Original Full Length Article

Increased fat mass is associated with increased bone size but reduced volumetric density in pre pubertal children

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A B S T R A C T

Recent studies have shown that obesity is associated with an increased risk of fracture in both adults and children. It has been suggested that, despite greater bone size, obese individuals may have reduced true volumetric density; however this is difficult to assess using two dimensional techniques such as DXA. We evaluated the relationship between fat mass, and bone size and density, in a population cohort of children in whom DXA and pQCT measurements had been acquired.

We recruited 530 children at 6 years old from the Southampton Women's Survey. The children underwent measurement of bone mass at the whole body, lumbar spine and hip, together with body composition, by DXA (Hologic Discovery, Hologic Inc., Bedford, MA, USA). In addition 132 of these children underwent pQCT measurements at the tibia (Stratec XCT2000, Stratec Biomedical Systems, Birkenfeld, Germany).

Significant positive associations were observed between total fat mass and both bone area (BA) and bone mineral content (BMC) at the whole body minus head, lumbar spine and hip sites (all $p < 0.0001$). When true volumetric density was assessed using pQCT data from the tibia, fat mass (adjusted for lean mass) was negatively associated with both trabecular and cortical density ($\beta = -14.6$ mg/mm^3 per sd, $p = 0.003$; $\beta = -7.7$ mg/mm^3 per sd, $p = 0.02$ respectively).

These results suggest that fat mass is negatively associated with volumetric bone density at 6 years old, independent of lean mass, despite positive associations with bone size.

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Introduction

Weight and body composition are major determinants of bone size and density throughout life, reflecting adaptation of skeletal model-

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Studies of children have yielded conflicting results with regard to the relationships between fat mass, and bone size, density and fracture risk. Thus some studies have shown positive relationships between fat mass and bone size[2,3], with others additionally demonstrating [4–7], suggesting a ptation to the excess ve shown associations which varied by the age and sex of the child and whether the relationships were assessed cross-sectionally or longitudinally [8,9]. Given the increasing prevalence of obesity and the epidemiological data suggesting a positive relationship between obesity and risk of fracture in children [10], it is important to understand these relationships more fully.

These data have been difficult to disentangle because of the imperfect correction for body size afforded by DXA, and the existence of few data from the use of pQCT. In this study we therefore aimed to evaluate the relationship between fat mass and bone size and volumetric density among pre-pubertal children within a narrow age range, recruited from a free-living population cohort, the Southampton Women's Survey (SWS) and who had undergone assessment with DXA and pQCT.

Methods

Participants

The Southampton Women's Survey is a prospective cohort study of 12,583 women aged 20–34 years recruited from the general population [11]. At enrolment the participants were characterised in detail in terms of diet, lifestyle, health, physical activity and anthropometric measurements. 3159 of these women were followed through a subsequent pregnancy and delivered a live born infant. The children are being followed and characterised at regular intervals. Of the 1268 eligible families contacted during the study period for a 6 year follow up 530 attended for DXA, forming the cohort presented in this paper.

6 year DXA assessment

The mother and child were invited to visit the Osteoporosis Centre at Southampton General Hospital for assessment of bone mass and body composition. At this visit written informed consent for the DXA scan was obtained from the mother or father. The child's height (using a Leicester height measurer, Seca Ltd, UK) and weight, using calibrated digital scales (Seca Ltd, UK) were measured. Whole body (including body composition) and lumbar spine scans were obtained, using a Hologic Discovery instrument (Hologic Inc., Bedford, MA, USA). To encourage compliance, a suitably bright sheet with appropriate pictures was laid on the couch and to help reduce movement artefact, the children were shown a suitable DVD. The total radiation dose for the scans were as followed: whole body (paediatric scan mode) 4.7 μSv, spine (L1–L4) 1.5 μSv and hip 7.3 μSv. The manufacturer's coefficient of variation (CV) for the instrument was 0.75% for whole body bone mineral density, and the experimental CV when a spine phantom was repeatedly scanned in the same position 16 times was 0.68%. All scans were checked for movement and clothing artefact resulting in 499 suitable for analysis.

pQCT

A consecutive subgroup of 172 children was invited back to the Osteoporosis Centre to have an additional assessment of bone mass using a pQCT peripheral quantitative computed tomography scanner (Stratec XCT 2000, Software version 6.00 B 00.61, threshold for cortical bone 710 mg/cm^3, Stratec Biomedical Systems, Birkenfeld, Germany) following the DXA visit. After written informed consent was obtained the child's lower leg length was measured from the medial malleolus to the tibial tuberosity in order to demarcate the correct scan position. The child was then asked to place their right lower leg into the pQCT instrument; the laser guidance system on the instrument was used to position the medial malleolus correctly. The foot was secured into place in order to reduce movement artefact. A suitable DVD was used in order to occupy the child and again reduce movement artefact.

A scout view was obtained to find the distal end of the tibia. A reference line was then placed; the 4% site was used to assess trabecular density and the 38% site for cortical density. The total radiation dose for the scans was 1.5 μSv. All scans were checked for movement, and excluded if the circumference was interrupted. 148 children underwent pQCT assessment. Of these, 132 scans were suitable for trabecular bone analysis (4% site); 125 were also suitable for analysis of cortical bone indices (38% site).

Statistical analysis

Bone outcomes from DXA at 6 years included: bone area (BA), bone mineral content (BMC), areal bone mineral density (aBMD) at the whole body minus head and lumbar spine. Bone indices from pQCT included total area, trabecular content and trabecular density at the

4% site; at the 38% site total area, cortical area, content, thickness and density were assessed, together with stress–strain index. Children were classified as either normal weight, overweight or obese using the method of Vidma et al. [12]. This classification incorporates height, weight, age and gender based on records derived from the 1990 British Growth Reference and the 2000 US CDC Growth Reference data, to give outcomes appropriate to growing children. All fat mass variables were positively skewed, and so were log-transformed. For ease of interpretation, and to allow comparison of relationships, these values, and those for lean mass variables, were converted to within group z-scores. T-test and Wilcoxon–Mann Whitney tests were used to explore differences in anthropometric characteristics, pQCT and DXA measurements between males and females. Linear regression models were fitted to explore the relationships between body composition and bone indices. Both age at DXA/pQCT and gender of the child were associated with bone indices, hence all bone indices were adjusted for age at scan, and gender. All analyses were also conducted unadjusted for gender, but incorporating a gender-predictor interaction term to explore the role of the child's sex in potentially modifying any relationships observed. Since more adipose children also tend to have greater lean mass (more muscle is required to enable locomotion in a heavier individual), and lean mass may have a positive effect on bone through loading, lean mass was considered to be a potential mechanistic mediator in any relationship between fat and bone. Analyses were therefore conducted unadjusted and adjusted for lean mass. Statistical analyses were performed using Stata 11.0 (Statacorp, Texas, USA).

The Southampton Women's Survey was approved by the Southampton and South West Hampshire Local Research Ethics Committee. Written consent was obtained from parents/carers of all participants.

Results

Characteristics of the children

Analyses are based on 499 children with complete DXA data at 6 years. Table 1 summarises the characteristics of the children. Despite similar height and weight at age 6 years, there were differences in bone indices by gender. Additionally, girls had a greater mean total fat mass compared with the boys (p<0.0001). 395 children were of normal weight (equivalent to adult BMI<25 kg/m^2), 50 were overweight (equivalent to adult BMI between 25 and 30 kg/m^2) and 17 were obese (equivalent to adult BMI>30 kg/m^2). All, apart from 18 children were of white Caucasian ethnicity. There was no difference in the anthropometric measures at birth and at age 1 year between those children who did or did not participate in this study; however study participants' mothers tended to be of higher social class (p=0.004) and were less likely to smoke (p=0.03). The subgroup of children who underwent pQCT were slightly younger than the overall group who underwent DXA (6.5 years versus 6.6 years in the overall DXA group, p<0.01), but otherwise were broadly similar.

Relationships between body composition and bone mass

Table 2 summarises the relationships between body composition and bone indices. Both total fat mass and total lean mass were positively associated with whole body minus head BA, BMC and aBMD. When lean mass was included in regression models, these relationships were somewhat attenuated, but remained statistically significant; the associations between fat mass and bone indices at the lumbar spine became non-significant after inclusion of lean mass. There was evidence of gender differences in the relationships between lean adjusted fat mass and the bone outcomes, which were stronger in male than female children (p value for the lean adjusted fat mass–gender interaction terms with whole body BA, BMC, aBMD all <0.05). Similar

Table 1
Childhood characteristics among the 253 boys and 246 girls at age 6 years.

Characteristic	Boys (n = 253)	Girls (n = 246)	P difference
Gestational age, weeks (median, IQR)	40.0 (38.9–40.9)	40.1 (39.1–41)	0.1
Birthweight, g (mean, sd)	3483.9 (525.3)	3416.6 (524.6)	0.16
Age at pQCT, years (mean, sd)	6.8 (0.2)	6.7 (0.2)	0.19
Age at DXA, years (mean, sd)	6.6 (0.2)	6.6 (0.2)	0.7
Height, cm (mean, sd)	120.4 (4.6)	120 (5.5)	0.32
Weight, kg (median, IQR)	22.8 (21.2–24.9)	23.2 (21.2–25.8)	0.23
BMI category			
Normal [n (%)]	215 (90.34)	180 (80.36)	
Overweight [n (%)]	18 (7.56)	32 (14.29)	
Obese [n (%)]	5 (2.10)	12 (5.36)	0.009
WB BMC, g (mean, sd)	531.7 (71.1)	532.3 (72.8)	0.92
WB bone area, cm^2 (mean, sd)	893.8 (62.3)	901.5 (67.0)	0.18
WB aBMD, g/cm^2 (mean, sd)	0.6 (0.05)	0.6 (0.05)	0.28
Total fat mass, g (median, IQR)	4605.1 (3795.2–5524.1)	5937.3 (4856.8–7518.2)	<0.0001
Total lean mass, g (median, IQR)	17604.7 (16270.6–18940.3)	16659.7 (15105.8–18055.1)	0.0001
LS BMC, g (mean, sd)	18.1 (2.8)	17.7 (2.7)	0.07
LS bone area, cm^2 (mean, sd)	34.0 (3.1)	32.3 (3.2)	<0.0001
LS aBMD, g/cm^2 (mean, sd)	0.53 (0.06)	0.55 (0.06)	0.01
4% total area mm^2 (mean, sd)	682.1 (101)	675 (93.2)	0.67
4% trabecular content mg/mm slice (mean, sd)	100.1 (27.2)	103.2 (22.5)	0.48
4% trabecular density mg/mm^3 (mean, sd)	321.1 (58.4)	337.8 (51.2)	0.08
38% area mm^2 (mean, sd)	213.2 (31.8)	217.8 (31)	0.42
38% cortical content mg/mm slice (mean, sd)	123 (17.7)	121 (17.2)	0.51
38% cortical density mg/mm^3 (mean, sd)	1036.4 (35.6)	1038.2 (33.7)	0.77

WB = whole body minus head; LS = lumbar spine; BA = bone area; BMC = bone mineral content; aBMD = areal bone mineral density; 4% and 38% tibial sites from pQCT.

gender differences were observed in the associations between lean-adjusted fat mass and bone indices at the lumbar spine.

Relationships between body composition and pQCT derived volumetric bone indices

The results from the subgroup of 132 children who had pQCT data available for the tibia are shown in Table 3. There was a negative relationship between total fat mass and cortical density and a suggestion of a negative association with trabecular density. After adjustment for lean mass, total fat was negatively associated with both trabecular and cortical density. Fat mass adjusted for lean mass was associated positively with total and cortical area but not cortical thickness or stress–strain index at the 38% site. When the pQCT outcomes were adjusted for the height of the child at six years, the relationships were broadly similar, but the association between total fat and total area at the 4% site became attenuated (unadjusted β = 26 mm^2/sd vs adjusted β = 7 mm^2/sd) and statistically non-significant (p = 0.3). The smaller number of children with pQCT than DXA assessments offered reduced power to explore interactions with the sex of the child but there was evidence of an interaction between lean adjusted fat and gender with trabecular density (p value for lean

adjusted fat–gender interaction term = 0.006). This suggested stronger associations between lean adjusted total fat mass and trabecular density in the male than female children.

Discussion

In this pre-pubertal, free-living population, fat mass, adjusted for lean mass, was associated positively with bone size but negatively with true volumetric density assessed by pQCT, across the whole fat mass distribution.

We recruited children from a free-living population cohort and used objective measures of body composition and bone size and density. However, there are several limitations to our study. We were only able to study a proportion of the original cohort. However the children who underwent the 6 year assessment did not differ at birth or 1 year old from those who did not. Mothers of children who underwent 6 year assessment were broadly similar to mothers of those children who did not, but were more likely to be of higher social class and less likely to smoke. However, as the analysis is based on internal comparisons it is difficult to envisage how this would have spuriously shown an association between fat mass and bone size and density. The study population included a very small number of non-

Table 2
The relationships between body composition at age 6 years and whole body and lumbar spine bone mass.

	Whole body			Spine		
	BA, cm^2	BMC, g	aBMD, g/cm^2	BA, cm^2	BMC, g	aBMD, g/cm^2
	β (CI)	β (CI)	β (CI)	β (CI)	β (CI)	β (CI)
Total fat (sd)	18.5 (13.2,23.8)[***]	27.5 (21.9,33.2)[***]	0.02 (0.01,0.02)[**]	0.5 (0.2,0.7)[***]	0.5 (0.2,0.7)[***]	0.007 (0.002,0.01)[**]
Total lean-adjusted fat (sd)	6.9 (2.4,11.4)[**]	11.4 (7.6,15.3)[***]	0.008 (0.006,0.01)[***]	−0.1 (−0.4,0.1)[*]	−0.1 (−0.3,0.03)	−0.002 (−0.007,0.002)
Total lean (sd)	39.2 (34.1,42.9)[***]	55.1 (50.7,58.4)[***]	0.04 (0.03,0.04)[***]	1.9 (1.6,2.1)[***]	2.0 (1.8,2.1)[***]	0.03 (0.02,0.03)[***]

Tables show regression coefficient and 95% CI.
BA = bone area; BMC = bone mineral content; aBMD = areal bone mineral density.
[***] p<0.001.
[**] p<0.01.
[*] p<0.05.

Table 3
Relationships between body composition (adjusted for age at DXA and sex) and tibial PQCT measurements (adjusted for age at PQCT and sex).

	4% total area mm²	4% trabecular content mg/mm slice	4% trabecular density mg/mm³	38% total area mm²	38% cortical area mm²	38% cortical thickness mm	38% cortical content mg/mm slice	38% cortical density mg/mm³	38% stress–strain index
Total fat (sd)	25.6(10.2,41.1)**	2 (−2.1,6.2)	−6.9 (−16.2,2.4)	12.7 (7.9,17.4)***	7.0 (4.5,9.5)***	0.1 (0.0,0.1)*	6.4 (3.8,8.9)***	−7.5 (−13.4,−1.7)*	30.2 (16.3,44.0)***
Total fat (adjusted for total lean) (sd)	0.1(−11.8,12.0)	−3.9 (−7.5,−0.2)*	−14.6 (−24.1,−5.1)**	6.8 (2.3,11.2)**	3.2 (1.1,5.3)**	0 (−0.0,0.1)	2.4 (0.3,4.6)*	−7.7 (−14.2,−1.3)*	10 (−2.2,22.3)
Total lean (sd)	69.3(58.0,80.6)***	14.5 (11.0,18.0)***	15.2 (6.0,24.5)**	18.6 (14.1,23.1)***	11.4 (9.2,13.5)***	0.2 (0.1,0.2)***	11.5 (9.3,13.6)***	−2.8 (−9.2,3.6)	57.6 (45.6,69.7)***

Tables show regression coefficient and 95% CI.
*** p<0.001.
** p<0.01.
* p<0.05.

white Caucasian children and therefore it is uncertain whether our findings may be generalisable across these other ethnic groups. Secondly we used DXA to measure bone mass. This technique is associated with technical limitations in children. Measurement of bone mineral in young children is hampered by their tendency to move and also by their low absolute BMC. However, we used specific paediatric software, and movement artefact was modest and uniform across the cohort; those few children with excessive movement were excluded from the analysis. DXA measures of bone mass have been shown to correlate well with whole body calcium content in ashing studies of piglets [13,14]. Finally, we used a number of adjustments in the analyses, for example adjusting fat mass for lean mass. There is a biological rationale for this approach, as described in the methods, but as a result of co-linearity between measurements, it is possible that some analyses were over-adjusted; our conclusions are supported, however, by the results from the unadjusted analyses.

Children who are overweight have approximately a twofold increased risk of forearm fractures compared with controls [15]. A recent study has shown that among obese children with a history of fracture, lumbar spine bone mineral apparent density was reduced by 2–3 sd compared with non-obese children with a history of fracture [16]. Thus at least part of the increased risk of fracture in obese children may be mediated via reduced bone density rather than other factors such as increased risk of falling. Our findings are in accord with some, but not all, studies of pre-pubertal children using DXA and pQCT. Thus fat mass adjusted for lean mass was positively associated with whole body bone area and bone mineral content in a large cohort in the South of England [2,3]. Volumetric density was not reported in this study however. Other studies with DXA have shown children with higher fat mass to have reduced BMC [4–6] for their body size. In a cohort of 239 children, aged 3 to 5 years old, percentage fat mass was positively associated with bone size but negatively with volumetric density measured by pQCT at the tibia [8]. A more recent study from the same group examined cross-sectional and then longitudinal relationships between body composition and pQCT measured bone indices. In this cohort of 370 children, aged 8 to 18 years, body composition was assessed by DXA at baseline and children were followed up with pQCT up to 90 months later [9]. In contrast to our study, pQCT measurements were obtained at the radius, a non-weight-bearing site, but longitudinally at the 4% site there were negative relationships between percentage fat mass and volumetric density. Interestingly in this study cross-sectional and some longitudinal relationships between fat mass and bone size were also negative, suggesting possible discordant effects of fat mass on upper and lower limbs (perhaps indicating differential importance of endocrine vs. mechanical mechanisms on non weight bearing and weight bearing limbs). This study also raises the possibility of differential influences of fat over time on childhood growth.

We observed that the relationships between lean adjusted total fat mass and the DXA indices and trabecular density measured by pQCT appeared stronger in the boys than in the girls. There are very few data in the literature pertaining to gender differences in the relationships between body composition and bone measures, particularly in young children. Associations between total fat mass and BMC measured at the lumbar spine, hip and radius appeared stronger in boys than girls in one population based study in children aged 10 to 17 years [17]. A larger study of 926 children aged 6 to 18 years, found similar relationships between total fat mass and bone mineral content in boys and girls before puberty but only in girls after puberty [18]. A further study observed opposing influences of age and menache on the fat-bone relationship in female children [9], supporting the notion that hormonal factors such as oestrogen might be important here, but clearly further work will be needed to elucidate any potential mechanisms that might underlie these observations.

There are several mechanisms whereby obesity might influence bone size and density: firstly by directly applying a greater load to the

skeleton; secondly via an increase in compensatory muscle mass and thirdly via modulation of physiological and biochemical parameters. The first two of these mechanisms would suggest a positive relationship between fat mass and bone and perhaps could explain the positive relationships with bone size, but not the negative associations with volumetric density. Additionally we found that the positive associations between fat mass and bone size and the negative associations between fat mass and volumetric density persisted after adjustment for lean mass, suggesting that the relationships were not mediated by muscle mass.

The emerging evidence that fat is not an inert tissue, but a highly active endocrine organ, yields some additional possible explanations. Adipocytes produce leptin, a peptide hormone involved in the regulation of fat metabolism and appetite through hypothalamic mechanisms [19]. Recent work in animals has suggested that the primary effect of leptin on bone formation is negative via hypothalamic action on the sympathetic nervous system [20]. How this relates to mechanisms in humans is as yet unclear. Conversely leptin may push mesenchymal stem cells towards differentiation to osteoblasts rather than adipocytes [21,22] and leptin receptors have been found on osteoblasts, chondrocytes and bone marrow stromal cells [23]. Thus it is possible that leptin may explain some of the relationship between fat mass and bone, both positive and negative. Adiponectin is another hormone released by adipocytes; in contrast to leptin it is negatively related to overall fat mass. Adiponectin is associated with increased insulin sensitivity and improved glucose tolerance. A recent study from a large UK cohort related adiponectin, measured at 9.9 years, cross-sectionally to bone indices measured by DXA, and longitudinally to those measured by pQCT at 15.5 years [24]. The direct relationships between fat mass and volumetric density were not reported but total fat mass was negatively related to adiponectin concentration, which in turn negatively predicted volumetric density at 15.5 years. It seems unlikely, therefore, that adiponectin could explain negative relationships between fat mass and volumetric bone density. Insulin has been shown to have positive effects on bone in animal studies [25], with insulin resistance (and higher levels of insulin, as might be found in obesity) associated with increased BMD [26–28] and reduced fracture risk in humans [29]. Finally, recent work has suggested a role for peroxisome proliferator-activated receptors (PPARs) in the regulation of bone mass; reduced osteoblast function [30,31], increased osteoclastogenesis [32] and altered adipocyte/osteoblast differentiation [33] have been demonstrated in animal studies; thiazolidinedione drugs, which activate PPAR-gamma, have been shown to increase fracture risk [34]. Subtypes of these nuclear receptors also have a role in regulating insulin sensitivity and lipid metabolism [35], and thus are likely to relate to obesity, but there are currently insufficient data to allow detailed conclusions regarding any bone-related role in humans to be made.

In summary we have demonstrated that increased lean-adjusted fat mass is related to increased bone size but decreased volumetric bone density at the tibia. These results suggest that there is a negative relationship between total fat mass and volumetric density of the tibia across the distribution of fat mass, independent of lean mass. Given the importance of peak bone mass for future fracture risk, obesity in childhood could be a major target for public health interventions aimed at optimising bone health.

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Conflict of interest

All authors report no conflict of interest.

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