Effect of cardiometabolic risk factors on the relationship between adiposity and bone mass in girls

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Abstract

Background/Objective—Childhood obesity has been separately associated with cardiometabolic risk factors (CMR) and increased risk of fracture. However, both augmented and compromised bone mass have been reported among overweight/obese children. Metabolic dysfunction, often co-existing with obesity, may explain the discrepancy in previous studies. The aim of this study was to examine whether the relationship between adiposity and dual-energy x-ray absorptiometry (DXA) derived bone mass differed in young girls with and without CMR(s).

Subjects/Methods—Whole body bone and body composition measures by DXA and measures of CMR (fasting glucose, high density lipoprotein cholesterol-HDL-C, triglyceride-TG, systolic and diastolic blood pressure, waist-circumference-WC) were obtained from 307, 9–12 year old girls. Girls with 1 or ≥ 2 CMR(s) were considered to be at risk (vs. no CMR). Multiple linear regression was used to test the relationship of total fat mass with total body bone mineral content (BMC) after controlling for height, lean mass, CMR risk, and other potential confounders.

Results—There was a significant interaction between CMR risk and total body fat mass. When girls were stratified by CMR group, all groups had a significant positive relationship between fat mass and BMC (p<0.05), however girls with ≥ 2 CMRs had a lower BMC for a given level of body fat. Total body fat was not significantly related to bone mineral density (p>0.05).

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Conclusion—Fat mass has a positive relationship with BMC even after controlling for lean mass. However, the positive relationship of fat mass with BMC may be attenuated if multiple CMRs are present.

Introduction

Childhood obesity is a major health concern leading to Type 2 Diabetes and cardiovascular disease.(1) A growing body of research suggests that childhood obesity may also impair bone development leading to decreased bone mineral content (BMC), density (BMD), strength and structural properties.(2) Although it has been reported that obese children are not more at risk of fracture than normal weight children(3), several studies have indicated the contrary, with findings of an increased prevalence and risk of certain fractures in overweight and obese children.(4–7) These findings challenge the past notion that excess adiposity is protective against fractures and enhances bone strength due to the increase in mechanical loading on the skeleton stimulating bone formation.(8).

Comparing bone mass of overweight and obese children to normal weight children is complicated, as overweight and obese children tend to be more advanced in maturation causing them to be taller and to reach peak height velocity sooner than children who are of normal weight.(2) In addition to having more fat mass, overweight and obese children also have higher amounts of lean mass, which is a major determinant of bone strength.(9) Thus, the higher bone mass seen in overweight and obese children may be primarily driven by the loading imposed by muscle forces on the skeleton instead of the extra weight of adipose tissue. Even after controlling for important confounders such as maturation, height, and lean mass, results are mixed with reports of augmented(10, 11) or compromised bone mass accrual in overweight and obese children.(12)

The conflicting results may be due to the presence of metabolic abnormalities in some but not all overweight and obese children.(2) These metabolic abnormalities may compromise gains in bone mass in overweight and obese children, despite the increased loading on bone with excess weight. Several possible mechanisms have been proposed to explain the consequences of obesity-related metabolic dysfunction for bone. Pre-diabetes and type 2 diabetes, which occur in a setting of low-grade inflammation, have negative associations with bone mass.(13, 14) Whether altered metabolic factors associated with these conditions directly alter bone or serve as biomarkers for other adverse systemic changes is not clear, although, for example, there is some pre-clinical evidence and clinical evidence in adults that high cholesterol can alter bone cell function leading to a decrease in bone mineral density.(15) Beyond the adverse metabolic effects of excess adiposity, lifestyle factors that contribute to obesity such as lack of physical activity and poor diet, can also contribute to impaired bone development.(16, 17)

Though methodological differences such as the skeletal site assessed, bone parameters reported, differences in confounders and covariates included in the statistical analysis, as well as differing demographics may contribute to the conflicting relationship reported between excess fat mass and bone in children, there is a paucity of research exploring the effects of CMRs.(18) Understanding how obesity and its metabolic consequences effect

bone development in children, especially during the two years surrounding the adolescent growth spurt when peak bone mineral density occurs, is an important clinical question as impaired bone growth during this time likely leads to suboptimal peak bone mass, increasing the risk for fractures and developing osteoporosis later in life.(13, 19) The aim of this study was to examine whether the relationship between whole body adiposity and DXA-derived total body bone mass in 9-to-12 year old girls differed depending on the number of CMRs present.

Subjects and Methods

Study population

Three hundred and thirty-two girls aged 9–12 years were recruited from local schools, pediatric clinics, and wellness community events in Tucson, Arizona to participate in the Soft Tissue And Bone Development in Young GiRls ("STAR") study, designed to assess the effects of adiposity and related metabolic risk factors on bone development in girls (Clinical trials # NCT02654262). The study protocol was approved by the University of Arizona Human Subjects Protection Committee. Written informed consent was obtained from all participants and their parents or legal guardians. Exclusion criteria included: diagnosis of diabetes, taking any medications that alter body composition, physical disability that limits physical activity, and learning disability that limited completion of questionnaires or otherwise made the participant unable to comply with assessment protocols.

Anthropometry

Anthropometric measures were obtained according to standardized protocols.(20) Body mass was measured to the nearest 0.1 kg using a calibrated scale (Seca, Model 881, Hamburg, Germany). Standing and sitting height were measured at full inhalation to the nearest mm using a stadiometer (Shorr Height Measuring Board, Olney, MD). Using a flexible tape and with the subjects standing, waist circumference (WC) was measured at the umbilicus in cm. Leg length was determined by subtracting sitting height from standing height. The mean of two measurements of weight, standing height, sitting height and WC were used in the analysis. Measurements were repeated if they differed by 0.3 kg for body mass, 0.5 cm for height, and 1 cm for WC. If repeat measures were required, the mean of the second set of measures was used.(21) BMI was calculated as weight (kg) divided by height (m) squared. Based on CDC growth charts, BMI percentiles specific for age and gender were used to categorize girls as either underweight (<5th percentile), normal weight (5th and <85th percentiles), overweight (85th and <95th percentiles), or obese (95th percentile).(22)

Physical Maturation

The use of chronological age as a proxy for maturation status is limited as there can be important differences in maturation in children of the same age, especially during the pre-pubertal and pubertal years.(23) Thus, in the "STAR" study, maturation was assessed several ways. First, a self-reported questionnaire was used where girls rated their breast and pubic hair development based on pictures illustrating the Tanner stages of pubertal maturation (24) in addition to self-reporting menarcheal status. Understanding that the ability of self-

reported Tanner staging to accurately assess maturation is limited(25, 26), maturation was also assessed using maturity offset, an estimate of years from peak height velocity (PHV) that is estimated from age and anthropometric measures (height, weight, sitting height, and leg length) using the Mirwald equation.(23) The maturity offset has been shown to explain 89% of the variance in years from PHV.(23) After PHV is reached maturity offset is positive, while a negative maturity offset represents years before PHV.

Dual-energy X-ray absorptiometry (DXA)

Measures of whole-body BMC, areal bone mineral density (aBMD, bone area (BA), fat mass, and lean mass were obtained from dual energy x-ray absorptiometry (DXA) using GE/ Lunar Radiation Corp (Madison, WI) Prodigy using software version 16.20.059 (n=287) and iDXA software version 13.60.033 (n=45) following standard subject positioning and data acquisition protocols. The within-subject variation for bone and soft tissue in our laboratory has been previously reported.(27, 28) The DXA was calibrated daily according to manufacturer guidelines. DXA scan analyses were performed by one certified technician.

Metabolic measures

Details regarding the collection and analysis of metabolic measures have previously been described.(29) In brief, serum from fasting blood samples was used to measure glucose, triglycerides (TG), and high density lipoprotein cholesterol (HDL-C) by a CLIA certified laboratory. Systolic and diastolic blood pressure was measured using an automated blood pressure monitor (Omron HEM-907XL). Blood pressure measurements were performed in triplicate with the subject in a seated position following 15 minutes at rest and using an appropriately sized BP cuff. The mean of the three measurements was used in analysis.

The criteria for CMR in this study were based on the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) definition of metabolic syndrome modified for age.(30) According to the modified NCEP ATP III definition, an individual is determined to have metabolic syndrome (MetS) if 3 of the 5 following criteria are met: a waist circumference 90th percentile for age and sex(31), HDL cholesterol 40 mg/dL(30), triglycerides 110 mg/dL(30), systolic or diastolic blood pressure 90th percentile for age, sex, and height(32), and fasting glucose 100 mg/dL.(33) Due to the low prevalence of MetS in this study (12%, n=36) using the modified NCEP ATP III criteria(30, 34) and to facilitate comparisons with previous work examining metabolic risk and BMC (35), girls were categorized into three groups: those having 1) no MetS risk factors, 2) 1 MetS risk factor, or 3) 2 MetS risk factors (i.e. CMR=0, CMR=1, CMR 2)

Physical Activity and Diet Assessment

Physical activity was assessed objectively using Actigraph GT3X+ (Pensacola, FL) accelerometers. All girls were instructed to wear the accelerometer on their hip for seven consecutive days. The accelerometers were initialized for data collection at a 30hz frequency. Data were saved in 60-second epochs with the "low frequency extension" option selected. Estimates of daily MVPA performed during the 7-day wear period were made using algorithms and cut-points developed by Evenson et al.(36)

Physical activity was also assessed using the past year physical activity questionnaire (PYPAQ), which has been validated in adolescents.(37) This questionnaire has been previously modified to include a comprehensive list of 41 common youth activities and used to survey all sport and leisure-time physical activity in which subjects had engaged at least 10 times in the past year outside of school physical education classes.(38) Participants were asked to record the average duration, weekly frequency, and the number of months of participation for each activity. The questionnaire was completed by the subject with the help of their parent(s)/guardian(s) and reviewed by a research technician during the measurement session to correct any issues regarding subjects' responses. Daily minutes of MVPA were calculated using the duration, frequency and number of months for each activity provided by the subject. Activities were determined to be MVPA if they had a metabolic equivalent (MET) value greater than or equal to 3.0 ml O_2/kg of bodyweight per minute according to the CDC.(39) All MET values to determine MVPA were obtained from the Compendium of Energy Expenditures for Youth.(40)

Dietary energy and nutrient intakes were assessed using the semi-quantitative Harvard youth/adolescent questionnaire (YAQ), which is a self-administered food-frequency questionnaire that has been validated in children and adolescents.(41) Participants filled out the questionnaire with assistance from parent(s)/guardian(s). Trained study staff reviewed the YAQs for completeness and coded them following standard coding procedures.(41) YAQs were then sent to Harvard T. H. Chan School of Public Health (Boston, MA) for nutrient analysis.

Statistical Analysis

Of the 332 participants recruited in the study, 307 were used in the analyses since 25 had missing data on DXA measures, fasting blood, waist circumference, or racial/ethnic status. The sample size justification was based on the relationship observed between BMC and adiposity in children ages 7 to 11 years.(14) Our sample size provided 80% statistical power to detect statistically significant relationships between measures of bone mass and total body fat mass, assuming that the percent of variation explained in the linear regression models was at least 82.5% (similar to that observed in (14)).

Descriptive statistics, including measures of central tendency (mean) and variability (standard deviation) for normally distributed variables and median (interquartile range) for skewed variables, were used to describe the characteristics of the full sample and the sample stratified by number of CMRs (CMR=0, CMR=1, and CMR $\geq$ 2). To compare differences in descriptive characteristics between the three CMR groups a one-way ANOVA with a Bonferroni adjustment for multiple comparisons was used to compare differences in continuous measures, a Kruskall-Wallis test for ordinal variables, and either a chi-square test or Fisher's exact test for categorical variables.

Multiple linear regression was used to test the relationship of total fat mass (kg) with BMC as dependent variables while controlling for CMR risk, maturity offset, height, total body lean mass, and ethnicity. Similar analyses using total body aBMD and BA as dependent variables were also performed and are presented in a supplementary table. Further adjustment of models for Tanner stage, MVPA assessed either by PYPAQ or objectively by

accelerometry, energy intake (kcal/day), and essential bone micronutrients including calcium (mg/day) and Vitamin D (IU/d), did not substantially alter the relationship of total body fat mass with BMC (β changed <0.001), nor did it alter the variance in BMC explained by the models (0.01 change in adjusted R^2) (data not shown). Thus, Tanner stage, physical activity, and diet were not included in the final models as covariates. Potential interactions between total body fat mass and CMR risk group (CMR=0, CMR=1, and CMR 2) as well as total body fat mass and ethnicity (Hispanic or non-Hispanic) were tested by inclusion of interaction terms in each model. There was no significant interaction between total body fat mass and ethnicity, thus Hispanic and non-Hispanic girls were included together in all regression models. Since there was a significant interaction between total body fat mass and CMR risk, regression models were stratified by CMR risk group. All models were checked for linearity, normality, and homoscedasticity and BMC was logarithmically transformed to meet linear regression assumptions. All results presented have been back-transformed for interpretability.

A p-value of <0.05 was considered statistically significant. All analyses were performed using STATA (StataCorp LLC, College Station, TX, USA) version 13.1.

Results

Descriptive characteristics are shown in Table 1 for the total sample (n=307) and by CMR group (CMR=0, CMR=1, and CMR 2). Of the 307 girls, 27% had 2 CMR, of whom n=47, 30, or 6 girls had 2, 3 and 4 risk factors respectively. Considering each risk factor separately, WC greater than the 90th percentile for age and sex (38% of girls) and high TG (32%) had the highest prevalence, followed by elevated glucose (14%) and low HDL-C (13%). Only 1% of girls had a systolic or diastolic blood pressure greater than or equal to the 90th percentile for age, sex, and height. A breakdown of the prevalence of each risk factor in girls with 1 and 2 CMR(s) is presented in Figure 1. Of the entire sample, 2% of girls were underweight, 58% normal weight, 16% overweight, and 24% were obese based on age and gender-specific established cut-points for percentiles of body mass index (BMI, kg/m^2)(42). More girls in the overweight/obese group had 2 CMR (85%) than girls in the normal weight group (14.5%). Girls with 2 CMRs had significantly more fat mass than girls with 1 and no CMR(s) (23.5 kg vs. 15.1 kg and 8.5 kg, respectively) with 91.6% of girls with 2 CMRs having a body fat percent greater than or equal to the 85th percentile for age (85th percentile range for girls aged 9–12 is 28.0–32.6% total body fat).(43) In addition to having more fat, girls with 2 CMR had a significantly higher absolute amount of lean mass (29.3 kg vs. 26.6 kg and 23.2 kg), but a lower percent of lean mass compared to girls with 1 and no CMR(s) (56% vs. 62% and 70% total body lean mass, respectively). There was no significant difference in age between girls with 1 CMR and those with 2 CMRs; however, girls with 1 CMR were slightly older than girls without risk factors. Despite only slight differences in age, the girls with CMRs were significantly taller and more mature (higher maturity offset and average Tanner breast stage (2.7 for CMR=1 and CMR 2 vs. 2.1 for CMR=0)) compared to girls with no CMRs (Table 1). Girls with 1 and 2 CMRs also had significantly higher BMC, aBMD, and BA (p<0.0001).

Results of linear regression models of total fat mass against total body BMC within the full sample and stratified by CMR group are presented in Table 2. There was a significant interaction between total fat and CMR group (p<0.05) when assessing the relationship between total body fat mass and BMC. After adjustment for height, maturity offset, lean mass, and ethnicity, total body fat mass had a significant positive association with BMC in the full sample and all three CMR groups, although the slopes of the 1 and no CMR group were significantly steeper (β=1.012 and 1.010, respectively) than the slope for the ≥2 CMR group (β=0.004) for BMC such that a one kilogram increase in total body fat mass resulted in a 1.1 and 1.2% increase in BMC when 1 or no CMRs were present, while a one kilogram increase in total body fat mass resulted in a 0.4% increase in BMC when ≥2 CMRs were present. The relationship of fat with bone was not significantly different between girls with 1 and no CMR(s). Similar results were observed for the relationship between BA with total body fat mass (Supplemental Table 1). There was no interaction effect of CMR group on the relationship of total body fat mass with aBMD and fat mass was not significantly associated with aBMD in any CMR group (p>0.05) (Supplemental Table 1).

Figure 2 displays the predicted BMC values for each girl from the regression models in Table 2. In all three groups, increased total body fat corresponded to increased BMC. For a given fat mass greater than ~10kg, predicted gains in BMC extrapolated from cross-sectional data were less in girls who had ≥2 CMR compared to girls with 1 or no CMR(s). Similarly, for a given fat mass greater than ~10kg, predicted increases in BA were less in girls who had ≥2 CMR (data not shown).

Discussion

The aim of this study was to assess the relationship between total body fat mass and DXA total body BMC in girls with and without CMR risk. Our results showed that higher fat mass was associated with higher BMC, which was independent of lean mass, height, maturation, and ethnicity. Our findings are consistent with those of other studies, which have investigated the relationship between fat mass and BMC in children controlling for important confounders (e.g., lean mass, maturation, and height).(11, 44) Although fat mass had a positive relationship with BMC in our sample of girls, the relationship significantly differed in girls having ≥2 CMRs compared to girls with 1 and no CMR(s) such that for a fat mass greater than ~10kg, girls with <2 CMRs had a higher BMC than girls with ≥2 CMRs. These findings suggest that the positive relationship between body fat and BMC as reported in other studies(11, 44), is attenuated by the presence of multiple CMRs. Our findings in this cohort of 9-to-12 year old predominately Hispanic girls with a wide range of body fat levels, are similar to those reported by Pollock et al. in two different cohorts of older overweight adolescents (boys and girls).(14, 35) Pollock et al. found that after controlling for age, sex, race, height, and fat free soft tissue, overweight male and female adolescents with either one or more CMRs as defined by the NECP adult treatment panel III definition modified by age, had significantly less total body BMC compared to the overweight adolescents with no CMRs.(35) In another study by Pollock et al., pre-pubertal overweight children with pre-diabetes had significantly lower total body BMC than pre-pubertal overweight children with normal glucose levels after adjusting for sex, race, height, and lean mass or body weight,

suggesting that impaired glucose regulation independent of weight has a negative effect on the skeleton.(14)

The mechanism(s) whereby a clustering of CMRs significantly attenuates bone mass is unclear. It has been suggested that many of the components of metabolic syndrome (MetS) are driven by the impaired storage capacity of hypertrophied, subcutaneous abdominal adipocytes and the resultant accumulation of visceral fat.(45) Dysfunctional subcutaneous and visceral adipose tissue are insulin resistant with increased lipolytic activity, secrete inflammatory cytokines into circulation, and together contribute to the development of insulin resistance of other tissues and the ensuing components of MetS (ie. glucose intolerance, dyslipidemia, and hypertension).(46) Plausible mechanisms have been proposed to explain the influence of inflammation, insulin resistance, and components of MetS on bone. For example, cell culture and human studies have provided evidence that increased secretion of pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-6, from visceral fat promote osteoclast activity and bone resorption through regulation of the RANKL/ RANK/OPG pathway.(47) Insulin resistance as well as increased levels of pro-inflammatory cytokines have been suggested to impair IGF-1 signaling. (48, 49) IGF-1 has been found to play a major role in bone mineral accrual and linear skeletal growth both directly through binding to its receptor on osteoblast and indirectly through increasing lean mass.(50) While we are unable to determine the mechanism underlying the attenuated BMC in girls with ≥ 2 CMRs, it is possible that the associated insulin resistance and low-grade chronic inflammation, as well as specific CMR factors themselves, are synergistically contributing to the compromised bone accrual.

In addition to contributing to the development of CMRs, excess fat can cause alterations in circulating hormones involved in bone metabolism, such as increased leptin and decreased serum 25-hydroxyvitamin D (25OHD) levels, which can lead to elevation in parathyroid hormone (PTH).(51) Despite the known anabolic effects PTH can have on bone through inducing IGF-1 expression by osteoblast(52, 53), high levels of PTH, such as those occurring in Vitamin D deficiency induced secondary hyperparathyroidism, can negatively affect bone mineralization through decreased calcium and phosphate absorption and enhanced bone resorption.(54) High leptin levels associated with excess fat can indirectly suppress bone formation through its actions in the hypothalamus(55) and can induce PTH secretion from parathyroid glands(56), which in the presence of Vitamin D deficiency can potentially exacerbate the negative effects of PTH on bone. One of the limitations of this study was the absence of measurements of serum PTH, 25OHD, and leptin, making us unable to determine if the reduced effect of fat on bone in girls with multiple CMRs in our study was partially due to greater alterations in the aforementioned hormones.

Although DXA is the most widely used technique for measuring bone in children due to its low radiation exposure, speed, and availability, DXA measures of BMC, BA, and aBMD provide information on the material properties of bone. DXA is unable to estimate true vBMD of cortical and trabecular bone and does not provide measures of bone geometry and strength.(13) However, predicting bone strength and fracture risk requires knowing not only the material properties of bone but also the geometric properties (eg. size and shape).(57) Thus, we are unable to comment on bone strength with any certainty, despite the important

contribution of BMC and BA. Three-dimensional imaging such as peripheral quantitative computed tomography (pQCT) would provide further information on the relationship of CMR to bone geometric properties and strength. Nevertheless, DXA measures of BMC are strongly correlated with bone strength and continue to be the standard for evaluating bone health.(58) Another limitation is the focus on only 9–12 year old girls, limiting the generalizability of our findings to boys and other age groups.

A major strength of this study was our large sample (n=307) of females, eliminating any confounding due to sex differences in the relationship between fat mass and bone. Furthermore, our sample was ~70% Hispanic. To our knowledge, no other study has addressed the relationship of fat with bone while considering multiple CMRs within Hispanic youth, which has been an understudied and at risk population. We found the relationship among fat, CMRs, and bone to be similar in Hispanic girls as shown for other ethnicities.

Our findings suggest that fat mass *per se* is not deleterious to bone, but the metabolic complications that often coexist with excessive fat accumulation do have a negative effect on bone. It is important to note that not all overweight and obese individuals suffer the traditional cardiometabolic risk factors (e.g., insulin resistance, dyslipidemia, and hypertension) that are often associated with excess body fat.(59) Indeed it has been demonstrated that about 1 in 5 to 1 in 3 children who are obese are metabolically healthy. (59) Mixed samples including so called metabolically healthy and at risk children likely explain many of the conflicting findings in the current literature. Future research should focus on these two distinct groups and prevention programs to promote bone health as well as cardiovascular health should be focused on overweight/obese children who present with cardiometabolic risk factors, as these risk factors could potentially compromise peak bone mass acquisition and increase the risk of current and future fracture risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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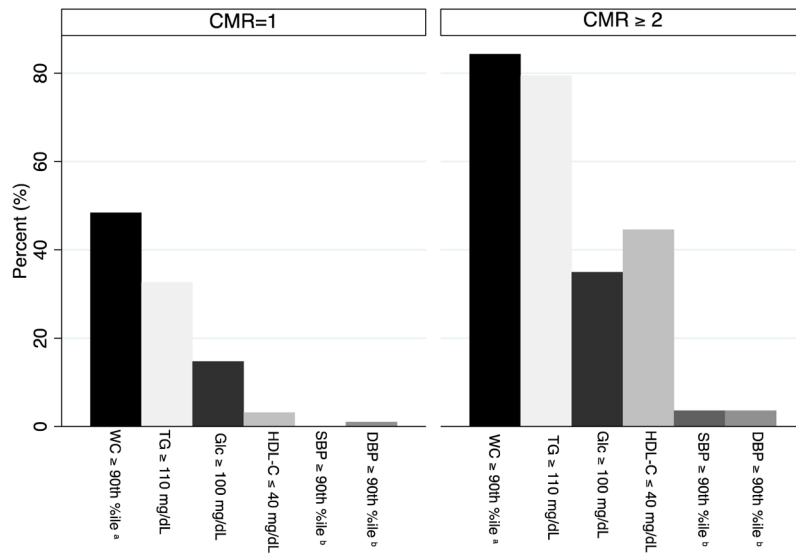
Figure 1.
Prevalence of risk factors by cardiometabolic risk (CMR) group
[a]specific for age and sex
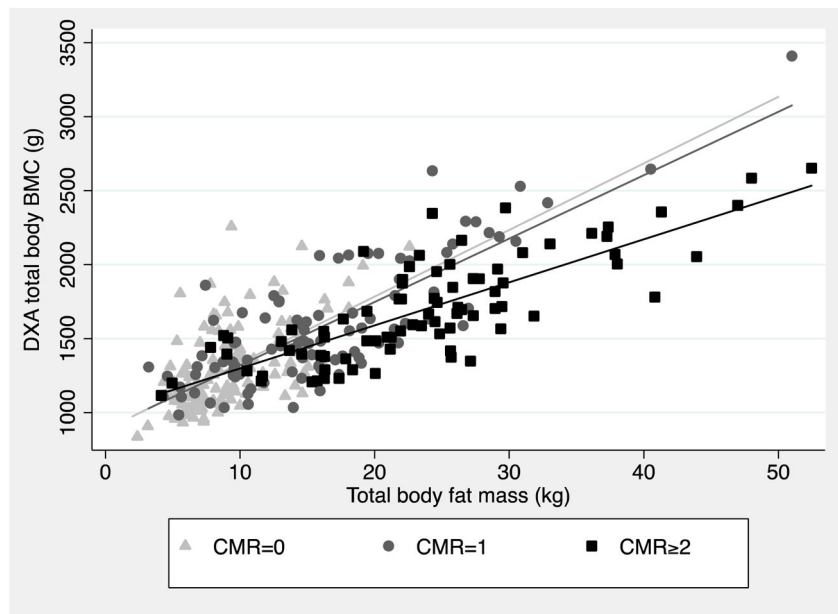[b]specific for age, sex, and height

Figure 2.
Relationship of DXA total body BMC with total body fat mass for girls with 0, 1, or ≥2 cardiometabolic risk factors (CMRs) adjusted for height (cm), maturity offset (yrs), lean mass (kg), and ethnicity (Hispanic vs. non-Hispanic).

Table 1

Participant Characteristics

	Full Sample (n=307)	CMR = 0[a] (n=129)	CMR = 1[a] (n=95)	CMR ≥ 2[a] (n=83)	p-value[e]
Age (years)	10.8 (1.1)	10.6 (1.0)	11.0 (1.1)[f]	10.8 (1.0)	0.006
Ethnicity [n(%)]					0.07
Hispanic	225 (73.3%)	87 (67.4%)	70 (73.7%)	68 (81.9%)	
Non-Hispanic	82 (26.7%)	42 (32.6%)	25 (26.3%)	15 (18.1%)	
Maturity Offset (years)	0.3 (1.2)	−0.2 (1.1)	0.6 (1.2)[f]	0.7 (1.0)[f]	0.00001
Tanner breast stage [n(%)]					0.0001
1	51 (16.6%)	35 (27.1%)	8 (8.4%)	8 (9.6%)	
2	109 (35.5%)	57 (44.2%)	30 (31.6%)	22 (26.5%)	
3	106 (34.5%)	25 (19.4%)	39 (41.1%)	42 (50.6%)	
4	33 (10.8%)	11 (8.5%)	14 (14.7%)	8 (9.6%)	
5	8 (2.6%)	1 (0.8%)	4 (4.2%)	3 (3.7%)	
Tanner pubic hair stage [n(%)]					0.0001
1	169 (55.1%)	92 (71.3%)	35 (36.8%)	42 (50.6%)	
2	100 (32.6%)	27 (20.9%)	39 (41.1%)	34 (41.0%)	
3	24 (7.8%)	4 (3.1%)	16 (16.8%)	4 (4.8%)	
4	10 (3.3%)	5 (3.9%)	5 (5.3%)	0 (0.0%)	
5	4 (1.1%)	1 (0.8%)	0 (0.0%)	3 (3.6%)	
Menarche [n(%)]	62 (20.2%)	17 (13.2%)	29 (30.5%)	16 (19.3%)	0.006
Weight (kg)	41.8 (19.7)[^]	34 (10.1)[^]	44.8 (16.8)[^,f]	54.9 (19.2)[^,f,g]	0.00001
Height (cm)	146.0 (9.6)	142.7 (9.6)	148.0 (9.3)[f]	148.9 (8.6)[f]	0.00001
BMI (kg/m²)	19.4 (6.9)[^]	17.0 (2.7)[^]	20.8 (5.8)[^,f]	25.4 (5.5)[^,f,g]	0.00001
BMI Percentile Status[b] [n(%)]					0.0001
Underweight (<5th)	7 (2.2%)	5 (3.9%)	2 (2.1%)	0 (0.0%)	
Normal (5th and <85th)	178 (58.0%)	119 (92.2%)	47 (49.5%)	12 (14.5%)	
Overweight (85th–<95th)	49 (16.0%)	5 (3.9%)	24 (25.3%)	20 (24.1%)	
Obese (95th)	73 (23.8%)	0 (0.0%)	22 (23.2%)	51 (61.4%)	

	Full Sample (n=307)	CMR = 0[a] (n=129)	CMR = 1[a] (n=95)	CMR 2[a] (n=83)	p-value[e]
Percentiles of Total body fat[c] [n(%)]					0.0001
<85th	140 (45.5%)	99 (76.7%)	34 (35.8%)	7 (8.4%)	
85th and <95th	66 (21.5%)	22 (17.1%)	27 (28.4%)	17 (20.5%)	
95th	101 (33.0%)	8 (6.2%)	34 (35.8%)	59 (71.1%)	
DXA measures					
aBMD (g/cm^2)	0.9 (0.09)	0.9 (0.08)	0.9 (0.09)[f]	1.0 (0.08)[f]	0.00001
BA (cm^2)	1565.0 (436.9)[^]	1415.8 (328.2)[^]	1606.2 (416.6)[^f]	1736.0 (379.4)[^f]	0.00001
BMC (g)	1428.7 (556.1)[^]	1225.4 (373.6)[^]	1539.2 (598.1)[^f]	1621.1 (544.2)[^f]	0.00001
Total fat mass (kg)	13.3 (13.0)[^]	8.5 (4.3)[^]	15.1 (11.3)[^f]	23.5 (12.7)[^,f]	0.00001
Total body fat (%)	32.6 (9.9)	26.1 (6.7)	33.9 (8.8)[f]	41.2 (7.7)[^,f]	0.00001
Total lean soft tissue mass (kg)	26.2 (8.0)[^]	23.2 (6.1)[^]	26.6 (7.5)[^f]	29.3 (8.7)[^,f]	0.00001
Total body lean (%)	63.9 (9.5)	70.2 (6.5)	62.6 (8.6)[f]	55.6 (7.4)[^,f]	0.00001
CMRs					
Waist circumference (cm)	73.0 (19.8)[^]	64.8 (7.9)[^]	76.6 (14.8)[^f]	87.9 (14.7)[^,f]	0.00001
Waist circumference percentile[d]	82.0 (42.0)[^]	56.0 (35.0)[^]	89.0 (25.0)[^f]	97.0 (5.0)[^,f]	0.00001
HDL–C (mg/dL)	50.0 (13.0)[^]	56.0 (12.0)[^]	49.0 (9.0)[^f]	42.0 (11.0)[^,f]	0.00001
TG (mg/dL)	89.0 (54.0)[^]	71.0 (34.0)[^]	91.0 (46.0)[^f]	143.0 (66.0)[^,f]	0.00001
Systolic blood pressure (mmHg)	99.7 (8.9)	97.7 (8.5)	99.9 (8.4)	102.6 (9.4)[f]	0.0004
Diastolic blood pressure (mmHg)	63.5 (7.2)	61.9 (6.6)	63.4 (7.2)	66.1 (7.4)[^,f]	0.0002
Fasting glucose (mg/dL)	92.9 (6.8)	89.5 (5.3)	94.1 (6.0)[f]	96.7 (7.4)[^,f]	0.00001

aBMD = areal bone mineral density; BA = bone area; BMC = bone mineral content; HDL–C= high density lipoprotein cholesterol (mg/dL); TG = triglycerides; CMR = cardiometabolic risk factors

[^] median (interquartile range)

[a] CMRs included: a waist circumference 90th percentile for age and sex, HDL cholesterol 40 mg/dL, triglycerides 110 mg/dL, systolic or diastolic blood pressure 90th percentile for age, sex, and height, and fasting glucose 100 mg/dL

[b] BMI percentiles specific for age and gender based on CDC growth charts[39]

[c] Fat percentiles based on body fat percentile curves for US children and adolescent[40]

[d]Percentile specific for age and gender

[e]Test of significance between groups were conducted using ANOVA for continuous variables, Kruskall-Wallis for ordinal variables, and either a χ2 test or Fisher's exact test for categorical variables

[f]Significantly different from CMR=0

[g]Significantly different from CMR=1

Table 2

Linear regression of total body fat mass against DXA bone mineral content (BMC) within full sample and by cardiometabolic risk (CMR) group

	BMC (g)
Full sample	n=307
total body fat mass (kg)	
β (95%CI)	1.006 (1.005, 1.008)
SE	0.0009
p-value	0.0001
Adjusted R^2	0.86
CMR = 0	n=129
total body fat mass (kg)	
β (95%CI)	1.012 (1.007, 1.017)
SE	0.002
p-value	0.0001
Adjusted R^2	0.84
CMR = 1	n=95
total body fat mass (kg)	
β (95%CI)	1.010 (1.007, 1.014)
SE	0.002
p-value	0.0001
Adjusted R^2	0.86
CMR ≥ 2	n=83
total body fat mass (kg)	
β (95%CI)	1.004 (1.000, 1.007)
SE	0.002
p-value	0.05
Adjusted R^2	0.80

β = regression coefficient adjusted for height (cm), maturity offset (yrs), lean mass (kg), and ethnicity (Hispanic vs. non-Hispanic); SE = standard error; BMC = bone mineral content; CMR = cardiometabolic risk factors