Association of Hip Bone Mineral Density and Body Composition in a Rural Indian Population: The Andhra Pradesh Children and Parents Study (APCAPS)

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Abstract

Background

Fat mass is variably associated with bone mass, possibly due to differential mechanical and biological effects of fat mass. We examined the association of fat mass with bone mass in a lean population.

Objective

To investigate association between hip bone mineral density and fat and lean mass in a cross-sectional study from southern India.

Design

The Andhra Pradesh Children and Parents Study is a prospective cohort study in Hyderabad, India. In 2009–2012, the study collected data on anthropometric measures, bone mineral density (BMD), fat mass, and lean mass measured by dual-energy x-ray absorptiometry, and socioeconomic data of the adult participants (n = 1760; mean age = 34.9 years old for women; 2130 and 32.3 for men).

Results

The median BMI (kg/m²) was 20.1 kg/m². Women had relatively higher fat mass as compared to men. In models adjusted for lean mass, there was an association between hip bone
mineral density and fat mass in women (β (95% confidence interval): premenopausal 0.025 (0.006 to 0.045); postmenopausal 0.045 (0.014 to 0.076)) but not in men (0.001 (-0.012 to 0.001)). The association between hip BMD and fat mass was stronger in postmenopausal than premenopausal women. Hip BMD was consistently associated with lean mass, in both men and women.

Conclusions
In this relatively lean population, lean mass was more consistently associated with hip BMD than fat mass. Weight gain through lean mass improvement may be a more reliable public health strategy for strengthening bone health in transitional settings.

Introduction
Osteoporotic fractures are associated with morbidity and mortality [1]. A study estimated that the number of hip fractures will rise to 6.3 million globally by 2050 [2]. Hip bone mineral density (BMD) is a predictor of overall risk of fractures in later life [3]. Body mass is one of the key determinants of hip bone mineral density [4].

Fat mass is one of the main components of body mass. On one hand, sheer mechanical loading of fat mass stimulates bone formation [5,6]. However, in vivo and in vitro studies have suggested negative effects of fat on bone mass accrual through several biological mechanisms [5–8]. Leptin, an adipocyte hormone, was shown to have an anti-osteogenic property in mice [9]. Shared precursor stromal cells suggest competitive cell lineages between osteogenesis and adipogenesis [10]. Peroxisome proliferator-activated receptor (PPAR) γ pathway is a key regulator of adipogenesis and also an inhibitor for osteoblastogenesis [7].

Epidemiological studies have shown inconsistent results on association between bone and fat mass [11–14]. Many studies examined this association in the context of obesity and osteoporosis partially because of concerns for higher fracture rates among obese individuals [15]. However, it is possible that the balance between mechanical and biological mechanisms may vary depending on the combination of body size and composition. Asians have been shown to have higher proportion of fat mass at lower body mass index (BMI) [16]. The patterns of association between fat and bone mass in the Indian population may therefore be distinct from the European and American populations. There have been few large-scale studies examining this association in the Indian population.

The Andhra Pradesh Children and Parents Study (APCAPS) is a prospective cohort study from southern India. The study population has been undergoing a nutritional and epidemiological transition due to urbanization over the past decade; as a result, this population has a wide variation in body sizes and compositions. The current study assessed how fat and bone mass may be associated in this transitional rural population.

Methods
Ethics statement
The study received approvals from the ethics committees of the NIN (Hyderabad, India), the Indian Council of Medical Research (ICMR), Centre for Chronic Disease Control, and London School of Hygiene and Tropical Medicine (London, UK). Approval was also sought from the village heads and their panchayats in each of the 29 villages. Written informed consent or
witnessed thumbprint if illiterate was obtained from the participants prior to their inclusion in the study.

Study design
The analyses in this study used data from two waves of data collection (2009–2010; 2010–2012) of the APCAPS study, a prospective cohort study established through long-term follow up of the Hyderabad Nutrition Trial (HNT). The HNT studied impact of the Integrated Child Development Services scheme, a national community outreach programme providing food supplementation along with health, hygiene, and nutrition education, immunization, anemia control, and basic health care to pregnant and lactating women and children under the age of six years [17]. A detailed description of the initial trial (HNT) and the first wave of data collection (the first follow-up of the HNT, 2003–2005) has previously been published [17,18].

Since the second and third waves of data collection (W2/3) were conducted within a relatively short period of time (2009–2012), the analyses in this manuscript used combined data from these two waves of data collection. W2/3 examined markers for chronic diseases affecting cardiovascular, musculoskeletal, and mental health. All consenting participants underwent dual-energy x-ray absorptiometry (DXA) measurements at the National Institute of Nutrition (NIN), Hyderabad and physical measurements at NIN (W2) or the village clinics (W3). In cases where participants attended both waves of data collection, the data from the third wave were used, unless there were major artifacts in the DXA scans from W3, which prompted the use of data from W2. The current manuscript analyzed data on the adult participants only (18 years old and above).

Of the 7375 participants of the second/third wave of data collection whose age, sex, height, and weight information were available, 4251 participants (58%) underwent DXA scans during W2/3. Of those, scans without major artifacts were available in 4243 (99.8%) participants for hip BMD. 97.5% of the DXA participants also had scans without major artifacts for whole-body estimation of fat and lean mass. Information on the other descriptive variables were available for ≥99% of the DXA participants. In total, 1200 premenopausal women, 560 postmenopausal women, and 2130 men who had complete data for hip BMD, whole body composition and demographic data were included in the regression models.

Measurements

**Questionnaire data.** A semi-structured questionnaire was administered to all participants by a trained interviewer. A subset of questions (14/29) from the Standard of Living Index (SLI) in the National Health Family Survey-2, a summary measure of household level asset-based scale devised for Indian surveys, was used to estimate socioeconomic position as joint family structures are common in rural India [19]. We collected information on the quality of house, toilet facilities, source of lighting and drinking water, ownership of clock, radio, television, bicycle, motorcycle, car, refrigerator, telephone, and agricultural land. These items were weighted to give a maximum score of 34, using weights developed by the International Institute of Population Science in India [19]. Occupation was classified into four categories: students, manual employment, professional employment, and unemployment. Current tobacco use was defined as smoking, chewing, or snuffing tobacco in the last 6 months. Menopausal status was set as a binary variable (yes or no).

**Anthropometric data.** Weight was measured to the nearest 0.1kg with a digital SECA balance and standing height was measured to the nearest 1 mm with a plastic stadiometer.
(Leicester height measure). Measurements were taken twice and the average of two values was used in the analysis. Body mass index (BMI) was calculated as weight (kg) / height (m²).

**DXA scanning.** Bone density and body composition measures were made by DXA using a Hologic Discovery A densitometer. The whole body scan was performed with the participant supine on the scanning bed with their arms resting by their sides. Women suspected of pregnancy were excluded from DXA scanning and the scans were taken only after confirming the negative pregnancy by conducting urine pregnancy test. Standard Hologic software options were used to define regions of the body (head, arms, trunk, and legs). The coefficients of variation were determined to be 0.7% for hip bone mineral density (BMD), 1.3% for LS BMD, and 0.9% for whole-body BMD (n = 30). Scans were coded for artifacts by a visual inspection and those hip scans with major movement and foreign objects as well as incomplete scans were excluded from the analyses of hip BMD (g/cm²). Whole body scans with major movements and incomplete scans were counted as artifacts and removed from analysis with fat and lean mass. Bone mineral content (BMC in g) was calculated from DXA-measured BMD (g/cm²) and bone area (cm²) for total hip. Fat to lean mass ratio (FLR: fat mass / lean mass) was calculated using the allometric coefficients from sex-stratified models regressing log-transformed fat mass upon log-transformed lean mass as x (x = 1.57 for women; 1.66 for men). FLR was multiplied by 100 to improve clarity. Total fat and lean mass (kg) were estimated from whole-body scans. Osteopenia and osteoporosis were defined based on the reference BMD values measured on Hologic DXA machines in healthy Indian young adults (Hip BMD mean (sd): Women = 0.901 (0.111); Men = 0.988 (0.131)) [20].

**Statistical analysis**

Descriptive statistics were calculated for premenopausal women, postmenopausal women, and men separately. Comparison between premenopausal and postmenopausal women and women and men were made using Student t-test for the continuous variables with normal distributions (height, weight, lean mass, wbPA, and SLI), Wilcoxon rank-sum test for the continuous variables with non-normal distributions (age, fat, FLR, and wbPA), and χ² test for the categorical variables (BMI categories, tobacco use, vegetarianism, and occupation) with appropriate degrees of freedom. Tukey’s honest significant difference test was also performed to assess BMD differences between age groups within each sex group.

The associations between hip BMD and fat mass, lean mass, and FLR were examined in multilevel regression models that accounted for household-level clusters. Three-level nested multilevel models to adjust for both village and household-level clusters were considered but the small intraclass correlations for the village level suggested that adjustment for the household-level clustering alone was sufficient. All models were stratified by sex. There was evidence of an interaction between fat mass and menopausal status; therefore, regression models for women were stratified by menopausal status. Fat mass (kg) was log-transformed as its distribution was positively skewed. Hat, PRESS, and Cook statistics were examined to remove outliers for regression models.

Model 1 assessed association between hip BMD and fat mass, adjusting for lean mass, age, height, SLI. Model 2 (Table S1) examined association between hip BMD and FLR, adjusting for age, height, and SLI. Model 3 (Table S1) further adjusted Model 2 for weight. Further adjustment for other potential confounders (vegetarianism and current tobacco use) did not materially change the results and therefore parsimonious models are presented.

All analyses were conducted using R, version 3.1.1 and multilevel modeling was done with nlme version 3.1–118.
Results

Table 1 summarizes the key characteristics of the participants. Although the average BMIs were similar between women and men, women had higher fat to lean mass ratio than men. Current tobacco use was more common among men. Premenopausal and postmenopausal women differed in all characteristics except for FLR. There were few vegetarians in this community. As shown in Table 2, young women (20–29 years old) in the APCAPS community had remarkably lower hip BMD in comparison Indian reference values for women in the same age group (0.901±0.111 g/cm^2) [20]. Men had higher hip BMD than women across all ages.

In the multilevel models adjusting for age, height, and SLI, fat mass (in women only) and lean mass were positively associated with hip BMD (Table 3). There was no clear evidence of

| Table 1. Characteristics of the participants of the Andhra Pradesh Parents and Children Study (2009–2012). |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                                | Premenopausal women (n = 1200) | Postmenopausal women (n = 560) | Men (n = 2130)                  |
|                                | mean(sd)                        | mean(sd)                        | mean(sd)                        |
| age (year)                     | 29.3(9.9)                       | 46.8(6.9)                       | 32.3(14.7)                      |
| height (cm)                    | 152.4(5.5)                      | 150.2(5.4)                      | 165(6.5)                       |
| weight (kg)                    | 47.6(9)                         | 48.5(9.2)                       | 55.3(9.9)                      |
| BMI (kg/m^2)                   | 20.5(3.6)                       | 21.5(3.7)                       | 20.3(3.2)                      |
| Underweight (n)                | 407                             | 136                             | 724                            |
| Normal (n)                     | 650                             | 335                             | 1209                           |
| Overweight/obesity (n)         | 143                             | 89                              | 197                            |
| Fat mass (kg)                  | 14.7(5.1)                       | 15.9(5.3)                       | 10.1(4.7)                      |
| Lean mass (kg)                 | 31.6(4.5)                       | 31.7(4.5)                       | 43.6(6.2)                      |
| FLR                            | 6.19(1.49)                      | 6.66(1.56)                      | 1.88(0.69)                     |
| wbPA (hours)                   | 92.6(122.4)                     | 156.9(143.4)                    | 143.5(119)                     |
| vegetarian (n)                 | yes 52                          | 10                              | 42                             |
| no                             | 1148                            | 550                             | 2088                           |
| tobacco use (n)                | current 63                      | 152                             | 683                            |
| never/former                  | 1137                            | 408                             | 1447                           |
| SLI                            | 17.7(4.8)                       | 16.6(4.7)                       | 18.2(4.5)                      |
| occupation (n)                 | student 170                     | 2                               | 444                            |
|                                | employed: manual 638            | 474                             | 1561                           |
|                                | employed: professional 20       | 0                               | 45                             |
|                                | unemployed 372                  | 84                              | 80                             |

BMI = body mass index; FLR = fat to lean mass ratio; SLI = Standard of Living Index; wbPA = weight-bearing physical activity.

All values are mean (standard deviation) except for occupation (%), vegetarian (n), menopause status (n), and tobacco use (%).

FLR for women: fat mass / lean mass $1.57 \times 100$; for men: fat mass / lean mass $1.66 \times 100$

Current tobacco use included smoking, chewing, or snuffing tobacco in the last 6 months; former users stopped using tobacco products 6 months ago or more.

Comparison between premenopausal and postmenopausal women and women and men were made using t-test for the continuous variables with normal distributions (height, weight, lean mass, wbPA, and SLI), Wilcoxon rank-sum test for the continuous variables with non-normal distributions (age, fat, FLR, and wbPA) and $\chi^2$ test for the categorical variables (BMI categories, tobacco use, vegetarianism, and occupation) with appropriate degrees of freedom. $p$-values for comparison between premenopausal and postmenopausal women were $<$0.001 for all variables except for weight ($p = 0.04$) and FLR ($p = 0.79$); $p$-values for comparison between women and men were all $<$0.001.

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association between hip BMD and fat mass in men adjusting for lean mass. There was clear and consistent evidence for a positive association between hip BMD and lean mass. In models examining association between hip BMD and fat to lean mass ratio (S1 Table), FLR was negatively associated with hip BMD upon adjustment for total weight. Adjusting models for other

Table 2. Description of mean hip bone mineral density, osteopenia (%), and osteoporosis (%) by sex and age groups for the participants of the Andhra Pradesh Parents and Children Study (2009–2012).

<table>
<thead>
<tr>
<th>Age</th>
<th>Women n</th>
<th>BMD (g/cm²)</th>
<th>Osteopenia (%)</th>
<th>Osteoporosis (%)</th>
<th>Men n</th>
<th>BMD (g/cm²)</th>
<th>Osteopenia (%)</th>
<th>Osteoporosis (%)</th>
<th>p *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20–29</td>
<td>666</td>
<td>0.84(0.1)</td>
<td>28.4</td>
<td>1164</td>
<td>0.95(0.11)</td>
<td>14.6</td>
<td>0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>30–39</td>
<td>204</td>
<td>0.86(0.1)</td>
<td>24.5</td>
<td>108</td>
<td>0.93(0.11)</td>
<td>25</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>40–49</td>
<td>648</td>
<td>0.84(0.11)</td>
<td>30.9</td>
<td>253</td>
<td>0.92(0.12)</td>
<td>29.2</td>
<td>1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>50≤</td>
<td>248</td>
<td>0.75(0.12)</td>
<td>51.2</td>
<td>14.9</td>
<td>516</td>
<td>0.89(0.12)</td>
<td>39.7</td>
<td>3.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BMD = bone mineral density (g/cm²).

All participants who had at least age and hip BMD data and were 20 years old or older were included in this table.

Osteopenia is defined as 1 to 2.5 standard deviations (sd) and osteoporosis as more than 2.5 sd below peak bone mass in a healthy Indian population for each sex (reference hip BMD: women = 0.901 ± 0.111; men = 0.988 ± 0.131 g/cm²).

* Student t-test comparing hip BMD between sex within each age group was conducted.

Tukey’s honest significant difference test showed that hip BMD in the 20–30 and 30–40 year old groups were similar and higher than the ≥50 year old groups for both men and women (p<0.001).

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Table 3. Association of hip bone mineral density (BMD) and fat and lean mass in the participants of the Andhra Pradesh Parents and Children Study (2009–2012).

<table>
<thead>
<tr>
<th>Model 1</th>
<th>β</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-menopausal women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.025</td>
<td>(0.006 to 0.045)</td>
<td>0.01</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.009</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Post-menopausal women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.045</td>
<td>(0.014 to 0.076)</td>
<td>0.008</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.001</td>
<td>(&lt;0.012 to 0.014)</td>
<td>0.92</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Sample size: n = 1200 (pre-menopausal women); n = 560 (post-menopausal women); n = 2130 (men).

CI = confidence interval.

All models are multilevel models adjusting for household level clustering. ε ij and υ j are errors terms for multilevel regression models accounting for individual and household level differences:

Model: HIP BMD = β0 + β1 FAT MASS + β2 LEANM ASS + β3 AGE + β4 HEIGHT + ε ij + υ j

Age (years); Height (cm); Fat and lean mass (kg)

Fat mass (kg) has been log-transformed.

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potential confounders such as serum calcium and phosphorus levels, vegetarianism, tobacco use, and parity (for women only) did not materially change the results on association between hip BMD and fat mass.

Discussion

Hip bone mineral density was low in this relatively lean population in rural India. There was a positive association between hip BMD and fat mass in women (who also had relatively higher fat to lean mass ratio), but not in men. Higher hip BMD was consistently associated with greater lean mass in this population.

Comparison to previous studies

A number of epidemiological studies have examined the association between bone and fat mass: some showed no clear evidence of association while both positive and negative associations were also suggested [12,13,21–26]. As with many of the previous studies, we saw more consistent evidence for a positive effect of lean mass than fat mass [24,27]. Previous studies on Asian populations have shown attenuation of the association between BMD and fat mass upon adjustment for lean mass, similarly to our findings [24,25,27–29]. Our study also showed that for a given body size, lower fat to lean mass ratio may be associated with higher hip BMD, which suggests that both body mass and composition may be important determinants of healthy bone mass accrual. A large DXA study from China examined association between bone mass and body composition in a similarly lean population [12]. In unadjusted models, fat mass was positively associated with hip BMD, but on adjustment for body weight, fat mass became negatively associated with hip BMD, similar to the findings from our study.

Fat may have opposing effects on bone mass accrual through mechanical and biological mechanisms. Positive association between mechanical loading and bone mass accrual have been shown consistently in studies examining the effects of body mass and weight-bearing physical activity [4,6,30–33]. The mechanostat theory suggests that weight causes structural adaptation through local mechanical strain, which was supported by direct measurement of mechanical strain in live animals and humans [5,33]. On the other hand, in vitro and in vivo studies have suggested several biological mechanisms underlying the association between fat and bone mass. Osteoblasts and adipocytes originate in common stromal cells in bone marrow, suggesting plasticity between these two cell lineages [34]. Insufficiency in PPAR γ, a key regulator for adipocyte differentiation, increased osteoblastogenesis in vitro and bone mass in vivo [7]. Leptin is a hormone produced by adipocytes, regulating both fat distribution and bone turnover through the hypothalamic neural networks [26].

The combined effect of mechanical and biological properties of fat on bone mass accrual is not well-established in epidemiological studies [35] and may differ depending on sex, menopausal status, and ethnicity. [36–38]. In our study, hip BMD was positively associated with fat mass in women, but not in men. Certain fat hormones suggested to be beneficial for bone mass accrual may be more strongly associated with bone mass accrual in women [23]. Another potential explanation may be that the amount of fat mass in men in this lean population was too low to detect its contribution to hip BMD in the presence of lean mass. In an Australian study, fat mass was also associated with hip BMD more consistently among women than men [39]. Interestingly, the positive association was only seen in lean men (BMI <25kg/m²); however, the male participants in this study, on average, had higher fat mass (24.9±9.3kg/m²) than our study population (10.3±9kg/m²) [39]. Such distinct combinations of body size and composition may have contributed to varying degrees of association
between bone and fat mass in our study population and the Australian population. A study from the United States, where the men on average had greater fat mass and BMI than the men in this study population, showed no strong evidence for a positive association between BMD and fat mass and leptin in men while in women, adjustment for leptin attenuated the association between BMD and fat mass [23]. These studies suggest that sexual dimorphism may contribute to the varying degrees of association between fat and bone mass between women and men.

The association between hip BMD and fat mass was also slightly greater in postmenopausal than premenopausal women. Several studies have shown greater positive effects of fat mass in postmenopausal women although underlying biological mechanisms are not well-understood [38,40]. Adipose tissue is a key site for estrogen production in postmenopausal women [41]. Since estrogen has been suggested to play a role in the association between bone and fat mass, this may partially explain the difference in the strengths of the association between premenopausal and postmenopausal women [41,42].

It is important to note that this population had low hip BMD when compared to the values in a healthier Indian population [20]. One potential explanation for this finding is that although this rural community has become more developed and has been experiencing a nutritional transition over the past decade, modest gain in weight and lean mass during mid to late adulthood may not be able to fully mitigate adverse effects of undernutrition at younger ages when the majority of bone mass accrual occurs. Our previous study showed that gain in weight and lean mass in late adolescence and young adulthood was positively associated with bone mass (unpublished data) but it is currently unknown how long the window of opportunity to improve bone mass lasts.

Strengths and limitations
This study is one of the few large-scale DXA studies examining the Indian population. The study subjects reside in an urbanizing rural community where there is a wide range of combinations in body sizes and compositions, allowing assessment of the association between hip BMD and fat mass in both underweight and overweight populations. The use of DXA provides more accurate estimation of fat and lean mass than anthropometric measurements.

There are some limitations in this study as well. The cross-sectional nature of the study does not allow causal inference. This community has been experiencing a nutritional transition due to urbanization over the past decade. There may be risk factors from the past that are contributing to current bone mass, although, in young adults, our previous studies did not find strong evidence for longitudinal effects of early life [43] and adolescent undernutrition [44] on bone mass during adulthood after controlling for current body mass. The study population was also lean compared to higher income countries [45]; therefore, our findings may not be generalizable to other populations but the findings may be of interest to other lean populations whose body compositions are more similar to this study population in India. Another limitation is the lack of data on fractures and fat hormones, which would be of clinical and biomedical importance.

Conclusions
In this relatively lean population, hip BMD was associated with lean mass in both men and women, but hip BMD was associated with fat mass in women only. Weight gain through lean mass improvement may be a more reliable public health strategy for strengthening bone health in transitional settings.
Supporting Information

S1 Table. Multilevel regression models examining association between hip bone mineral density and fat to lean mass ratio in the participants of the Andhra Pradesh Parents and Children Study (2009–2012).

(PDF)

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Project administration: HK SK KVR PP VG GKW AA.
Supervision: SK.
Writing – original draft: MM.
Writing – review & editing: MM BK HK JCW GBP PP VG GKW AA DP GDS KVR YBS SK.

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