Percent Fat Mass Is Inversely Associated With Bone Mass and Hip Geometry in Rural Chinese Adolescents

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ABSTRACT
This study was an attempt to examine the phenotypic, genetic, and environmental correlations between percent fat mass (PFM) and bone parameters, especially hip geometry, among 786 males and 618 females aged 13 to 21 years from a Chinese twin cohort. PFM, bone area (BA), bone mineral content (BMC), cross-sectional area (CSA), and section modulus (SM) were obtained by dual-energy X-ray absorptiometry. Multiple linear regression models were used to assess the PFM-bone relationships. A structural equation model for twin design was used to estimate genetic/environmental influences on individual phenotype and phenotypic correlations. After controlling for body weight and other pertinent covariates, we observed inverse associations between PFM and bone parameters: Compared with the lowest age- and gender-specific tertile of PFM, males in the highest tertile of PFM had lower measures of whole-body-less-head BA (WB-BA), lumbar spine BA (L2–L4-BA), total-hip BA (TH-BA), total-hip BMC, CSA, and SM (p < .005 for all, adjusted p < .05). Similar inverse associations were observed in females for all the preceding parameters except WB-BA and L2–L4-BA. These associations did not vary significantly by Tanner stages. In both genders, the estimated heritabilities were 80% to 86% for BMC, 67% to 80% for BA, 74% to 77% for CSA, and 64% for SM. Both shared genetics and environmental factors contributed to the inverse PFM-bone correlations. We conclude that in this sample of relatively lean Chinese adolescents, at a given body weight, PFM is inversely associated with BA, BMC, and hip geometry in both genders, and such associations are attributed to both shared genetic and environmental factors.

KEY WORDS: PERCENT FAT MASS; HIP GEOMETRY; BONE MINERAL CONTENT; ADOLESCENCE; COHERITABILITY

Introduction

The increasing prevalence of obesity and its health consequences have become a major public health challenge worldwide. Obesity is a well-established risk factor for excess cardiovascular disease, stroke, and type 2 diabetes in both adolescents and adults.1,2 Previously, we observed that percent fat mass (PFM), an index of obesity, was inversely related to bone mineral density (BMD) and the risk of osteoporotic fractures in an adult populations,3 which has also been observed in some other but not all studies.4 There is still an important knowledge gap on the association between fat mass and an array of bone parameters such as bone mass, bone area, and hip geometry, especially among adolescents.

Delineating the association between fat mass and bone parameters in adolescents is important but particularly challenging. In contrast to mature adulthood, adolescence is a period of rapid physical growth and functional maturation.
Adolescence is also a critical stage for bone growth, including bone length, width, area, material, and geometry. Factors or conditions that alter bone formation or enhance bone resorption during adolescence will lead to suboptimal bone growth, presumably putting the person at greater risk of osteoporotic fracture later in life. Methodologically, one has to take into account the normal growth and development of fat mass during adolescence and to tease out the positive mechanical loading effect of body weight on bone from the non-weight-bearing effect of fat mass. One also has to be able to adequately account for many other factors that may affect bone parameters, such as age, gender, and physical activity. Furthermore, the type of bone varies by skeletal region of the body (ie, vertebra, hip, etc.), and both bone material properties and bone geometry are independent determinants of bone strength. It has been suggested that changes in bone geometry can occur that may affect bone mechanical strength but will not necessarily be apparent in bone material. Thus the association between fat mass and bone parameters may vary by skeletal regions and/or by specific bone parameters (ie, bone material versus bone geometry) being studied.

Longitudinal studies in childhood and adolescence have shown that higher body weight is a strong predictor of higher bone mass later in life. However, the associations between body weight and bone parameters may not necessarily represent the correlation between fat mass and bone. Body weight consists of lean mass, fat mass, and bone mass. It is commonly believed that lean mass mediates the positive effect of body weight on bone mass. In contrast, fat tissue is a metabolically active organ, and thus it may influence the skeleton not only through the weight-bearing pathways but also through the non-weight-bearing pathways, including the hormonal metabolism of adipocytes. To date, only a few studies have explored the associations between fat mass (or obesity) and bone in adolescents, and they yielded inconclusive results. One important reason is that some of the available studies did not take into account the positive mechanical loading effect of body weight on bone parameters. Of note, most of these previous studies focused on bone area and bone mass, whereas limited data are available on bone strength or hip geometry, although the latter has been recognized as an important factor for hip fracture. Furthermore, few studies among adolescents have explored the effects of Tanner stage on the fat mass–bone relationship. Finally, fat mass and bone parameters are complex traits that are influenced by environmental factors, genetic factors, and their interactions. Currently, there are few data examining the genetic influence on the relationship between fat mass and bone parameters.

This study sought to examine the association of PFM with an array of bone parameters, including bone area (BA) and bone mineral content (BMC), at different skeletal regions, as well as two hip geometry indices at the femoral neck region, in rural Chinese adolescents after controlling for the mechanical loading effect of body weight, Tanner stage, and other pertinent covariates. We also explored whether the PFM-bone associations vary by gender, Tanner stage, bone parameters, and skeletal region. Additionally, we estimated to what degree the PFM-bone associations were contributed by the shared genetic factors using a twin design.

Methods

Study population and procedures

The study populations were part of a community-based prospective twin cohort that was recruited during 1998–2000 in the rural area of Anqing, China (baseline study). Since 2005, twins who participated in the baseline study have been followed, including clinical measurement of height, weight, body composition, and bone mass using the same study protocols as in the baseline. Detailed information on enrollment criteria of the twins at the baseline and follow-up studies was described previously. This study was approved by the institutional review boards of Children’s Memorial Hospital and the University of Illinois at Chicago and the Ethics Committee of Anhui Medical University. Written informed consent was obtained from each participant. In this report we used data collected at follow-up for subjects aged 13 to 21 years, which met the definition of adolescence by the American Academy of Pediatrics (http://www.aap.org/healthtopics/stages.cfm).

A comprehensive questionnaire was used to collect each participant’s demographic, occupational, and lifestyle information, as well as dietary information. The short version of the International Physical Activity Questionnaire (IPAQ Short) (www.ipaq.ki.se) was applied to evaluate physical activity level using the “last 7 days” as a reference period. Detailed definition for low, moderate, or high physical activity was described previously. In brief, each type of activity was weighted by its metabolic-equivalent (MET) level, and a score in MET-minutes (MET-min) was produced for each subject. “High” physical activity level was defined as (1) vigorous-intensity activity on 3 or more days and accumulating 1500 or more MET-min/week, or (2) 7 or more days of any combination of walking, moderate-intensity, or vigorous-intensity activities, achieving 3000 or more MET-min/week. “Moderate” physical activity level was defined as meeting any one of the following criteria: (1) 3 or more days of vigorous-intensity activity for 20 minutes/day or more, (2) 5 or more days of moderate-intensity activity or walking for at least 30 minutes/day, or (3) 5 or more days of any combination of walking or moderate- or vigorous-intensity activities achieving 600 or more MET-min/week. Individuals who did not meet criteria for high or moderate physical activity were considered to have a “low” physical activity level.

Anthropometry

Height was measured without shoes to the nearest 0.1 cm on a portable calibrated stadiometer. Similarly, weight was measured without shoes to the nearest 0.1 kg with the subject standing motionless in the center of a calibrated scale. In addition, Tanner stage (I through V) was determined by visual inspection by a trained physician.

Measurement of BMC, BA, and body composition

Dual-energy X-ray absorptiometry (DXA; GE-Lunar Prodigy, Waukesha, WI, USA, with enCORE 6.0 software) was used to
measure soft tissue body composition, BMC (in grams), and BA (in square centimeters) through whole-body, lumbar spine, and total-hip scans. All the bone densitometry readings were performed in a single study center in China by an experienced technologist who received training from the manufacturer. The machine was calibrated daily with a phantom. Seventy-one individuals have been measured repeatedly at different skeletal sites. The coefficient of variability (CV%) is 1.3% at the whole body and 2.1% at the total hip, respectively. Whole-body fat mass (FM) and lean mass (LM) were expressed in terms of weight (kilograms). PFM was calculated as FM × 100/weight in kilograms.

Hip structure analysis (HSA)

With the manufacturer’s HSA program that is commercially available, hip geometry variables were calculated automatically from the scan image and bone distribution variables derived from information contained within DXA X-ray absorption curves. In this study, two variables were studied: (1) cross-sectional area (CSA, square centimeters) of the minimum cross-sectional moment of inertia (CSMI) section within the femoral neck region and (2) section modulus (SM, cubic centimeters), which was calculated as the minimum CSMI within the femoral neck region divided by distance from the center of mass to the superior neck margin for the section of minimum CSA.

Zygosity identification

Twin zygosity was determined using DNA fingerprint technology by genotyping 10 microsatellite markers with high heterozygosity (>70%) and located on different autosomes.

Statistical analysis

The primary outcomes were whole-body-less-head BA (WB-BA) and BMC (WB-BMC), lumbar-spine BA (L1–L4-BA) and BMC (L1–L4-BMC), total-hip BA (TH-BA) and BMC (TH-BMC), and two hip geometric indices, CSA and SM. During the analyses, each BMC was adjusted for the corresponding BA by including it in the linear regression model. We excluded outliers (n = 44) that were 4 SD away from the respective average obtained from the analyses. All the analyses were conducted by gender in the remaining 1404 subjects.

First, we plotted the relationships between PFM and each bone parameter, adjusting for the potentially confounding effects of age, Tanner stage, weight, height, menarche status (for females only), physical activity, passive or active smoking (yes/no), and occupation. For BMCs, the corresponding BA also was adjusted. Second, the gender- and age-specific tertiles of PFM was created by tertiling PFM within each 1-year strata of age in males and females, separately. Multiple linear regressions, with adjustment of the pertinent covariates, were used to assess the associations of PFM tertiles with each of the bone parameters. To examine the influence of Tanner stage on the PFM-bone associations, these regression models were stratified by Tanner stage, and the interaction effect between PFM tertiles and Tanner stage was tested by including a product term in the model. Generalized estimating equations (GEEs) were used in all models to accommodate intrapair correlations. SAS Version 9.1 (SAS Institute, Cary, NC, USA) was used for all analyses. Further, the Bonferroni correction, with the significant level α = 0.00625 (＝0.05/8 phenotypes), was applied to control for multiple tests.

Structural equation modeling was applied to estimate the additive genetic component (a²) and shared (c²) and individual-specific (e²) environmental components for the phenotypic variance using the twin design. We evaluated three different models, including a model encompassing additive genetic influences (A), common (C), and individual-specific (E) environment influences (the full ACE model), a model encompassing A and E (AE model), and a model encompassing C and E (CE model). Under the principle of parsimony, the best-fitting model was defined as the one not having a significantly worse fit compared with the full ACE model (ie, the chi-square test was not significant with p > .05). To estimate the genetic/environmental influence on the phenotypic correlations between PFM and bone parameters, the best-fitted bivariate Cholesky decomposition model, defined based on the same criteria mentioned earlier, was applied to calculate genetic (rG), shared environmental (rC), and individual-specific environmental correlations (rE) between each pair of phenotypes. Then the genetic (Cagr), common (Cagr), and individual-specific (Cagr) environmental contributions to the phenotypic correlations were calculated as rG × √a² × a², rC × √c² × c², and rE × √e² × e², respectively. To adjust for important confounding factors, PFM and bone parameters were modeled on age, Tanner stage, weight, height, menarche status (for females only), physical activity, passive or active smoking (yes/no), occupation, and the corresponding BA (for all the BMC measures only), and the residuals from these models were used to estimate the genetic/environmental contribution to the phenotypic variations as well as their correlations using the Mx software (http://www.vcu.edu//mx/).

Results

Epidemiologic and clinical characteristics

A total of 786 males and 618 females, from a same-sex twin cohort, with a mean age of 16.6 ± 2.0 years, were included in this study. About 84.0% of the subjects had available zygosity information: In males, there were 200 monozygotic (MZ) and 128 dizygotic (DZ) pairs, and in females, there were 179 MZ and 83 DZ pairs. The epidemiologic characteristics of the study population are shown in Table 1. Males had significantly higher values in height, weight, lean mass, BA and BMC at different skeletal sites, CSA and SM (p < .0001) at the femoral neck, and a higher percentage of high physical activity. However, PFM in males (mean ± SD 11.5% ± 4.9%) was less than half that in females (27.2% ± 5.6%). In general, our study subjects, especially males, were relatively lean compared with a Western population.

Attained level of hip geometry variables by age and Tanner stage

Previously, we reported gender differences in BMC/BA by either age or Tanner stage in a subset of this cohort. We observed a similar trend for CSA and SM in this study. As shown in Fig. 1, CSA and SM increased with age linearly until approximately 17 years of age (or Tanner stage IV) in males or approximately 15 years of age (or Tanner stage III) in females and slowed thereafter. From
the age of 13, there was an increasing gender difference in CSA and SM, with CSA and SM significantly higher in males than in females across Tanner stages II through V.

Relationship between BMC and hip geometry

We examined the relationship of BMC with hip geometric variables after adjusting for age, Tanner stage, weight, height, menarche status (for females only), physical activity, passive or active smoking, occupation, and the corresponding BA. We found that BMC at the whole body, lumbar spine, and total hip was significantly and positively associated with CSA and SM ($p < .0001$) in males and females, respectively (Supplemental Table S1). These positive associations remained unchanged when age- and gender-specific tertile of PFM was further adjusted in the model.

Table 2 summarizes the associations between age- and gender-specific tertiles of PFM and each bone parameter. In males, the crude mean values for L2–L4-BA and TH-BA were the lowest in the top tertile of PFM, whereas the crude mean values for the other bone parameters did not differ significantly across tertiles of PFM. In females, bone parameters did not change with increasing PFM for PFM < 0.25. However, for PFM ≥ 0.25, all bone parameters, except for WB-BA and L2–L4-BA, decreased with increasing PFM.

Association of PFM with bone parameters

Each bone parameter, after adjustment for age, Tanner stage, weight, height, physical activity, menarche status, active or passive smoking, and occupation, was plotted against PFM by gender (Fig. 2). In males, WB-BA, L2–L4-BA, TH-BA, L2–L4-BMC, TH-BMC, CSA, and SM tended to decrease with increasing PFM; in females, bone parameters did not appear to change with increasing PFM when PFM < 0.25. However, for PFM ≥ 0.25, all bone parameters, except for WB-BA and L2–L4-BA, decreased with increasing PFM.

### Table 1. Characteristics of 1404 Chinese Adolescents From the Anqing Twin Cohort (Mean ± SD)

| Variable                        | Male (n = 786) | Female (n = 618) | p Value$^a$ |
|---------------------------------|---------------|-----------------|-------------|
| Age, years                      | 16.6 ± 2.0    | 16.6 ± 2.0      | .750        |
| Weight, kg                      | 48.7 ± 8.1    | 46.1 ± 6.5      | <.001       |
| Height, cm                      | 160.7 ± 7.9   | 152.6 ± 5.3     | <.001       |
| Body composition, kg            |               |                 |             |
| Whole-body fat mass             | 5.7 ± 3.2     | 12.8 ± 4.0      | <.001       |
| Total lean mass                 | 41.5 ± 6.5    | 31.6 ± 3.2      | <.001       |
| Percent fat mass (%)            | 11.5 ± 4.9    | 27.2 ± 5.6      | <.001       |
| Bone area (BA), cm$^2$          |               |                 |             |
| Whole body less head            | 1659.5 ± 248.2| 1517.0 ± 179.1  | <.001       |
| Lumbar spine                    | 39.0 ± 5.7    | 35.7 ± 3.8      | <.001       |
| Total hip                       | 31.0 ± 3.3    | 27.0 ± 2.0      | <.001       |
| Bone mineral content (BMC), g   |               |                 |             |
| Whole body less head            | 1505.6 ± 357.3| 1319.3 ± 230.3  | <.001       |
| Lumbar spine                    | 37.2 ± 9.9    | 35.6 ± 6.7      | <.001       |
| Total hip                       | 28.4 ± 5.8    | 23.9 ± 3.5      | <.001       |
| Hip geometry indices            |               |                 |             |
| Cross-sectional area, cm$^2$    | 1.4 ± 0.3     | 1.2 ± 0.2       | <.001       |
| Section modulus, cm$^3$         | 0.55 ± 0.14   | 0.42 ± 0.08     | <.001       |

| Physical activity               |               |                 |             |
| Low                             | 234 (29.8)    | 237 (38.3)      |             |
| Moderate                        | 278 (35.4)    | 207 (33.5)      |             |
| High                            | 191 (24.3)    | 105 (17.0)      |             |
| Unknown                         | 83 (10.5)     | 69 (11.2)       | <.001       |
| Tanner stage                    |               |                 |             |
| I                               | 65 (8.2)      | 9 (1.4)         |             |
| II                              | 116 (14.8)    | 98 (15.9)       |             |
| III                             | 154 (19.6)    | 235 (38.0)      |             |
| IV                              | 161 (20.5)    | 160 (25.9)      |             |
| V                               | 155 (19.7)    | 103 (16.7)      |             |
| Unknown                         | 135 (17.2)    | 13 (2.1)        | <.001       |
| Passive smoking, yes            | 551 (70.1)    | 400 (64.7)      | .060        |
| Current smoker                  | 77 (9.9)      | 0               | <.001       |
| Occupation, student             | 580 (73.8)    | 405 (65.5)      | <.001       |
| Menarche, yes                   | —             | 550 (89.0)      |             |

$^a$A t test was performed to compare the difference of continuous variables, and a chi-square test was performed to compare categorical variables between males and females, respectively.
Fig. 1. The attained level of hip geometry variables by age and Tanner stage among 1404 Chinese adolescents from the Anqing twin cohort.

Fig. 2. The gender-specific relationship of percent fat mass with an array of adjusted bone parameters among 1404 Chinese adolescents from the Anqing twin cohort.
Table 2. Associations of Age- and Gender-Specific Tertile of Percent Fat Mass (PFM) With Bone Parameters in 1404 Chinese Adolescents From the Anqing Twin Cohort

| Phenotype     | PFM Tertile | Male          |              | Female         |              | Total population |              |
|---------------|-------------|---------------|--------------|----------------|--------------|------------------|--------------|
|               |             | Mean ± SD     | β ± SE<sup>a</sup> | p Value<sup>a</sup> | Mean ± SD     | β ± SE<sup>b</sup> | p Value<sup>b</sup> | Mean ± SD     | β ± SE<sup>ab</sup> | p Value<sup>ab</sup> |
| WB-BA         | Low         | 1659.1 ± 219.1 | ref          | 1447.1 ± 169.2 | ref          | 1566.1 ± 224.8 | ref          |
|               | Middle      | 1663.4 ± 259.6 | −12.7 ± 7.0  | .072           | 1505.2 ± 164.7 | −17.2 ± 7.7   | .025           | 1630.6 ± 229.2 | −25.4 ± 6.9   | .0003<sup>c</sup>    |
|               | High        | 1655.9 ± 263.9 | −39.3 ± 9.0  | <.0001<sup>c</sup> | 1598.5 ± 170.7 | −9.9 ± 10.0  | .322           | 1630.6 ± 229.2 | −25.4 ± 6.9   | .0003<sup>c</sup>    |
| L₂₋₇-BA       | Low         | 39.5 ± 5.2    | ref          | 34.9 ± 4.0     | ref          | 37.5 ± 5.3     | ref          |
|               | Middle      | 39.3 ± 5.9    | −0.23 ± 0.26 | .379           | 35.8 ± 3.6   | −0.11 ± 0.27  | .688           | 37.8 ± 5.3     | −0.22 ± 0.19  | .239           |
|               | High        | 38.1 ± 5.9    | −1.34 ± 0.32 | <.0001<sup>c</sup> | 36.5 ± 3.6   | −0.25 ± 0.35  | .463           | 37.4 ± 5.1     | −0.99 ± 0.24  | <.0001<sup>c</sup>    |
| TH-BA         | Low         | 31.3 ± 2.9    | ref          | 26.9 ± 1.9     | ref          | 29.4 ± 3.3     | ref          |
|               | Middle      | 31.3 ± 3.6    | −0.15 ± 0.16 | .354           | 27.0 ± 2.0   | −0.46 ± 0.17  | .006<sup>c</sup> | 29.4 ± 3.7     | −0.33 ± 0.12  | .005<sup>c</sup>    |
|               | High        | 30.4 ± 3.4    | −1.14 ± 0.20 | <.0001<sup>c</sup> | 27.2 ± 2.0   | −1.00 ± 0.21  | <.0001<sup>c</sup> | 29.0 ± 3.3     | −1.14 ± 0.15  | <.0001<sup>c</sup>    |
| WB-BMC        | Low         | 1496.5 ± 318.9 | ref          | 1233.4 ± 227.6 | ref          | 1381.1 ± 311.0 | ref          |
|               | Middle      | 1507.2 ± 375.6 | −1.2 ± 8.0  | .882           | 1312.1 ± 219.5 | −0.6 ± 7.3   | .933           | 1421.0 ± 330.5 | −2.1 ± 5.7   | .707           |
|               | High        | 1513.1 ± 375.2 | −8.6 ± 10.0 | .394           | 1411.3 ± 209.4 | −31.2 ± 9.9  | .002<sup>c</sup> | 1468.3 ± 317.0 | −21.4 ± 7.2  | .003<sup>c</sup>    |
| L₂₋₇-BMC      | Low         | 37.7 ± 9.3    | ref          | 33.7 ± 7.0     | ref          | 36.0 ± 8.6     | ref          |
|               | Middle      | 37.3 ± 10.2   | −0.46 ± 0.33 | .173           | 35.8 ± 7.0   | −0.01 ± 0.36  | .969           | 36.6 ± 9.0     | −0.19 ± 0.25  | .439           |
|               | High        | 36.5 ± 10.2   | −0.35 ± 0.44 | .421           | 37.2 ± 5.6   | −0.54 ± 0.46  | .239           | 36.8 ± 8.5     | −0.32 ± 0.32  | .327           |
| TH-BMC        | Low         | 28.6 ± 5.2    | ref          | 23.3 ± 3.5     | ref          | 26.3 ± 5.2     | ref          |
|               | Middle      | 28.6 ± 6.3    | −0.42 ± 0.29 | .148           | 24.0 ± 3.7   | −0.55 ± 0.28  | .052           | 26.5 ± 5.8     | −0.38 ± 0.21  | .071           |
|               | High        | 27.9 ± 5.8    | −1.06 ± 0.34 | .002<sup>c</sup> | 24.4 ± 3.1   | −1.76 ± 0.37  | <.0001<sup>c</sup> | 26.4 ± 5.1     | −1.24 ± 0.26  | <.0001<sup>c</sup>    |
| CSA           | Low         | 1.38 ± 0.24   | ref          | 1.13 ± 0.16    | ref          | 1.27 ± 0.24    | ref          |
|               | Middle      | 1.35 ± 0.27   | −0.05 ± 0.02 | .001<sup>c</sup> | 1.17 ± 0.16  | −0.02 ± 0.01  | .073           | 1.27 ± 0.25    | −0.04 ± 0.01  | .0003<sup>c</sup>    |
|               | High        | 1.35 ± 0.25   | −0.08 ± 0.02 | <.0001<sup>c</sup> | 1.20 ± 0.15  | −0.08 ± 0.02  | <.0001<sup>c</sup> | 1.28 ± 0.23    | −0.08 ± 0.01  | <.0001<sup>c</sup>    |
| SM            | Low         | 0.56 ± 0.13   | ref          | 0.40 ± 0.08    | ref          | 0.49 ± 0.13    | ref          |
|               | Middle      | 0.55 ± 0.15   | −0.02 ± 0.01 | .030           | 0.41 ± 0.08  | −0.01 ± 0.01  | .152           | 0.49 ± 0.14    | −0.02 ± 0.01  | .004<sup>c</sup>    |
|               | High        | 0.54 ± 0.14   | −0.04 ± 0.01 | .0004<sup>c</sup> | 0.44 ± 0.08  | −0.02 ± 0.01  | .005<sup>c</sup> | 0.49 ± 0.13    | −0.03 ± 0.01  | <.0001<sup>c</sup>    |

<sup>a</sup>WB-BA = whole-body-less-head bone area (BA); L₂₋₇-BA = lumbar-spine BA; TH-BA = total-hip BA; WB-BMC = whole-body-less-head bone mineral content (BMC); L₂₋₇-BMC = lumbar-spine BMC; TH-BMC = total-hip BMC; CSA = cross-sectional area; SM = section modulus.

<sup>b</sup>The model adjusted for age, Tanner stage, weight, height, menarche status (for females only), physical activity, passive or active smoking, occupation, and the corresponding bone area (for BMC only).

<sup>c</sup>Gender also was included in the model.

<sup>d</sup>Adjusted p < .05 after the Bonferroni correction.
PFM tertiles. However, after adjustment for body weight and the other covariates, those in the top tertile of PFM had significantly lower WB-BA (β ± SE = −39.3 ± 9.0 cm², p < .0001), lower L2–L4-BA (−1.34 ± 0.32 cm², p < .0001), lower TH-BA (−1.14 ± 0.20 cm², p < .0001), lower TH-BMC (−1.06 ± 0.34 g, p = .002), lower CSA (−0.08 ± 0.02 cm², p < .0001), and lower SM (−0.04 ± 0.01 cm³, p = .0004) than those in the bottom PFM tertile. No significant relationships between PFM and WB-BMC or L2–L4-BMC were observed in males.

In females, the crude mean values for each bone parameter were the highest in the top tertile of PFM, but after adjustment for the covariates, those in the top tertile of PFM had significantly lower TH-BA (β ± SE = −1.00 ± 0.21, p < .0001), WB-BMC (−31.2 ± 9.9, p = .002), TH-BMC (−1.76 ± 0.37, p < .0001), CSA (−0.08 ± 0.02, p < .0001), and SM (−0.02 ± 0.01, p = .005) than those in the bottom tertile. The relationships between PFM and the other bone parameters, including WB-BA, L2–L4-BA, and L2–L4-BMC, were insignificant.

We performed the same analyses in the total population and found similar inverse relationships between PFM tertiles and each bone parameter (Table 2). The inverse associations in males, females, and the total population remained significant after the Bonferroni correction (Table 2). Additional analyses of age- and gender-specific tertiles of fat mass in relation to bone parameters showed that their associations were comparable with the associations between PFM tertiles and bone parameters (data not shown). We also repeated our analyses by removing 99 subjects older than 19 years of age (56 males and 43 females) who did not meet the World Health Organization (WHO) definition of adolescence and found that the inverse relationship between PFM and each bone parameter remained unchanged (data not shown).

Effect of Tanner stage on the PFM-bone associations

To examine the effect of Tanner stage on the association between PFM tertiles and bone measures, least-squares means and standard errors of TH-BMC, TH-BA, CSA, and SM across PFM tertiles in each Tanner stage, after adjustment of age, weight, height, physical activity, menarche status, active or passive smoking, occupation, are presented in Supplemental Figures S1 and S2. In males, those in the higher tertile group had lower TH-BMC values in Tanner stages I, IV, and V and had lower TH-BA, CSA, and SM values in each Tanner stage, with the strongest associations in Tanner stages IV and V. No significant interactive effect was detected between PFM and Tanner stage on these four phenotypes. In females, Tanner stage I was excluded from this subset of analyses owing to very limited sample size (n = 9). In Tanner stage II and above, females in the higher tertile group of PFM also had lower TH-BMC values in Tanner stages II through IV and had lower TH-BA, CSA, and SM values in all Tanner stages, with the strongest associations in Tanner stages III and IV. A modest interactive effect between PFM and Tanner stage was observed on CSA only (p = .045), and it became insignificant after the Bonferroni correction. In males, patterns similar to TH-BA were found for WB-BA and L2–L4-BA, and in females, a pattern similar to TH-BMC was observed for WB-BMC (data not shown).

**Table 3.** Heritability Estimates\(^a\) of Bone Parameters and Percent Fat Mass in 590 Same-Sex Chinese Twins From the Anqing Twin Cohort

| Outcome\(^b\) | Male(200 MZ and 128 DZ pairs) | Female (179 MZ and 83 DZ pairs) |
|--------------|--------------------------------|---------------------------------|
|              | \(a^2\) (95% CI)\(^c\) | \(e^2\) (95% CI)\(^c\) | \(a^2\) (95% CI)\(^c\) | \(e^2\) (95% CI)\(^c\) |
| WB-BA        | 0.80 (0.74–0.84) | 0.20 (0.16–0.26) | 0.74 (0.67–0.79) | 0.26 (0.21–0.33) |
| L2–L4-BA     | 0.76 (0.69–0.80) | 0.24 (0.20–0.31) | 0.67 (0.60–0.74) | 0.33 (0.26–0.40) |
| TH-BA        | 0.71 (0.64–0.77) | 0.29 (0.23–0.35) | 0.80 (0.74–0.84) | 0.20 (0.16–0.26) |
| WB-BMC       | 0.83 (0.78–0.86) | 0.17 (0.14–0.22) | 0.80 (0.75–0.84) | 0.20 (0.16–0.25) |
| L2–L4-BMC    | 0.86 (0.83–0.89) | 0.14 (0.11–0.17) | 0.85 (0.80–0.88) | 0.15 (0.12–0.20) |
| TH-BMC       | 0.81 (0.76–0.85) | 0.19 (0.15–0.24) | 0.85 (0.80–0.88) | 0.15 (0.12–0.20) |
| Cross-sectional area | 0.74 (0.68–0.79) | 0.26 (0.21–0.32) | 0.77 (0.71–0.82) | 0.23 (0.18–0.29) |
| Section modulus | 0.64 (0.56–0.71) | 0.36 (0.29–0.44) | 0.64 (0.55–0.71) | 0.36 (0.29–0.45) |
| Percent fat mass | 0.83 (0.78–0.86) | 0.17 (0.14–0.22) | 0.77 (0.71–0.82) | 0.23 (0.18–0.29) |

\(^a\)Heritability (\(a^2\)) was estimated using the best-fit AE model.

\(^b\)Adjusted for age, Tanner stage, weight, height, menarche status, physical activity, passive or active smoking, occupation, and the corresponding bone area (for BMC only).

\(^c\)95% confidence intervals (CI) were calculated for each parameter estimate. These parameters were statistically insignificant if their CIs include 0.
bone pair were negative and significant. The proportions of phenotypic correlations between PFM and various bone parameters explained by shared genetics were as follows: 87% (−0.23 to −0.10) for WB-BA, 70% for L2–L4-BA, 92% for TH-BA, 76% for TH-BMC, 84% for CSA, and 84% for SM. The rest of these phenotypic correlations were explained by individual-specific environmental factors. Similarly, in females, we found that the phenotypic correlations between PFM and the five bone parameters (including TH-BA, WB-BMC, TH-BMC, CSA, and SM) could be explained by both shared genetics and individual-specific environmental factors, although the genetic correlation (rG) for the PFM–WB-BMC pair, as well as individual-specific environmental correlations (rE) for the PFM-CSA and PFM-SM pairs, did not attain statistical significance (Table 4).

**Discussion**

This study in lean, healthy Chinese adolescents has demonstrated that PFM is inversely associated with hip geometry (CSA and SM), as well as with BA and BMC, in both genders, after accounting for body weight (mechanical-loading effect). The inverse effect of PFM on BMC mainly focuses on the total hip bone rather than the lumbar spine bone. Such relationships did not vary substantially by Tanner stage. We report for the first time that this may be a general property of human biology.

Epidemiologic studies exploring the effects of fat mass or adiposity on adolescent bone health, most of which have limited sample size (n < 400), have yielded conflicting results, ranging from protective effects, to no effects, to detrimental effects. It is possible that these discrepancies in previous studies may be due in part to such factors as differences in age, gender, bone phenotype, and study design. Another important explanation for these discrepancies is that different authors have chosen different ways to account for the confounding of mechanical-loading effect in their studies. For example, some authors presented unadjusted data, whereas others presented adjusted data for lean mass only. Our study, together with some others, has adjusted the full mechanical-loading effect by including body weight in the regression models. We observed inverse relationships between PFM and bone parameters, which is consistent with findings from previous studies in adolescents in New Zealand, in adolescent females in the United States, and in adolescent females in Canada. These consistent findings across multiple populations raise the possibility that this may be a general property of human biology.

Our study suggested that the PFM-bone relationship may vary by skeletal regions, for which PFM was associated with BMC at...
the hip (total hip) but not at the lumbar spine region in both genders. Notably, the quantity of cortical bone at the hip region is much higher than that at the lumbar spine region. A previous study by Pollock and colleagues also reported that areas consisting predominantly of cortical bone were affected more than areas consisting predominantly of trabecular bone by PFM.\(^{(14)}\) These findings raised the possibility that PFM may have a differential effect on cortical versus trabecular bones. However, the underlying biologic mechanisms are not yet known and need additional research.

We observed that the magnitude of the inverse PFM-bone relationships was greater in males than in females (Fig. 2). Such gender-specific associations have been reported previously.\(^{(15,28)}\) For example, Ackerman and colleagues suggested that BMC was lower in children with higher FM for a given sex and weight, which was more pronounced in pubertal boys.\(^{(28)}\) Although the underlying mechanisms remain unclear, one possible explanation for the gender-specific effect is that males have a higher proportion of visceral fat than females.\(^{(29)}\) Previous studies showed that visceral fat was associated with a higher risk of metabolic syndrome than subcutaneous fat.\(^{(30)}\) Visceral fat also was associated with increased levels of interleukin 6 (IL-6),\(^{(31)}\) which may be involved in bone loss and resorption.\(^{(32)}\) A recent study has found that visceral fat is inversely associated with the structure and strength of bone.\(^{(33)}\) Subcutaneous fat, in contrast, is positively associated with bone structure and strength.\(^{(32)}\) Further studies are needed to investigate the molecular and functional differences of visceral and subcutaneous adipocytes and how they interact with bone.

Puberty is a time of great fluctuations in body composition and bone growth. We found no significant interaction between Tanner stage and PFM on bone parameters in our population. However, we found that in females, PFM and BMC tended to be negatively related in Tanner stages II through IV but not in Tanner stage V. This finding needs to be confirmed in a future study given the limited sample size and statistical power of this study (n = 103 in Tanner stage V).

It has been suggested that hip geometry is an important factor for subsequent hip fracture.\(^{(19)}\) Studies on the relationship between fat mass and hip geometry, especially SM (an estimate of bone bending strength), might provide some additional insight into the prevention of hip fractures later in life. Petit and colleagues demonstrated that proximal femur bone geometry, especially in adolescents. We, for the first time, provide estimates of the heritability of CSA and SM in Chinese adolescent twins. In our study, both CSA and SM are highly heritable in both genders, although the estimated heritability for SM (64%) appears to be lower than that for BMC. These estimates are slightly higher than those estimated from a Chinese adult family-based cohort (55% to 57%).\(^{(36)}\) We also observed that shared genetics significantly contributed to the observed inverse PFM-bone relationships, indicating a set of genes shared by both PFM and bone parameters. These findings are consistent with a previous study in white adults\(^{(37)}\) and may be explained by current understanding about the reciprocal differentiation of adipocytes (the main origin of adipokines) and osteoblasts, which each originate from the same mesenchymal stem cells in a mutually exclusive way.\(^{(38)}\) This process is regulated by two key transcription factors, Runx2 [also called Cbfa(\(^{(39)}\))] and peroxisome proliferator-activated receptor γ (PPAR-γ2).\(^{(40)}\) Thus genetic factors influencing the expression of Runx2 and/or PPAR-γ2 potentially may contribute to the inverse correlation between adiposity and bone health. Genetic association/linkage studies also have observed that polymorphisms on a set of genes, such as insulin-like growth factor 1,\(^{(41)}\) leptin receptor,\(^{(42)}\) and IL-6\(^{(43)}\) have common effects on both osteoporosis and fat mass (or obesity).

There are several limitations to this study. First, the cross-sectional design does not allow for determining the causal effect between fat mass and bone parameters, although it is difficult to consider reverse causation at play. Second, both fat mass and bone mass were derived from the same DXA measurements, which do not provide a means for distinguishing between cortical and trabecular bone and between subcutaneous and visceral fat, and this study did not account for the confounding effect of fat on bone measures when using DXA. Also, our hip geometry measurements are subject to certain technical limitations, including axial asymmetry of cross sections and the tissue mineralization assumption.\(^{(44)}\) Third, although the twin design allows us to calculate the genetic influence on each phenotype and their correlations, it is possible that this twin cohort is not wholly representative of nontwin populations. However, we used a community-based twin cohort, and our previous reports demonstrated that this twin cohort was similar to the local general pediatric and adolescent populations with regard to socioeconomic characteristics, lifestyles, and anthropometric measurements.\(^{(45)}\) Fourth, the number of female DZ twin pairs in this study was relatively small (n = 83), and the phenotypic correlations between PFM and bone in females are nonlinear (as shown in Fig. 2), which could limit our power to accurately estimate the genetic and environmental contributions to the mild to moderate phenotypic correlation we observed among the females in our cohort.

In summary, our study provides strong evidence that PFM has an inverse relationship with BMC, BA, and hip geometry for a given body weight in this sample of relatively lean Chinese adolescents and that the relationship was not affected substantially by Tanner stage. Both genetic and environmental factors contributed significantly to each of the bone parameters and to the inverse phenotypic correlation between PFM and the bone parameters. Continued follow-up of this cohort will provide further insight into the temporal relationship between PFM and
bone health and the utility of PFM during adolescence as a predictor of bone mass, hip geometry, and fractures in later years.

**Disclosures**

All the authors state that they have no conflicts of interest.

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