetes youth and reference population could explain the lower z-scores observed in this study. Nevertheless, this result is comparable to other studies that have shown decreased bone growth in adolescents with type 1 diabetes compared to controls, matched for pubertal stage (4, 7).

The results of this study are subject to certain limitations. The participants were children and adolescents at different stages of puberty, and thus, skeletal maturity. Although pubertal stage was not significantly associated with BMD in the current sample (data not shown), it is important to confirm the findings of this study in adults with type 1 diabetes who have reached their peak bone mass. The predominantly white sample, eligibility criteria for the primary study, and 22% recruitment rate limit generalizability of the study findings. Nevertheless, this study is strengthened by the longitudinal design utilizing repeated measures of exposures and outcomes, assessment of BMD at different regions (lumbar spine, pelvis, leg, arm and rib), and adjustment of other potentially important covariates in the analyses.

In conclusion, this study demonstrates that CRP and adiposity are inversely related to BMD in youth with type 1 diabetes. Given findings suggesting that greater body fat may limit bone acquisition during adolescence in youth with type 1 diabetes, it may be important to screen bone density in those with increased adiposity. The results also provide evidence of a potentially catabolic effect of inflammation on bone in this sample. Investigating the impact of inflammation and adiposity on bone turnover markers could provide further insights on mechanisms that relate adiposity to lower BMD in type 1 diabetes.

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Highlights

1. Nearly 1/5th of type 1 diabetes youth in the study had low or low normal bone mineral density (BMD)
2. Inflammation was inversely related to BMD of the total body, pelvis and leg
3. Percentage of body fat was negatively associated with BMD of the total body and pelvis
4. Elevated body fat and inflammation could induce low BMD, and increase fracture risk in patients with type 1 diabetes

Table 1.

Baseline sample characteristics and bone mineral density (BMD) measurements of the overall group and by treatment assignment.

	Overall (N=136)	Treatment (N=66)	Control (N=70)	p-value
Age, years	12.7±2.6	12.5±2.7	13.0±2.5	0.27
Body mass index, kg/m ²	21.3±4.2	21.0±4.1	21.6±4.3	0.37
Weight status [*]				
Underweight	1 (0.7)	1 (1.5)	0 (0)	0.55
Normal weight	91 (66.9)	42 (63.6)	49 (70.0)	
Overweight	28 (20.6)	17 (25.8)	11 (15.7)	
Obese	16 (11.8)	6 (9.1)	10 (14.3)	
HbA1c, (% [mmol/mol])	8.1±1.0 [65±11]	8.1±1.1 [65±12]	8.1±1.0 [65±12]	0.95
Duration of diabetes, years	6.0±3.1	5.6±2.5	6.4±3.6	0.15
Insulin dose, Units/day	49.8±26.8	46.9±23.9	52.7±29.3	0.24
Tanner stage	2.5±1.4	2.4±1.4	2.6±1.5	0.38
Youth race/ethnicity				
Non-Hispanic white	123 (90.4)	58 (87.9)	65 (92.9)	0.17
Non-Hispanic black	5 (3.7)	2 (3.0)	3 (4.3)	
Hispanic	7 (5.2)	6 (9.1)	1 (1.4)	
American Indian/Alaska Native	1 (0.7)	0 (0)	1 (1.4)	
Total BMD, g/cm ²	0.93±0.12	0.93±0.12	0.94±0.11	0.84
Subtotal BMD, g/cm ²	0.82±0.13	0.82±0.14	0.82±0.12	0.94
Spine BMD, g/cm ²	0.86±0.16	0.85±0.17	0.86±0.16	0.75
Pelvis BMD, g/cm ²	1.03±0.19	1.03±0.20	1.03±0.19	0.96
Arm BMD, g/cm ²	0.65±0.10	0.65±0.10	0.65±0.08	0.92
Leg BMD, g/cm ²	0.97±0.15	0.97±0.17	0.97±0.14	0.99
Rib BMD, g/cm ²	0.58±0.09	0.57±0.09	0.58±0.09	0.51
C-reactive protein, mg/L	1.14±1.92	0.86±1.44	1.41±2.25	0.09
Percent body fat, %	27.5±7.9	25.9±3.4	28.9±8.2	0.03
Percent trunk fat, %	23.7±8.7	22.2±7.8	25.1±9.4	0.06
Serum vitamin D, ng/ml	29.7±8.6	30±8.7	29.5±8.5	0.73
Mean calcium intake	988.4±434.3	1009.3±377.1	968.6±484.0	0.59
Physical activity, hours/week	12.5±13.3	13.9±13.3	10.9±13.3	0.26

Data are presented as mean ± SD or n (%)

^{*} Underweight = BMI%ile < 5; Normal weight = 5 BMI%ile < 85; Overweight = 85 BMI%ile < 95; Obese = 95 BMI%ile

Table 2.

Associations of C-reactive protein (CRP) and adiposity with total and subtotal bone mineral density (BMD) in youth with type 1 diabetes participating in an 18-month behavioral nutrition intervention trial.

	Total BMD [*]			Subtotal BMD [†]		
	β	SE	p	β	SE	p
CRP (mg/l) [‡]	-0.02	0.01	0.02	-0.02	0.01	0.02
% body fat (%)						
Model 1 [§]	-0.002	0.001	0.006	-0.0003	0.001	0.50
Model 2 ^δ	-0.001	0.001	0.01	-0.0002	0.001	0.78
Trunk % fat (%)						
Model 1 [§]	-0.001	0.001	0.01	-0.0002	0.001	0.69
Model 2 ^δ	-0.001	0.001	0.03	-0.00002	0.001	0.98

^{*} BMD presented as g/cm²

[†] Subtotal BMD calculated as Total body BMD – Head BMD

[‡] Linear mixed model estimating associations of log-transformed CRP with bone outcomes, adjusting for time-varying age, sex, treatment assignment, height, weight, tanner stage, duration of diabetes diagnosis, glycemic control, insulin dose and regimen, serum vitamin D, physical activity, and ratio of calcium intake to age-based recommendations.

[§] Linear mixed model estimating associations of adiposity indicators with bone outcomes, adjusting for time-varying age, sex, treatment assignment, height, tanner stage, duration of diabetes diagnosis, glycemic control, insulin dose and regimen, serum vitamin D, physical activity, and ratio of calcium intake to age-based recommendations.

^δ Linear mixed model estimating associations of adiposity indicators with bone outcomes, adjusting for Model 1 covariates and log-transformed CRP

Table 3.

Associations of C-reactive protein (CRP) and adiposity with regional bone mineral density (BMD) in youth with type 1 diabetes participating in an 18-month behavioral nutrition intervention trial.

	Lumbar spine BMD [*]			Pelvis BMD			Leg BMD			Arm BMD			Rib BMD		
	β	SE	p	β	SE	p	β	SE	p	β	SE	p	β	SE	p
CRP (mg/l) [†]	-0.06	0.02	0.005	-0.02	0.02	0.31	-0.04	0.01	0.002	-0.01	0.01	0.11	-0.02	0.01	0.14
% body fat (%)															
Model 1 [‡]	-0.0004	0.001	0.71	-0.003	0.001	0.01	0.0005	0.001	0.58	-0.001	0.001	0.05	0.001	0.001	0.13
Model 2 [§]	-0.00002	0.001	0.99	-0.003	0.001	0.006	0.001	0.001	0.32	-0.001	0.001	0.06	0.001	0.001	0.08
Trunk % fat (%)															
Model 1 [‡]	0.0003	0.001	0.73	-0.001	0.001	0.14	0.0004	0.001	0.55	-0.001	0.0005	0.16	0.001	0.001	0.05
Model 2 [§]	0.001	0.001	0.49	-0.002	0.001	0.11	0.001	0.001	0.30	-0.001	0.0005	0.18	0.001	0.001	0.03

^{*} BMD presented as g/cm²

[†]Linear mixed model estimating associations of log-transformed CRP with bone outcomes, adjusting for time-varying age, sex, treatment assignment, height, weight, tanner stage, duration of diabetes diagnosis, glycemic control, insulin dose and regimen, serum vitamin D, physical activity, and ratio of calcium intake to age-based recommendations.

[‡]Linear mixed model estimating associations of adiposity indicators with bone outcomes, adjusting for time-varying age, sex, treatment assignment, height, tanner stage, duration of diabetes diagnosis, glycemic control, insulin dose and regimen, serum vitamin D, physical activity, and ratio of calcium intake to age-based recommendations.

[§]Linear mixed model estimating associations of adiposity indicators with bone outcomes, adjusting for Model 1 covariates and log-transformed CRP