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# Relationship of cardiometabolic risk biomarkers with DXA and pQCT bone health outcomes in young girls

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ABSTRACT

*Background:* Excess weight exerts the positive effect of mechanical loading on bone during development whereas obesity-related metabolic dysfunction may have a detrimental impact. In adults, the presence of metabolic syndrome and type 2 diabetes has been associated with compromised bone density, quality, and strength, and an increased incidence of fractures. The few studies that have investigated the role of cardio-metabolic disease risk biomarkers (CMR) on bone strength in children have given conflicting results. The aim of this study was to assess the combined and independent relationships of cardio-metabolic biomarkers with total body and regional bone parameters in young girls.

*Methods:* In 306, 9–12 year old girls, measures of whole body fat and lean mass, areal bone mineral density (aBMD), bone mineral content (BMC), and bone area (BA) were obtained by dual-energy x-ray absorptiometry (DXA). Bone mineral density (vBMD), geometry, and strength of metaphyseal and diaphyseal regions of the femur and tibia and a diaphyseal region of the radius were measured using peripheral quantitative computed tomography (pQCT). Fasting serum measures of CMRs included, fasting glucose, insulin, homeostatic model assessment for insulin resistance (HOMA-IR), high-density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglyceride (TG), systolic and diastolic blood pressure (SBP and DBP), and C-reactive protein (CRP). Multiple linear regression was used to assess the independent associations of a single CMR with total body and peripheral measures of bone strength after controlling for the other CMRs, plus total body soft tissue, and other relevant covariates. Also, a standardized total CMR composite score, calculated by standardizing to z-scores and then summing z-scores of each CMR biomarker, was regressed with total body and regional bone measures to assess the relationship of a cluster of risk factors with bone health.

*Results:* Total CMR composite score had inverse associations ($p < 0.001$) with DXA total BMC and BA. Inverse associations ($p < 0.05$) of CMR risk score with pQCT regional bone measures occurred with total and trabecular BA at the 4% tibia. Of the individual CMRs, HOMA-IR and CRP were significant predictors of total body bone measures by DXA accounting for ~1–5% of the variance in BMC, BA, and/or aBMD. HOMA-IR was the main predictor of regional pQCT bone outcomes, accounting for the most variance in trabecular vBMD (2.6%) and BSI (3.8%) at the 4% tibia. Most markers of dyslipidemia (TG, HDL-C, LDL-C) and hypertension (SBP, DBP) were not

*Abbreviations:* MetS, metabolic syndrome; aBMD, areal bone mineral density; CMR, cardiometabolic risk; DXA, dual-energy x-ray absorptiometry; BMC, bone mineral content; pQCT, peripheral quantitative computed tomography; CRP, C-reactive protein; PHV, peak height velocity; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; vBMD, volumetric bone mineral density; Tot A, total bone area; Cort vBMD, cortical vBMD; Cort BMC, coritical BMC; Cort area, cortical area; EC, endosteal circumference; PC, periosteal circumference; Crt Thk, cortical thickness; Trab vBMD, trabecular vBMD; Trab area, trabecular area; SSI, strength-strain index; BSI, bone strength index

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associated ($p > 0.05$) with any total body or regional bone outcomes with the exception of the inverse relationship of LDL-C with total and trabecular BA and the positive relationship of DBP with cortical vBMD at the radius.

*Conclusion:* Of the obesity-related metabolic impairments, insulin resistance and chronic inflammation may compromise whole body bone development in young girls. In particular, trabecular bone, such as that found at the metaphysis of long bones, may be more susceptible to the detrimental effects associated with obesity-related metabolic dysfunction.

## 1. Introduction

Excess adiposity has been linked to the development of insulin resistance, chronic inflammation, and the ensuing metabolic syndrome (MetS) components (i.e. glucose intolerance, dyslipidemia, and hypertension) [1]. These metabolic alterations are known risk factors for cardiovascular disease [1], type 2 diabetes [2], non-alcoholic fatty liver disease [3] and certain cancers [4]. Recent evidence in animal [5,6] and human studies [6–10] has suggested that these obesity-derived metabolic alterations may also be risk factors for compromised bone strength. For example, von Muhlen et al. reported that fracture incidence was 2.6 times more likely to occur in older adults with MetS compared to older adults without MetS [8]. Moreover, the number of MetS components was negatively associated with femoral neck and total hip areal bone mineral density (aBMD) after adjustment for BMI [8]. Kim et al., also observed significantly lower femoral neck aBMD in participants with MetS after adjusting for body weight; however, when assessing associations of the individual MetS components with femoral neck BMD, waist circumference in both men and women and fasting triglycerides in women were the only MetS components having significant inverse relationships with aBMD [10]. Hence, not all components of MetS have similar associations with skeletal outcomes.

Despite evidence in adults of an association between cardiometabolic risk (CMR) factors and compromised bone health, less research has been focused around the effect of obesity-induced metabolic dysfunction on bone development in children. Recently, in a cohort of 9-to-12 year-old girls participating in the Soft Tissue and Bone Development (STAR) study, we have shown that the positive relationship between total body adiposity and whole body bone mineral content (BMC) was attenuated by the presence of 2 or more CMRs defined by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) definition of MetS modified for age [11]. In that analysis, the relationship of individual CMRs representative of inflammation, insulin resistance, dyslipidemia, and hypertension with bone outcomes independent of each other was not assessed. It is possible that the relationship of dyslipidemia with bone may be explained by insulin resistance, which underlies many of the MetS components [12]. Currently, few studies have evaluated single CMR biomarkers with bone in children, with inconsistent results [13–23]. In addition, most previous studies in children have focused on the relationship of CMRs with dual-energy x-ray absorptiometry (DXA) measured total body BMC and aBMD. However, BMC and BMD are just two among several indicators of bone health. Bone strength is also comprised of other bone properties such as the microarchitecture at the bone tissue level [24]. With newer 3-dimentional imaging techniques, such as peripheral quantitative computed tomography (pQCT), both the material properties (i.e. density and mass) and the geometric properties (i.e. size and shape) of bone can be measured [25]. Obesity-related CMRs may have differential relationships with these different components of bone strength. For instance, Viljakainen et al. found that C-reactive protein (CRP), a biomarker of inflammation, was not significantly associated with whole body aBMD, but did have a significant inverse relationship with cortical density, periosteal circumference, and overall bone strength at the tibia in young male adults [26]. No study to our knowledge has assessed the associations of obesity-related CMR biomarkers of inflammation, insulin resistance, dyslipidemia, and hypertension together with measures of bone geometry and strength in children. Given the paucity of research assessing the effects of CMR biomarkers with bone indices in children, which is an important age for bone development, and an estimated prevalence of up to 7% of overweight children and 30% of obese adolescents having MetS [27], the aim of this study was to assess the combined and independent relationships of CMR biomarkers with total body and regional bone parameters in young girls.

## 2. Methods

### 2.1. Study population

The study participants consisted of 344 girls aged 9-to-12 years-old who participated in the cross-sectional arm of the Soft Tissue And Bone Development in Young Girls (“STAR”) study, a study designed to assess the effects of adiposity and related metabolic risk factors on bone development in girls (Clinical trials #NCT02654262). Detailed descriptions of the study protocol with inclusion and exclusion criteria have been published previously [11,28–30]. 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. Following informed consent, participants' guardians were asked to complete a demographic and health history questionnaire.

### 2.2. Anthropometry

Anthropometric measures were obtained according to standardized protocols [31]. In brief, 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 millimeter using a stadiometer (Shorr Height Measuring Board, Olney, MD). Leg length was determined by subtracting sitting height from standing height. Femur, tibia, and radius lengths were measured to the nearest millimeter on the non-dominant limb. Upper leg (femur) length was recorded as the distance from the proximal aspect of the patella to the inguinal crease. The distance from the proximal end of the medial border of the tibial plateau to the distal edge of the medial malleolus was used for tibia length. Forearm (radius) length was measured from the posterior edge of the olecranon to the distal end of the styloid process of the ulna. The mean of two measurements of each anthropometric variable was used in the analysis. BMI was calculated as weight (kg) divided by height (m) squared. Based on U.S. CDC growth charts, BMI percentiles specific for age and gender were used to categorize girls as either underweight ($<$ 5th percentile), normal weight ($\geq$5th and $<$ 85th percentiles), overweight ($\geq$85th and $<$ 95th percentiles), or obese ($\geq$95th percentile) [32].

### 2.3. Physical maturation

Maturation was determined using a self-reported questionnaire with pictures illustrating the Tanner stages of pubertal maturation [33] and using maturity offset, an estimate of years from peak height velocity (PHV). PHV is a maturational indicator that reflects the maximum growth during adolescence. Maturity offset is estimated from age and anthropometric measures (height, weight, sitting height, and leg

length) using the sex- and gender-specific algorithms developed by Mirwald et al. The maturity offset equation for girls has been shown to explain 89% of the variance in years from PHV [34]. After PHV is reached maturity offset is positive; a negative maturity offset represents years before PHV.

### 2.4. Cardiometabolic measures

Details regarding the collection and analysis of metabolic measures have previously been described [28]. In brief, fasting glucose and lipids were measured by a CLIA certified clinical laboratory. Serum glucose was analyzed by a hexokinase-based automated assay with intra- and inter-assay variability of 0.54% and 3.22%, respectively. Total triglycerides (TG) were measured in serum with an automated enzymatic method using a modified Trinder reaction. The intra- and inter-assay variability were 0.48% and 2.85%, respectively. Total high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured in serum with an automated enzymatic assay utilizing a homogeneous method. Intra- and inter-assay variability for HDL-C were 1.25% and 4.12% and for LDL-C were 0.46% and 5.61%. Using stored serum samples, fasting insulin was measured utilizing a human insulin specific RIA that does not cross-react with pro-insulin (EMD Millipore, Darmstadt, Germany). The intra- and inter-assay variability were 2.9% and 7.5%, respectively. Insulin resistance was estimated using the original homeostatic model assessment of insulin resistance (HOMA- IR1) developed by Matthews et al. [35] HOMA-IR1 is defined by the product of the fasting glucose and fasting insulin divided by a constant and is represented by the following equation: HOMA-IR = (insulin [μU/L] × glucose [mM/L])/22.5 [35]. Fasting CRP was analyzed using a Beckman Coulter AU5812 Clinical Chemistry Analyzer, which uses a high-sensitivity CRP latex reagent and nephelometric assay to measure CRP levels in serum. The inter-assay variability was 3.46% at 0.69 mg/L for low concentration and 1.28% at 5.6 mg/L for high concentration controls. Lower limit of detection for the high sensitivity CRP assay was ≤0.07 mg/L. Systolic (SBP) and diastolic blood pressure (DBP) were measured using an automated blood pressure monitor (Omron HEM-907XL) as previously described [11]. Due to the moderately high correlation between SBP and DBP ($r = 0.70$), SBP and DBP were combined into a single variable called mean arterial blood pressure (MAP) in order to reduce the potential for co-linearity in regression models. MAP was calculated by applying the following formula: MBP = [(SBP-DBP/3) + DBP] [36].

For each metabolic measure, a *Z*-score was calculated based on the study sample, i.e. Z = ([individual value − mean]/standard deviation). Due to the skewed nature of HOMA-IR, CRP, TG, HDL-C, and LDL-C, z-scores were created from the logarithmic transformed form of these variables. Each of the individual CMR z-scores was summed together to create a continuous CMR risk z-score (CMR z-score = logHOMA-IR z-score + logCRP z-score + logTG z-score + (logHDL-C z-score X − 1) + logLDL-C z-score + MAP z-score), where a higher CMR z-score is indicative of greater metabolic dysfunction. The z-score for HDL-C was multiplied by −1, since a high HDL-C value is inversely related to metabolic risk.

### 2.5. Dual energy X-ray absorptiometry (DXA)

Measures of whole-body BMC, aBMD, BA, fat mass, and lean mass were obtained from DXA using GE/Lunar Radiation Corp (Madison, WI) Prodigy using software version 13.60.033 (n = 287). Due to an upgrade at the facility performing the scans, the remaining 57 girls were measured using iDXA software version 16.20.059 following standard subject positioning and data acquisition protocols. Each of the models used in this study has been independently validated for assessing lean and fat mass and high agreement has been found between the two DXA systems ($R^2 = 0.85$ to 0.99) [37]. In addition, because this study did not involve a longitudinal analysis, where serial measurements on a subject might be measured using two different machines, no practical implications were anticipated when merging the data from both instruments. DXA scan analyses were performed by one certified technician. The within-subject variation for bone and soft tissue in our laboratory are < 1 to 3% [38]. The DXA was calibrated daily according to manufacturer guidelines.

### 2.6. Peripheral quantitative computed tomography (pQCT)

STRATEC, XCT 3000 pQCT (Medizintechnik GmbH, Pforzheim, Germany, Division of Orthometrix; White Plains, NY) was used to assess cortical (diaphyseal) weight bearing (tibia and femur 66% and 20% sites, respectively, relative to the distal growth plates), and non-weight bearing (radius 66%) bones, and a trabecular (metaphyseal) weight bearing bone (tibia 4%) of the non-dominant limb. All pQCT scans were analyzed using Stratec XCT software, Version 6.0 and operators were trained for pQCT data acquisition and analyses following guidelines provided by Bone Diagnostic LLC (Spring Branch, TX). A detailed description of the instrument and imaging processing and analysis protocols used in our laboratory has been published [29], as have coefficients of within-subject variation for pQCT bone measurements [39].

### 2.7. Physical activity and diet assessment

Physical activity was assessed objectively using Actigraph GT3X+ (Pensacola, FL) accelerometers. A description of accelerometry protocols and data acquisition for the STAR study has been published [11]. Recorded data were analyzed using moderate-to-vigorous physical activity (MVPA) cut-points and algorithms developed by Evenson et al. [40], which are included with Actigraph's Actilife software.

The semi-quantitative Harvard Youth/Adolescent Questionnaire (YAQ), a self-administered food-frequency questionnaire that has been validated in children and adolescents [41], was used to assess Vitamin D (IU/day) and calcium (mg/day) intake. Participants were instructed to fill 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.

### 2.8. Statistical analysis

Of the 344 participants recruited in the study, 306 had complete measures for all DXA, pQCT, anthropometric, and metabolic variables and were used in the analyses. The mean and standard deviation for normally distributed variables and median and interquartile range for skewed variables, were used to characterize the sample.

Using multiple linear regression, CMR z-score was regressed with total body and regional bone measures to assess the relationship of a cluster of risk factors with bone health after adjusting for soft tissue composition (total body lean and fat mass), height, maturity offset, and ethnicity.

Additionally, multiple linear regression was used to assess the independent effects of biomarkers of insulin resistance (HOMA-IR), inflammation (CRP), dyslipidemia (TG, LDL-C, HDL-C) and hypertension (MAP) on DXA total body bone measures (i.e. BMC, aBMD, and BA) and pQCT regional measures of bone geometry, vBMD, and bone strength as the dependent variables. All linear regression models contained each of the CMR biomarkers together in order to be able to assess associations of individual CMRs independent of each other. In addition, each model was controlled for soft tissue composition (total body lean and fat mass), height, maturity offset, and ethnicity. Further adjustment of all models for dietary intake of calcium and Vitamin D and MVPA was also performed. All p-values are unadjusted for multiple comparisons.

Given the known limitations associated with self-reported Tanner staging [42,43] and since maturity offset was more highly correlated

with the bone outcomes compared to Tanner stage, maturity offset was used as the covariate in all models to control for maturation. Substitution of Tanner stage for maturity offset did not substantially alter results (change in adjusted $R^2 \leq 0.02$ with the exception of Crt Thk of Femur where adjusted $R^2$ differed by 0.07).

## 3. Results

Descriptive characteristics of the sample are presented in Table 1. Based on U.S. age and gender-specific established cut-points for percentiles of body mass index (kg/m$^2$) [44], the majority of girls had a healthy weight (59%). Only 2% of girls were underweight, while 15% were overweight, and 24% were obese. The majority of girls identified as Hispanic (74%). On average girls were 11 years-old and had just reached their estimated peak height velocity (maturity offset = 0.3 year). Self-reported calcium and vitamin D intakes were below the recommended dietary allowances for 9–12 year old girls, which are 1300 mg/day for calcium and 600 IU/day for Vitamin D [45]. The median physical activity, as measured by an accelerometer, was 21 min, which is 40 min less than the 60 min of moderate-to-vigorous physical activity per day recommended by the U.S. Department of Health and Human Services 2008 Physical Activity Guidelines for children and adolescents [46].

Biomarkers of inflammation, insulin resistance, dyslipidemia, and hypertension for the study cohort are given in Table 2. Cut-points for fasting glucose, TG, HDL-C, SBP, and DBP were based on the NCEP ATP III definition of MetS modified by age [27] in order to facilitate comparisons with study samples from previous work examining metabolic risk and bone [14]. The cut-point for LDL-C was based on the National Heart, Lung, and Blood Institute (NHLBI) guidelines for cardiovascular health in Children and Adolescents [47]. One third of girls had elevated levels of TG and LDL-C, 13% had elevated glucose and low HDL-C, and only 1% had SBP and DBP values that were ≥ 90th percentile for sex, age, and height (Table 2). There is currently no established risk cut-off for CRP for children and adolescents; however according to the CRP cut-point derived from the literature in adults indicating elevated cardiovascular disease risk (≥3 mg/L) [48], ~18% of girls had elevated levels. There are currently no standard accepted cut-offs for insulin and HOMA-IR in children or adults due to the lack of standardization of insulin assays, which is needed when determining cut-points. Proposed cutoffs for HOMA-IR determined using HOMA-IR1 developed by Matthews et al. [35] have ranged from 1.14–5.56 for children between the ages of 3-to-18 years [49].

Results of linear regression of CMR z-score with DXA and pQCT bone outcomes after adjusting for total body fat and lean mass, height, maturity offset and ethnicity are presented in Table 3. CMR z-score had significant ($p < 0.001$) inverse associations with DXA total body BMC and BA. Inverse associations ($p < 0.05$) of CMR z-score with pQCT regional bone measures occurred only with total and trabecular BA at the 4% tibia, the sole metaphyseal (trabecular) site analyzed.

The independent contributions of each CMR biomarker with DXA and pQCT bone outcomes after adjusting for total body fat and lean mass, height, maturity offset and ethnicity are also presented in Table 3. Of the individual CMRs, HOMA-IR and CRP were consistent, significant (negative) predictors of DXA total body bone measures accounting for ~1–5% of the variance in BMC, BA, and/or aBMD. HOMA-IR was the main consistent predictor of pQCT measures of bone geometry and strength, having significant ($p < 0.05$) negative associations with total area at the radius, cortical BMC at the femur, cortical BMC and cortical thickness at the diaphyseal (66% site) tibia, and trabecular vBMD and bone strength index (BSI) of the metaphyseal (4% site) tibia. HOMA-IR, however, accounted for the most variance in trabecular vBMD (2.6%) and BSI (3.8%) at the metaphyseal tibia. Biomarkers of dyslipidemia (TG, HDL-C, LDL-C) and hypertension (MAP) were not consistently associated with any total body or regional bone outcomes.

Further adjustment for MVPA and dietary intake of calcium and Vitamin D did not significantly alter relationships between CMRs and bone outcomes (Table 4 Supplement). Thus, due to missing diet and physical activity data on a portion of our cohort, we did not include these measures in the final models as covariates in order to maximize sample size. In addition, all analyses were performed eliminating girls with CRP concentrations > 10 mg/L, as levels higher than 10 mg/L could be indicative of recent infection and are suggested to be repeated when used for assessing cardiometabolic risk [50]. Elimination of girls with CRP > 10 mg/L did not alter the observed relationships between CRP and bone outcomes with the exception of the inverse relationships of CRP with radial bone strength (SSI) becoming significant ($p < 0.05$) (data not shown).

## 4. Discussion

The aim of this study was to assess the combined and independent relationships of CMR biomarkers with total body bone mass and density and regional bone parameters of geometry and strength in young girls. Our results showed that greater metabolic dysfunction as indicated by a higher CMR z-score was associated with decreases in total body BMC and BA. These findings are similar to those of Pollock et al., who observed that overweight adolescents with a clustering of 2 or more cardio-metabolic risk components had significantly less DXA total body BMC compared to overweight adolescents without metabolic impairment [14]. Hence, consistent with previous studies in adults [6–10], increased metabolic dysfunction as indicated by the presence of multiple abnormal CMRs, may have negative effects on overall bone mass in children and adolescents.

As associations between CMR risk and measures of regional bone geometry and strength have not, to our knowledge, previously been analyzed in children, our finding of an inverse association between CMR z-score and total and trabecular bone area at a metaphyseal (but not cortical) site only, is novel. Unlike the diaphysis of long bones composed of predominately cortical bone, bone at the metaphyseal

**Table 1**
Descriptive characteristics.

| | Full sample (n = 306) |
|---|---|
| | Mean ± SD |
| Demographics | |
| Age (years) | 10.8 ± 1.1 |
| Ethnicity [n(%)] | |
| Hispanic | 226 (73.9%) |
| Non-Hispanic | 80 (26.1%) |
| Maturity offset (years) | 0.3 ± 1.2 |
| Body composition | |
| Weight (kg) | 41.6 (19.9)[a] |
| Height (cm) | 145.9 ± 9.7 |
| BMI (kg/m$^2$) | 19.3 (6.9)[a] |
| BMI percentile status [n(%)][b] | |
| Underweight (< 5th) | 7 (2.2%) |
| Normal (≥5th and < 85th) | 181 (59.2%) |
| Overweight (≥85th < 95th) | 45 (14.7%) |
| Obese (≥95th) | 73 (23.9%) |
| Total fat mass (kg) | 13.1 (13.2)[a] |
| Total body fat (%) | 32.5 ± 9.9 |
| Total lean soft tissue mass (kg) | 26.2 (8.1)[a] |
| Total body lean (%) | 64.0 ± 9.6 |
| Physical activity and diet | |
| MVPA (min/day)[c] | 20.7 (18.8)[a] |
| Total energy (kcals/day)[d] | 2050.6 (1155.2)[a] |
| Calcium (mg/day)[d] | 1093.3 (777.5)[a] |
| Vitamin D (IU/day)[d] | 259.6 (276.4)[a] |

MVPA, moderate to vigorous physical activity.
[a] Median (interquartile range).
[b] Percentiles specific for age and gender based on CDC growth charts [32].
[c] n = 287.
[d] n = 301.

**Table 2**
Summary of cardiometabolic measures.

| | Mean ± SD |
|---|---|
| Glucose (mg/dL) | 92.8 ± 6.9 |
| Glucose ≥100 mg/dL.[n(%)] [27] | 42 (13.7%) |
| Insulin (μU/mL) | 17.0 (10.3)[a] |
| HOMA-IR[b] | 3.9 (2.5)^ |
| TG (mg/dL) | 89 (53)^ |
| TG ≥ 110 mg/dL [27] | 95 (31.1%) |
| HDL-C (mg/dL) | 51 (13)[a] |
| HDL-C ≤ 40 mg/dL [27] | 40 (13.1%) |
| LDL-C (mg/dL) | 96.5 (38)[a] |
| LDL-C ≥ 110 mg/dL [47] | 101 (33.0%) |
| SBP (mmHg) | 99.7 ± 8.9 |
| SBP ≥90th percentile for sex, age, and height [27] | 4 (1.3%) |
| DBP (mmHg) | 63.5 ± 7.2 |
| DBP ≥90th percentile for sex, age, and height [27] | 4 (1.3%) |
| MAP (mmHg) | 75.6 ± 7.2 |
| CRP (mg/L) | 0.5 (1.4)[a] |
| CRP ≥ 3 mg/L [n(%)] [48] | 54 (17.7%) |
| CMR z-score[c] | 0.0 ± 3.7 |

n = 306.
HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; CRP, C-reactive protein (mg/L); TG, triglyceride (mg/dL); HDL-C, high density lipoprotein cholesterol (mg/dL); LDL-C, low density lipoprotein cholesterol (mg/dL); SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); MAP, mean arterial blood pressure; CMR, cardiometabolic risk marker.
[a] Median (interquartile range).
[b] HOMA-IR = (insulin [μU/L] × glucose [mM/L])/22.5.
[c] CMR z-score = logHOMA-IR z-score + logCRP z-score + logTG z-score + (logHDL z-score *-1) + logLDL z-score + MAP z-score.

region of long bones is made up of highly vascularized trabecular bone [51]. Thus, our findings indicate that trabecular bone may be more susceptible to metabolic alterations than cortical bone, which is likely due to the generally higher bone turnover occurring in trabecular bone [51,52]. In mice, diet-induced MetS caused a deterioration of bone architecture and strength in the trabecular region but not in the cortical regions [53].

When assessing the relationship of each individual CMR biomarker with bone, HOMA-IR, and CRP were the CMRs having significant inverse associations with total body bone indices. In a similar analysis including CMR biomarkers of insulin resistance, dyslipidemia, and hypertension, Pollock et al. also found that the marker of insulin resistance (i.e. HOMA-IR) was the CMR significantly associated with total body BMC, accounting for ~4.1%, which is similar to the variance in total body BMC explained by HOMA-IR in our study (~3.9%) [14]. A biomarker of inflammation, such as CRP, was not assessed in the study by Pollock et al. [14]. However, a prospective study evaluating girls at age 13 and again at age 17 found that CRP was significantly and inversely associated with forearm aBMD in overweight girls at 13 and both overweight and normal weight girls at 17 years-old [23]. On the contrary, Soininen et al. reported that neither CRP nor HOMA-IR had significant associations with total body less head BMD in 6-to-8 year-old children after controlling for age, sex, height, and total body fat mass [16]. However, only 14% of the cohort in the Soininen et al. [16] study was overweight or obese, which may be contributing to differences in findings from our study and those of others [14,15], whom have assessed children of slightly older ages with a range of BMIs.

Beyond total body bone measures, HOMA-IR was also a significant negative predictor of regional measures of bone density, geometry and strength, having the strongest relationships with total and trabecular vBMD at the metaphyseal tibia. Similarly, in a study assessing the relationship of insulin with pQCT bone parameters at the diaphyseal tibia, Sayers et al. found insulin to have negative relationships with bone density and geometry in an older cohort of adolescents [22]. We found that the biomarkers of dyslipidemia, inflammation, and hypertension did not significantly contribute to the prediction of regional bone measures with the exception of LDL-C at the metaphyseal tibia.

Overall, results from this study suggest that the presence of multiple CMRs may not only be a risk factor for cardiovascular complications, but may also be a risk factor for compromised bone development in girls, particularly at bone sites higher in trabecular bone. This is of concern given osteoporosis is a disease mainly of trabecular bone, especially in individuals of < 65 years of age [54]. Hence, having obesity related metabolic impairment during childhood may put young girls at a higher risk for developing osteoporosis as adults. Although assessment of a clustering of CMRs together may be an indicator of negative bone outcomes, our results indicate that not all CMRs were equally predictive of total body and regional bone outcomes in girls. The detrimental skeletal effects associated with a state of metabolic dysfunction, as indicated by the inverse association of CMR z-score with total body and regional bone strength in our study and MetS in previous studies [6–10,14,17], are most likely a result of insulin resistance and chronic low-grade inflammation underlying the common biomarkers for cardiovascular disease risk (i.e. triglycerides, lipids, and blood pressure).

It is not surprising that one of the CMRs found to have a significant inverse association with total body bone was CRP, a biomarker of inflammation, given the known direct negative effect inflammation has on bone at the cellular level [55], and the presence of compromised bone mass and osteoporosis accompanying inflammatory conditions, such as rheumatoid arthritis, systemic lupus erythematosus, and ankylosing spondylitis [56]. Although HOMA-IR also had significant inverse associations with both total body and regional bone sites, the exact mechanism for its potential negative effects on bone remains unclear. It has been suggested that obesity-related insulin resistance impairs the influence of insulin-like-growth-factor-1 (IGF-1) mediated lean mass development on bone [18,19]. In addition, the bone-forming osteoblasts are insulin-dependent and thus susceptible to impaired insulin signaling and the resulting altered downstream signaling process that occur in a state of insulin resistance [47].

A limitation of this study is its observational design, which limits the ability to make conclusions on causal mechanisms between CMRs and bone outcomes. Moreover, since this study included only cross-sectional measures, we were unable to determine if CMR z-score or individual CMRs have differential effects on bone depending on duration of exposure. For example, Lucas et al. found that overweight adolescent girls who were above the median CRP at 13 (0.4 mg/L) and remained above the median CRP at 17 (0.7 mg/L) had significantly lower BMD than overweight girls below the median at 13 who remained low at 17 [23]. In addition, we did not assess relationships among CMRs with serum bone turnover markers, and thus do not know if the negative associations observed between CMR z-score, HOMA-IR, and CRP with measures of total body and/or regional bone strength are due primarily to decreases in bone formation or increases in bone resorption. Lastly, it should be noted that during puberty there are marked changes in lipid levels, which can potentially affect the relationships observed between lipid measures with bone. However, we attempted to adjust for these pubertal differences by inclusion of a measure of maturational status other than age in our regression models (i.e. maturity offset or Tanner stage).

Despite limitations, this study has several strengths. First, the use of a CMR z-score allowed for greater statistical power to detect associations between a group of increasing metabolic biomarker levels and bone health outcomes in girls compared to the use of a dichotomous variable indicative of CMR risk. Secondly, our use of pQCT to assess the relationship of CMRs to bone geometric properties and strength at both predominately cortical and trabecular bone sites is novel as most previous studies in children have focused on assessing the relationship of CMRs with total body bone as measured by DXA, which has inherent limitations when assessing bone strength in growing children due to its two-dimensional nature [57]. Nevertheless, it is important to understand how CMR's are related to DXA bone measures, as from a clinical

**Table 3**
Relationships of CMR z-score and individual risk biomarkers independent of each other with DXA and pQCT bone outcomes.

| | HOMA-IR[a] | CRP[a] | TG[a] | HDL | LDL | MAP | Model adjusted $R^2$ | zCMR[b] | Model adjusted $R^2$ |
|---|---|---|---|---|---|---|---|---|---|
| DXA | | | | | | | | | |
| BMC[a] | **−0.10*** | **−0.07*** | 0.01 | 0.01 | −0.03 | −0.01 | 0.88 | **−0.11*** | 0.88 |
| BA[a] | **−0.08*** | **−0.10**** | −0.007 | 0.02 | −0.03 | −0.02 | 0.88 | **−0.13**** | 0.88 |
| aBMD | **−0.12*** | −0.02 | 0.05 | 0.001 | −0.02 | 0.004 | 0.60 | −0.05 | 0.60 |
| pQCT | | | | | | | | | |
| Radius 66% | | | | | | | | | |
| Cort BMC | −0.11 | −0.06 | −0.002 | 0.04 | 0.03 | 0.07 | 0.53 | −0.05 | 0.52 |
| Cort vBMD[a] | −0.12 | −0.01 | 0.006 | 0.07 | 0.04 | **0.13*** | 0.13 | −0.01 | 0.11 |
| Tot A[a] | **−0.11*** | **−0.10*** | 0.007 | −0.02 | −0.01 | 0.004 | 0.61 | −0.08 | 0.61 |
| PC | −0.13 | −0.04 | 0.02 | 0.02 | 0.03 | −0.01 | 0.42 | −0.06 | 0.43 |
| EC | −0.10 | −0.01 | −0.009 | 0.02 | 0.03 | −0.08 | 0.11 | −0.06 | 0.12 |
| Crt Thk | −0.05 | −0.05 | 0.004 | 0.02 | 0.02 | 0.09 | 0.30 | 0.007 | 0.34 |
| SSI[a] | −0.11 | −0.06 | 0.03 | 0.02 | 0.01 | 0.01 | 0.58 | −0.06 | 0.58 |
| Femur 20% | | | | | | | | | |
| Cort BMC[a] | **−0.09*** | −0.02 | −0.02 | −0.02 | −0.02 | 0.03 | 0.74 | −0.05 | 0.74 |
| Cort vBMD | −0.10 | −0.04 | −0.12 | −0.05 | 0.09 | 0.07 | 0.14 | −0.02 | 0.13 |
| Tot A[a] | −0.03 | −0.02 | 0.07 | 0.04 | −0.06 | −0.02 | 0.72 | −0.05 | 0.72 |
| PC[a] | −0.04 | −0.005 | 0.04 | 0.04 | −0.03 | −0.05 | 0.70 | −0.06 | 0.70 |
| EC[a] | −0.01 | 0.002 | 0.05 | 0.05 | −0.03 | −0.08 | 0.51 | −0.06 | 0.51 |
| Crt Thk[a] | −0.08 | −0.02 | −0.05 | −0.06 | −0.02 | 0.09 | 0.25 | −0.02 | 0.25 |
| SSI[a] | −0.05 | −0.002 | 0.008 | 0.008 | −0.03 | −0.02 | 0.81 | −0.06 | 0.82 |
| Tibia 66% | | | | | | | | | |
| Cort BMC[a] | **−0.11*** | 0.04 | 0.008 | 0.01 | −0.02 | 0.04 | 0.71 | −0.03 | 0.71 |
| Cort vBMD | −0.10 | −0.07 | −0.06 | 0.003 | 0.05 | 0.10 | 0.23 | −0.04 | 0.22 |
| Tot A[a] | −0.03 | 0.02 | 0.03 | 0.001 | −0.03 | −0.007 | 0.73 | −0.01 | 0.73 |
| PC[a] | −0.01 | 0.002 | 0.03 | 0.03 | −0.02 | −0.02 | 0.62 | −0.02 | 0.62 |
| EC[a] | 0.05 | −0.01 | 0.03 | 0.03 | −0.006 | −0.05 | 0.28 | −0.01 | 0.29 |
| Crt Thk | **−0.15*** | 0.03 | −0.002 | 0.01 | −0.03 | 0.05 | 0.35 | −0.04 | 0.33 |
| SSI[a] | −0.07 | 0.02 | 0.01 | 0.02 | −0.01 | 0.01 | 0.78 | −0.03 | 0.78 |
| Tibia 4% | | | | | | | | | |
| Trab vBMD | **−0.21*** | −0.006 | 0.11 | −0.04 | −0.08 | 0.06 | 0.19 | −0.01 | 0.18 |
| Trab A | −0.11 | 0.04 | 0.03 | 0.10 | **−0.10*** | −0.08 | 0.39 | **−0.19*** | 0.38 |
| Tot vBMD | −0.13 | −0.04 | 0.002 | −0.10 | 0.007 | 0.09 | 0.17 | 0.05 | 0.17 |
| Tot A[a] | −0.11 | 0.03 | 0.03 | 0.08 | **−0.10*** | −0.06 | 0.52 | **−0.18*** | 0.52 |
| BSI[a] | **−0.18*** | −0.002 | 0.03 | −0.03 | −0.07 | 0.03 | 0.60 | −0.07 | 0.59 |

n = 306.
β = standardized β adjusted for log fat mass (kg), log lean mass (kg), maturity offset (years), height (cm), and ethnicity (1 = Hispanic, 0 = non-Hispanic).
HOMA-IR, homeostasis model assessment of insulin resistance; CRP, C-reactive protein (mg/L); TG, triglyceride (mg/dL); HDL-C, high density lipoprotein cholesterol (mg/dL); LDL-C, low density lipoprotein cholesterol (mg/dL); MAP, mean arterial blood pressure (mmHg); zCMR, cardiometabolic risk z-score; BMC, bone mineral content (g); BA, bone area ($cm^2$); aBMD, areal bone mineral density ($g/cm^2$); Cort vBMD, cortical volumetric bone mineral density ($mg/cm^3$); Cort BMC, cortical bone mineral content (mg/mm); Cort A, cortical area ($mm^2$); Tot A, total bone area ($mm^2$); PC, periosteal circumference (mm); EC, endosteal circumference (mm); Crt Thk, cortical thickness (mm); SSI, strength-strain index ($mm^3$); BSI, bone-strength index ($mg/cm^4$).
[a] Logrithmic transformed.
[b] zCMR = logHOMA-IR z-score + logCRP z-score + logTG z-score + (logHDL z-score *-1) + logLDL z-score + MAP z-score.
* $p < 0.05$.
** $p < 0.001$.

prospective, DXA measures of bone mass are strongly correlated with bone strength and continue to be the standard means for evaluating bone health [58].

## 5. Conclusion

In conclusion, greater cardio-metabolic dysfunction is associated with impaired total body bone strength and regional bone strength, especially that of trabecular bone in young girls. In particular, inflammation and insulin resistance are likely the CMR biomarkers underlying the negative associations observed between obesity-related cardio-metabolic dysfunction and negative bone health outcomes. Whether biomarkers of inflammation and insulin resistance can be used for not only assessing CVD risk but also risk for compromised bone development in children warrants further investigation. Future studies focusing on examining the relationship of CMRs with changes in the skeleton of children over time using advanced bone imaging techniques such as pQCT and high resolution pQCT (HR-pQCT) along with the assessment of bone turnover biomarkers may help provide further insight into whether possible deficits in bone development and peak bone mass accrual are a consequence of obesity related CMRs.

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## Declaration of interest

None.

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