JOURNAL OF BONE AND MINERAL RESEARCH
Volume 24, Number 3, 2009
Published online on November 3, 2008; doi: 10.1359/JBMR.081109

How Does Body Fat Influence Bone Mass in Childhood? A Mendelian Randomization Approach

Nicholas J Timpson,[1] Adrian Sayers,[2] George Davey-Smith,[1] and Jonathan H Tobias[2]

ABSTRACT: Fat mass may be a causal determinant of bone mass, but the evidence is conflicting, possibly reflecting the influence of confounding factors. The recent identification of common genetic variants related to obesity in children provides an opportunity to implement a Mendelian randomization study of obesity and bone outcomes, which is less subject to confounding and several biases than conventional approaches. Genotyping was retrieved for variants of two loci reliably associated with adiposity (the fat mass and obesity-related gene *FTO* and that upstream of the *MC4R* locus) within 7470 children from the Avon Longitudinal Study of Parents and Children (ALSPAC) who had undergone total body DXA scans at a mean of 9.9 yr. Relationships between both fat mass/genotypes and bone measures were assessed in efforts to determine evidence of causality between adiposity and bone mass. In conventional tests of association, both with and without height adjustment, total fat mass was strongly related to total body, spinal, and upper and lower limb BMC (ratio of geometric means [RGM]: 1.118 [95% CI: 1.112, 1.123], 1.110 [95% CI: 1.102, 1.119], 1.101 [95% CI: 1.093, 1.108], 1.146 [95% CI: 1.143, 1.155]; $p < 10^{-10}$ [adjusted for sex, height, and sitting height]). Equivalent or larger effects were obtained from instrumental variable (IV) regression including the same covariates (1.139 [95% CI: 1.064, 1.220], 1.090 [95% CI: 1.010, 1.177], 1.142 [95% CI: 1.049, 1.243], 1.176 [95% CI: 1.099, 1.257]; $p = 0.0002$, 0.03, 0.002, and 2.3^{-6} respectively). Similar results were obtained after adjusting for puberty, when truncal fat mass was used in place of total fat, and when bone area was used instead of bone mass. In analyses where total body BMC adjusted for bone area (BA) was the outcome (reflecting volumetric BMD), linear regression with fat mass showed evidence for association (1.004 [95% CI: 1.002, 1.007], $p = 0.0001$). IV regression also showed a positive effect (1.031 [95% CI: 1.000, 1.062], $p = 0.05$). When *MC4R* and *FTO* markers were used as instruments for fat mass, similar associations with BMC were seen to those with fat mass as measured by DXA. This suggests that fat mass is on the causal pathway for bone mass in children. In addition, both directly assessed and IV-assessed relationships between fat mass and volumetric density showed evidence for positive effects, supporting a hypothesis that fat effects on bone mass are not entirely accounted for by association with overall bone size.
J Bone Miner Res 2009;24:522–533. Published online on November 3, 2008; doi: 10.1359/JBMR.081109

Key words: Avon Longitudinal Study of Parents and Children, BMD, DXA, *FTO*, *MC4R*

INTRODUCTION

There is considerable interest in understanding the factors that influence skeletal development, because this may enable population-based interventions aimed at reducing the burden of osteoporotic fractures in later life by optimizing bone mass accrual in childhood. Weight, body composition, and particularly lean mass are among the strongest determinants of bone mass throughout life, largely reflecting adaptation of skeletal modeling to loading. However, whether fat mass affects skeletal development independently of lean mass remains controversial. For example, obese children have been reported to have a lower bone mass for a given weight in several previous studies.[1–5] In contrast, in a study in which indices of proximal femur geometry were derived from hip DXA scans in overweight adolescents, fat mass was not found to influence any skeletal parameter independently of lean mass.[6] In a study of 18 obese and 30 nonobese children, bone age in the former group was more advanced, but BMD was similar.[7]

Cohort studies of the relationship between fat and bone mass in children and young adults have likewise yielded conflicting results. In 1068 men ~19 yr of age, fat mass was positively correlated with tibial cross-sectional area as assessed by pQCT, whereas a negative association was observed at the radius, suggesting that adipose tissue acts to stimulate growth of weight-bearing bones only.[8] On the other hand, in the Avon Longitudinal Study of Parents and Children (ALSPAC), fat mass was positively related to bone mass as reflected by BMC in 3032 children at age ~10 yr as measured at the total body and upper and lower limbs.[9] In contrast, in a study based on 300 subjects 13–21 yr of age, fat mass was not found to be related to either leg

The authors state that they have no conflicts of interest.

[1]MRC Centre for Causal Analyses in Translational Epidemiology, Department of Social Medicine, University of Bristol, Bristol, United Kingdom; [2]Department of Clinical Science at North Bristol, University of Bristol, Bristol, United Kingdom.

or lumbar spine bone mass or femoral or spinal cross-sectional area as measured by pQCT.[10] These apparently conflicting reports into the relationship between fat and bone mass in childhood are mirrored by findings in adults.[11]

A possible explanation for these conflicting results is that the relationship between fat and bone mass is subject to confounding, which distinct studies may adjust for to differing degrees. Diet, physical activity, socio-economic factors, puberty, lean mass, and illness are among many factors that may influence both fat and bone mass and may therefore act as possible confounding factors. In terms of the true nature of any functional relationship between fat and bone mass in childhood, theoretically, fat might exert both positive and negative effects. For example, adipose tissue is known to express aromatase enzymes that convert steroid precursors to estrogen, which has variously been reported to stimulate[12] and suppress periosteal[13] bone growth in childhood. Furthermore, increased leptin levels secondary to higher fat mass have been suggested to mediate the negative association between fat mass and periosteal growth observed at non–weight-bearing sites.[8] Conversely, fat mass may stimulate bone growth through a direct mechanical action of increased load,[14] by association with increased lean mass that occurs in obese subjects,[14] by association with the secretion of bone active hormones from pancreatic β cells,[15] or by an indirect action on timing of pubertal events.[16]

Mendelian randomization, whereby genetic variation associated with the risk exposure of interest is used as a nonconfounded proxy for that exposure,[17,18] represents a potential approach for nonconfounded assessment of the relationships that may exist between variables such as fat and bone mass in childhood. The application of this approach has been made possible by the recent discovery of common markers related to the *FTO* and *MC4R* genes, which can act as independent genetic associates of a small, but detectable, portion of the variance in human adiposity.[19,20] In this study, we aim to use a Mendelian randomization approach to explore the relationship between fat and bone mass in childhood, by using *FTO* and *MC4R* markers as instrumental variable(s) (IV) for fat mass.

MATERIALS AND METHODS

Study population

ALSPAC is a prospective study, which recruited pregnant women with expected delivery dates between April 1991 and December 1992 from Bristol, UK. The cohort is population based and broadly representative at the point of recruitment.[21] Individuals of known nonwhite ethnic origin were excluded from all analyses. DNA was collected from mothers and children as described previously.[22] Of these births, 13,988 were alive at 12 mo. This study is based on results for total body DXA scans obtained at a research clinic to which the whole cohort was invited at mean age of 9.8 yr. Ethical approval was obtained from the ALSPAC Law and Ethics Committee and local research ethics committees. Parental consent and child's assent were obtained for all measurements made.

Measurement of height, weight, and DXA-derived parameters

Of the 7725 children who attended the research clinic at age 9, 7470 agreed to undergo a whole body DXA scan, which was performed using a Lunar Prodigy with pediatric scanning software. At the same time, sitting and standing height were measured using a Harpenden Stadiometer, as was weight using a Tanita Body Fat Analyser and leg length. After exclusion of scans with anomalies (e.g., movement artefacts, artefacts caused by jewelry), complete scans were available for 7336 children. These were evaluated and reanalyzed as necessary, to ensure that borders between adjacent subregions were optimally placed. Fat mass was expressed as total and truncal fat mass. Bone variables comprised whole body minus head (hereafter referred to as total body), upper limb and lower limb BMC, bone area (BA), and areal BMD (obtained by dividing BMC by BA). A more rigorous method for adjusting for skeletal size, area-adjusted BMC (ABMC), was also derived to provide a measure of volumetric BMD, by using linear regression to adjust BMC for BA. The CV for total body BMD was 0.8%, based on analysis of results from 122 children who had two scans performed on the same day. DXA variables for the spine were derived from spine subregional analysis of whole body scans, excluding scans showing any spinal curvature, as previously described.[23]

Genotyping

Genotyping of the *FTO* SNP rs9939609 was undertaken in 8480 children.[19] Genotyping was performed by KBiosciences (Hoddesdon, UK) using their own system of fluorescence-based competitive allele-specific PCR (KASPar). Details of assay design are available from the KBiosciences website (http://www.kbioscience.co.uk). Genotyping of the *MC4R* SNP rs17782313 was undertaken in the same manner within the ALSPAC cohort. For this variant, 9024 individuals had recorded genotype information. Together with DXA data for both rs9939609 and rs17782313, this yielded an approximate working sample size of $n = 5300$ individuals for total body measures and 3000 for spine values.

Other variables

Sex was obtained from birth notifications. A puberty questionnaire was completed by the child's carer (usually the child's mother), which included questions on pubertal stage; Tanner staging subsequently was based on pubic hair distribution.[24] Maternal education was used as a proxy measure for social class as previously described.[25]

Statistical analysis

Relationships with the *FTO* and *MC4R* loci, including adjustments for sitting height, height, and sex, were assessed using linear regression. In efforts to account for non-Gaussian traits, log transformations were undertaken, and results were shown as geometric means and ratios of change between groups. Analyses assumed an additive genetic model[19] and included analysis of the variants

rs9939609 (*FTO)* and rs17782313 (*MC4R*) with basic anthropometric traits and measures of both fat mass and bone composition/area. In all basic analyses, twins and non-Europeans were excluded, and covariates were centered for the generation of adjusted means by groups defined by either quantile of fat mass or genotype. Hardy-Weinberg equilibrium was tested using the exact test using the command ''genhwi'' (part of the ''genassoc'' suite of commands, www-gene.cimr.cam.ac.uk/clayton/). All analyses were undertaken using the data analysis software STATA, version 10.

In a Mendelian randomization framework, we used IV methods to obtain estimates of the association between fat mass and bone-related continuous outcomes.[26] We used two-stage least squares to fit the IV models in the main analyses.[27] Within this model, we used both of the independent, fat mass–associated loci (rs9939609 and rs17782313) to explain the largest, nonconfounded, and nonreverse caused portion of variance in fat mass possible. Within these models, sex, sitting height, and height were included to generate adjusted observational estimates of fat mass effect and equivalent estimates derived from IV analysis. This aimed to provide a comparison of best observational relationships between fat mass and bone measures and to compare these against those known to be uninfluenced by confounding or reverse causation.

We compared the IV estimates to those from ordinary linear regression using the Durbin form of the Durbin-Wu-Hausman statistic but checked results using the Hansen J statistics (limited information maximum likelihood) and the Anderson-Rubin statistics (generalized method of moments). We examined *F*-statistics from the first-stage regressions to evaluate the strength of the instruments. Values >10 are often taken to indicate sufficient strength to ensure the validity of IV methods.[28,29]

RESULTS

Linear regression showed an expected, positive association between total fat mass tertile, as measured by DXA, and total body, spinal, and upper and lower limb BMC after adjustment for sitting height, height, and sex (Tables 1 and 2). For total body, spine, and upper and lower limb measurements, respectively, the ratios of geometric means (RGMs) between tertiles total fat mass were 1.118 [95% CI: 1.112, 1.123], 1.110 [95% CI: 1.102, 1.119], 1.101 [95% CI: 1.093, 1.108], and 1.146 [95% CI: 1.143, 1.155] ($p < 10^{-10}$). At all sites, fat mass had a similar association with BA to that seen for BMC. These effects are similar to those reported previously in this cohort.[9] In addition, fat mass was positively related to both spinal and total body ABMC, again after adjustment for sex, sitting height, and height (RGM: 1.004 [95% CI: 1.002. 1.007], $p = 0.0001$; and 1.041 [95% CI: 1.035, 1047], $p < 10^{-10}$).

Comparison of anthropometric and DXA data between those with and without genetic data showed no substantive differences between these groups with the exception of fat mass, which was slightly higher in those not genotyped (Table 3). Allele frequencies for the loci rs99439609 and rs17782313 (*FTO* and *MC4R,* respectively) have been

TABLE 1. INSTRUMENTAL VARIABLE ANALYSIS OF THE EFFECT OF FAT MASS TERTILE ON TOTAL BODY BONE VARIABLES WITHIN THE ALSPAC COHORT (ADJUSTED)

	Tertile of fat mass			*Effect (linear regression)*	*IV effect (IV regression)*	*ffirst*	*p*	*p′*	*WH_p″*
Bone measure (n)	*0*	*1*	*2*						
BMC (g) (*n*_reg = 6411) (*n*_IV = 4957)	826.62 (822.6, 830.7)	860.5 (856.6, 864.4)	940.1 (935.5, 944.6)	1.118 (1.112, 1.123)	1.139 (1.064, 1.220)	37.82	$<10^{-10}$	0.0002	0.6
BA (cm^2) (*n*_reg = 6411) (*n*_IV = 4957)	1086.4 (1083.1, 1089.6)	1112.7 (1109.6, 1115.8)	1185.1 (1181.6, 1188.7)	1.079 (1.076, 1.083)	1.072 (1.029, 1.118)	37.82	$<10^{-10}$	0.001	0.8
BMD (g/cm^2) (*n*_reg = 6411) (*n*_IV = 4957)	0.761 (0.759, 0.763)	0.773 (0.772, 0.775)	0.793 (0.791, 0.795)	1.035 (1.033, 1.038)	1.062 (1.025, 1.101)	37.82	$<10^{-10}$	0.001	0.1
ABMC (cm^2) (*n*_reg = 6411) (*n*_IV = 4957)	890.5 (888.7, 892.3)	893.9 (892.2, 895.5)	895.3 (893.6, 897.1)	1.004 (1.002, 1.007)	1.031 (1.000, 1.062)	37.82	0.0001	0.05	0.07

Means (95% CI) and effects derived from regression of DXA scan data on tertiles of total fat mass, values adjusted for sex, height, and sitting height. IV effect taken using both *MC4R* (rs17782313) and *FTO* (rs9939609) as instruments for truncal fat mass, adjusting for sex (centered). Means are back-transformed, presented as geometric means, and effects are shown as ratios of geometric means. *p* represents *p* value from basic linear regression of DXA scan variables on tertile of truncal fat mass, *p′* represents that of instrumental variable analysis using *FTO* and *MC4R* genotypes as instruments for truncal fat mass, and p″ represents the Wu/Hausmann test for difference between these estimates.

TABLE 2. INSTRUMENTAL VARIABLE ANALYSIS OF THE EFFECT OF FAT MASS TERTILE ON SPINE AND UPPER AND LOWER LIMB BONE VARIABLES WITHIN THE ALSPAC COHORT (ADJUSTED)

	Tertile of fat mass								
Bone measure (n)	*0*	*1*	*2*	*Effect (linear regression)*	*IV effect (IV regression)*	*fffirst*	*p*	*p′*	*WH_p″*
Spine BMC (g) (*n*_reg = 3572) (*n*_IV = 2744)	72.99 (72.48, 73.51)	75.05 (74.57, 75.53)	82.08 (81.53, 82.65)	1.110 (1.102, 1.119)	1.090 (1.010, 1.177)	34.32	$<10^{-10}$	0.03	0.6
Spine BA (cm^2) (*n*_reg = 3572) (*n*_IV = 2744)	97.51 (97.14, 97.89)	98.65 (98.31, 98.99)	102.64 (102.27, 103.02)	1.045 (1.040, 1.049)	1.041 (0.998, 1.086)	34.32	$<10^{-10}$	0.06	0.8
Spine BMD (g/cm^2) (*n*_reg = 3572) (*n*_IV = 2744)	0.749 (0.745, 0.752)	0.761 (0.757, 0.764)	0.800 (0.796, 0.804)	1.063 (1.057, 1.069)	1.047 (0.990, 1.109)	34.32	$<10^{-10}$	0.1	0.6
Spine ABMC (g) (*n*_reg = 3572) (*n*_IV = 2744)	76.54 (76.12, 76.95)	76.96 (76.59, 77.34)	79.55 (79.14, 79.97)	1.041 (1.035, 1.047)	1.020 (0.961, 1.084)	34.32	$<10^{-10}$	0.5	0.5
Upper limb BMC (g) (*n*_reg = 6411) (*n*_IV = 4957)	109.66 (109.01, 110.31)	115.42 (114.80, 116.05)	123.62 (122.90, 124.34)	1.101 (1.093, 1.108)	1.142 (1.049, 1.243)	37.82	$<10^{-10}$	0.002	0.4
Lower limb BMC (g) (*n*_reg = 6411) (*n*_IV = 4957)	404.96 (402.99, 406.94)	432.14 (430.20, 434.09)	478.51 (476.20, 480.83)	1.149 (1.143, 1.155)	1.176 (1.099, 1.257)	37.82	$<10^{-10}$	$2.30\text{x}10^{-6}$	0.5

Means (95% CI) and effects derived from regression of DXA scan data on tertiles of total fat mass, values adjusted for sex, height, and sitting height. IV effect taken using both *MC4R* (rs17782313) and *FTO* (rs9939609) as instruments for truncal fat mass, adjusting for sex (centered). Means are back-transformed, presented as geometric means and effects are shown as ratios of geometric means. *p* represents *p* value from basic linear regression of DXA scan variables on tertile of truncal fat mass, *p′* represents that of instrumental variable analysis using *FTO* and *MC4R* genotypes as instruments for truncal fat mass, and p″ represents the Wu/Hausmann test for difference between these estimates.

reported for this cohort elsewhere,[19] and both loci were seen to adhere to Hardy-Weinberg equilibrium within this population. As previously reported,[19,30] variation at the *FTO* and *MC4R* was consistently associated with fat mass as assessed through a series of body region–specific, DXA-derived measurements (Table 4).

In the analysis of measures of stature, which are strongly associated with bone mass, both sitting height and height showed strong relationships with fat mass (RGM: 1.023 [95% CI: 1.022, 1.024], $p < 10^{-10}$ and 1.024 [95% CI: 1.022, 1.025], $p < 10^{-10}$). Similarly, lean mass was strongly related to fat mass (RGM: 1.074 [95% CI: 1.070, 1.078], $p < 10^{-10}$). Socio-economic status, reflected by level of maternal education, which we previously found to be related to bone mass,[25] was also related to fat mass ($p = 0.003$; Table 5). In contrast to these results, the *FTO* and *MC4R* loci used in this study as proxies for fat mass measurement were unrelated to any of these factors (Table 6), with the exception of weak associations between the *FTO* marker, sitting height, and lean mass.

Variation at rs9939609 (*FTO*) and rs17782313 (*MC4R*) was positively related to total body BMC (Table 7), as found previously.[30] These associations partly reflected genetic effects on bone size. For example, total body BA showed a broadly similar relationship to total body BMC, with variation at rs9939609 and rs17782313 (RGM: rs9939609/BA, 1.008 [95% CI: 1.002, 1.013], $p = 0.006$ and rs9939609/BMC, 1.013 [95% CI: 1.005, 1.020], $p = 0.002$; rs17782313/BA, 1.007 [95% CI: 1.000, 1.013], $p = 0.04$ and rs17782313/BMC, 1.011 [95% CI: 1.002, 1.020], $p = 0.01$). Although there was some evidence that these genotypes were also related to total body ABMC (measure of volumetric BMD), these associations were relatively weak (RGM: rs9939609/ABMC, 1.002 [95% CI: 1.000, 1.003], $p = 0.07$ and rs17782313/ABMC, 1.002 [95% CI: 1.000, 1.004], $p = 0.07$).

Equivalent associations were observed with respect to regional BMC as measured at the spine and upper and lower limbs, with the exception that relationships between rs9939609 variation and spinal BMC and rs17782313 variation and lower limb BMC were relatively weak (RGM: 1.011 [95% CI: 1.000, 1.0211], $p = 0.05$ and 1.011 [95% CI: 1.001, 1.020], $p = 0.03$ respectively; Table 8). For spinal ABMC, effects were stronger for rs17782313 than for rs9939609 (RGM: 1.008 [95% CI: 1.003, 1.014], $p = 0.002$ and 1.002 [95% CI: 0.998, 1.007], $p = 0.4$, respectively).

In tests for association between *FTO* and *MC4R* genotypes and bone mass and other skeletal parameters, adjusting for total fat mass largely attenuated associations between these genotypes and total body, spinal, and upper and lower limb BMC and BA (Tables 9 and 10). The relatively weak associations between *FTO* and *MC4R* genotypes and total body ABMC described above remained after adjusting for fat mass (RGM: rs9939609/ABMC, 1.002 [95% CI: 1.000, 10.03], $p = 0.05$ and rs17782313/ABMC, 1.002 [95% CI: 1.000, 1.004], $p = 0.06$).

IV regressions based on *FTO* and *MC4R* genotypes showed similar associations with skeletal parameters to those found for measured fat mass. For total body, spinal, and upper and lower limb BMC, effects of a tertile increase in total fat mass as predicted by IV regression, shown as

TABLE 3. DESCRIPTIVE CHARACTERISTICS FOR KEY VARIABLES FROM THE ALSPAC COHORT

Variable (units)	*n*	*Geometric mean (with genotype data)*	*n*	*Geometric mean (without genotype data)*	*p(diff)*
Height (cm)	5283	139.4 (139.3, 139.7)	1662	139.3 (139.0, 139.6)	0.3
Sitting height (cm)	5325	73.25441 (73.17, 73.34)	1674	73.11 (72.96, 73.26)	0.1
Leg length (cm)	5325	66.26 (66.16, 66.37)	1674	66.21 (66.02, 66.40)	0.7
Total fat mass (g)	5330	7216.7 (7106.3, 7328.9)	1676	7443.7 (7233.5, 7660.1)	0.007
Truncal fat mass (g)	5330	2724.5 (2675.3, 2774.5)	1676	2819.4 (2725.6, 2916.5)	0.01
Total lean mass (g)	5330	24395.7 (24311.8, 24479.9)	1676	24257.0 (24100.1, 24414.9)	0.2
Total BMC (g)	5192	876.7 (872.0, 881.5)	1653	868.3 (859.5, 877.2)	0.2
Total BA (cm^2)	5192	1129.0 (1124.7, 1133.4)	1653	1122.8 (1114.8, 1130.8)	0.3
Total BMD (g/cm^2)	5192	0.777 (0.775, 0.778)	1653	0.773 (0.771, 0.776)	0.05
Total ABMC (g)	5192	893.6 (892.5, 894.6)	1653	892.6 (890.7, 894.6)	0.4
Spinal BMC (g)	2878	76.7 (76.13, 77.25)	932	76.09 (75.11, 77.08)	0.3
Spinal BA (cm^2)	2878	99.54 (99.11, 99.98)	932	99.18 (98.42, 99.94)	0.4
Spinal BMD (g/cm^2)	2878	0.770 (0.767, 0.773)	932	0.767 (0.762, 0.772)	0.3
Spinal ABMC (g)	2878	77.78 (77.53, 78.04)	932	77.68 (77.25, 78.11)	0.6
Upper limb BMC (g)	5192	116.5 (115.8, 117.2)	1653	115.1 (113.9, 116.3)	0.09
Lower limb BMC (g)	5192	438.3 (435.7, 440.8)	1653	435.4 (430.7, 440.2)	0.6

p values shown above are derived from a simple Student's *t*-test for the difference between arithmetic means.

RGM, were 1.139 [1.064, 1.220], 1.090[1.010, 1.177], 1.142 [1.049, 1.243], and 1.176 [1.099, 1.257] ($p = 0.0002$, 0.03, 0.002, and 2.3×10^{-6}, respectively; Tables 1 and 2). Overall, tests for difference between those estimates derived from adjusted observational associations and IV estimates did not yield consistent evidence for shifts in effect. If anything, IV estimates of fat mass effects on total and limb BMC, BMD, and ABMC were equal to or larger than those derived from observational analysis, suggesting possible negative confounding or overadjustment. The opposite was the case for spinal measurements. These differences were not statistically robust. Relationships between fat mass and bone parameters, and IV regressions, were also examined without height adjustment, as shown in Tables 11 and 12.

DISCUSSION

Previous studies of the relationship between fat and bone mass in childhood have yielded conflicting results, possibly reflecting an influence of confounding factors. In this study, we confirmed that *FTO* and *MC4R* genotypes, which are known to be independent determinants of fat mass,[19] are also related to bone mass.[20] Moreover, the relationship between these genetic markers and bone mass seem to be directed through their effects on fat mass, because the associations between *FTO* and *MC4R* genotypes and bone mass were largely attenuated by adjustment for fat mass. In subsequent IV analyses, in which markers of common variation in these loci were used as instruments for fat mass, a similar positive relationship with bone mass was observed to that between fat mass as measured by DXA and bone mass. Mendelian randomization approaches using IV analyses as described here has previously been used to make causal inferences in a range of epidemiological studies.[18] However, a noteworthy aspect of this study is that IV analyses were based on two independent genetic markers located on separate chromosomes.

Compared with measured fat mass, *FTO* and *MC4R* polymorphisms can be thought of as (and are shown to be) largely independent of factors that might confound the relationship with bone mass. As such, these findings suggest that at least part of the observed variance in fat mass is likely to bear a causal relationship with accrual of bone mass in childhood. However, genetic determinants of obesity may still be related to other variables, such as those derived from the possible pleiotropic action of *FTO* or *MC4R*. Although associations between fat and bone mass based on IV analyses are less likely to be explained by confounding factors, this possibility cannot be excluded completely. Nevertheless, to the extent that a causal relationship exists between fat and bone mass, results from IV analysis would seem to exclude reverse causality, whereby bone mass might influence fat mass, because it would seem unlikely that bone mass influences the risk of having genetic markers of obesity. This conclusion would seem to go against recent observations from animal studies suggesting that bone mass may be a determinant of fat mass, based on findings that osteoblast-derived osteocalcin stimulates adipocyte gene expression.[30]

The suggestion from our results that a causal relationship exists between fat and bone mass in childhood is consistent with our previous observations in the ALSPAC cohort of a strong association between fat and bone mass, despite adjusting for potential confounding factors such as height and lean mass.[9] Moreover, in longitudinal analyses, fat mass was found to predict gain in bone mass over the following 2 yr.[9] The magnitude of this fat mass effect, as confirmed by our Mendelian randomization study, is large enough to have significant implications for public health. For example, there was an ~1 SD difference in BMD between children whose fat mass was in the lower versus upper tertile, which, if translated to an adult population, equates with a 2-fold increase in fracture risk.[31] Therefore, population-based strategies intended to reduce fat mass in childhood would seem to run the risk of increasing fracture risk in later life, unless these can be combined with

TABLE 4. RELATIONSHIP BETWEEN GENOTYPES KNOWN TO BE ASSOCIATED WITH FAT MASS AND DXA SCAN RESULTS IN THE ALSAC COHORT

	Genotype			*Effect summary*	
Fat measure (n)	*0*	*1*	*2*	*Effect (linear regression)*	*p*
FTO total fat mass (g) (5282)	6857.9 (6691.9, 7028.0)	7311.2 (7155.0, 7470.9)	7883.0 (7593.6, 8183.4)	1.071 (1.048, 1.094)	$<10^{-10}$
FTO truncal fat mass (g) (5282)	2555.2 (2482.5, 2630.0)	2770.0 (2700.4, 2841.4)	3041.7 (2910.6, 3178.8)	1.090 (1.062, 1.117)	$<10^{-10}$
MC4R total fat mass (g) (5387)	7070.8 (6934.1, 7210.1)	7422.3 (7243.6, 7605.4)	7853.7 (7361.7, 8378.5)	1.052 (1.026, 1.078)	0.00008
MC4R truncal fat mass (g) (5387)	2653.7 (2593.4, 2715.3)	2829.9 (2749.9, 2912.3)	3003.2 (2783.0, 3240.9)	1.065 (1.034, 1.097)	0.00002
FTO total fat mass (g) (5282)*	6866.5 (6719.0, 7017.2)	7288.5 (7150.2, 7429.4)	7807.3 (7552.7, 8070.4)	1.065 (1.045, 1.086)	$<10^{-10}$
FTO truncal fat mass (g) (5282)*	2558.7 (2493.1, 2626.1)	2760.4 (2697.9, 2824.4)	3009.4 (2892.3, 3131.1)	1.083 (1.059, 1.108)	$<10^{-10}$
MC4R total fat mass (g) (5387)*	7049.3 (6928.5, 7172.1)	7434.5 (7275.8, 7596.6)	7693.2 (7265.0, 8146.6)	1.050 (1.027, 1.074)	0.00001
MC4R truncal fat mass (g) (5387)*	2644.7 (2590.7, 2699.9)	2835.1 (2762.9, 2909.1)	2935.1 (2741.0, 3143.0)	1.064 (1.036, 1.092)	4.6×10^{-6}

Means (95% CI) and effects derived from regression of DXA fat mass scan data on *FTO* (rs9939609) and *MC4R* (rs17782313) genotypes. Regression results are adjusted for sex (centered). Variables are log-transformed for analysis. Means are back-transformed, presented as geometric means, and effects are shown as ratios of geometric means.

* Analyses also adjusted for height at age 9.

TABLE 5. RELATIONSHIP BETWEEN FAT MASS AND BASIC ANTHROPOMETRY IN THE ALSAC COHORT

	Tertile of total fat mass			*Effect summary*	
Anthropometric trait (n) corrected	*0*	*1*	*2*	*Effect (linear/logistic regression)*	*p*
Height (cm) (6568)*	136.2 (135.945, 136.427)	139.6 (139.098, 139.337)	142.7 (142.455, 142.955)	1.024 (1.022, 1.025)	$<10^{-10}$
Sitting height (cm) (6622)*	71.59 (71.48, 71.71)	73.14 (73.02, 73.26)	74.91 (74.78, 75.03)	1.023 (1.022, 1.024)	$<10^{-10}$
Leg length (cm) (6622)*	64.62 (64.46, 64.77)	66.27 (66.11, 66.42)	67.81 (67.65, 67.97)	1.024 (1.023, 1.026)	$<10^{-10}$
Lean mass (g) (6629)*	22799.6 (22692.3, 22907.5)	24089.5 (23979.2, 24200.3)	26281.4 (26158.8, 26404.8)	1.074 (1.070, 1.078)	$<10^{-10}$
Mother's highest educational achievement	0.447 (0.428, 0.466)	0.428 (0.415, 0.440)	0.405 (0.386, 0.425)	0.907 (0.851, 0.967)	0.003

Means (95% CI) and effects derived from regression of anthropometric trait and social class data on tertile of total body fat mass (age 9). Regression results are adjusted for sex (centered). Means are back-transformed, presented as geometric means, and effects are shown as ratios of geometric means. Mother's highest educational achievement is a binary variable derived from the groups 0 = CSE + O level + vocational and 1 = a level + degree. Proportion in upper group is shown with OR (95% CI) for association.

* Log-transformed for analysis.

TABLE 6. RELATIONSHIP BETWEEN GENOTYPES KNOWN TO BE ASSOCIATED WITH FAT MASS AND BASIC ANTHROPOMETRY IN THE ALSAC COHORT

	FTO genotype			*Effect summary*	
Anthropometric trait (n)	*0*	*1*	*2*	*Effect (linear/logistic regression)*	*p*
Height (cm) (5337)*	139.3 (139.01, 139.57)	139.4 (139.18, 139.67)	139.6 (139.20, 140.04)	1.001 (0.999, 1.003)	0.2
Sitting height (cm) (5379)*	73.12 (72.99, 73.26)	73.26 (73.14, 73.38)	73.36 (73.15, 73.57)	1.002 (1.000, 1.003)	0.04
Leg length (cm) (5379)*	66.21 (66.04, 66.38)	66.20 (66.06, 66.35)	66.25 (65.99, 66.51)	1.000 (0.998, 1.002)	0.8
Lean mass (g) (5139)*	24255.8 (24122.3, 24390.0)	24393.9 (24275.9, 24512.5)	24580.8 (24375.2, 24788.1)	1.006 (1.002, 1.011)	0.009
Mother's highest educational achievement	0.432 (0.412, 0.453)	0.423 (0.409, 0 0.437)	0.413417 (0.387, 0.440)	0.960 (0.888, 1.040)	0.3
	MC4R genotype				
	0	*1*	*2*		
Height (cm) (5449)*	139.5 (139.23, 139.67)	139.3 (138.99, 139.53)	140.0 (139.28, 140.74)	1.000 (0.998, 1.002)	0.9
Sitting height (cm) (5491)*	73.20 (73.105, 73.31)	73.19 (73.06, 73.33)	73.52 (73.16, 73.88)	1.001 (0.999, 1.003)	0.4
Leg length (cm) (5491)*	66.27 (66.14, 66.41)	66.09 (65.93, 66.26)	66.58 (66.14, 67.03)	0.999 (0.997, 1.002)	0.7
Lean mass (g) (5249)*	24342.3 (24236.9, 24448.2)	24368.6 (24237.1, 24500.5)	24766.8 (24412.2, 25126.5)	1.004 (0.999, 1.010)	0.1
Mother's highest educational achievement	0.428 (0.411, 0.445)	0.42302 (0.405, 0.441)	0.419 (0.384, 0.456)	0.982 (0.896, 1.075)	0.7

Means (95% CI) and effects derived from regression of anthropometric trait data on *FTO* (rs9939609) and *MC4R* (rs17782313) genotypes. Regression results are adjusted for sex (centered). Means are back-transformed, presented as geometric means, and effects are shown as ratios of geometric means. Mother's highest educational achievement is a binary variable derived from the groups 0 = CSE + o level +vocational and 1 = a level + degree. Proportion in upper group is shown with OR (95% CI) for association.

* Log-transformed for analysis.

TABLE 7. RELATIONSHIP BETWEEN TOTAL BODY BONE VARIABLES AND FTO AND MC4R GENOTYPES WITHIN THE ALSPAC COHORT

	FTO genotype (n = 5282)					*MC4R genotype (n = 5387)*				
Bone measure (n)	*0*	*1*	*2*	*Effect (linear regression)*	*p*	*0*	*1*	*2*	*Effect (linear regression)*	*p*
BMC (g)	868.1 (860.5, 875.9)	878.9 (872.0, 885.8)	890.3 (878.3, 902.4)	1.013 (1.005, 1.020)	0.002	872.8 (866.7, 879.0)	880.3 (872.6, 888.1)	898.7 (878.0, 919.9)	1.011 (1.002, 1.020)	0.01
BA (cm^2)	1122.7 (1115.6, 1129.7)	1130.2 (1124.0, 1136.5)	1140.8 (1129.9, 1151.7)	1.008 (1.002, 1.013)	0.006	1126.2 (1120.6, 1131.8)	1132.2 (1125.2, 1139.2)	1145.7 (1127.0, 1164.7)	1.007 (1.000, 1.013)	0.04
BMD (g/cm^2)	0.773 (0.771, 0.776)	0.778 (0.776, 0.780)	0.780 (0.777, 0.784)	1.005 (1.002, 1.007)	0.0004	0.775 (0.773, 0.777)	0.778 (0.775, 0.780)	0.784 (0.778, 0.791)	1.004 (1.001, 1.008)	0.004
ABMC (g)	892.0 (890.3, 893.7)	894.3 (892.7, 895.8)	894.4 (891.7, 897.0)	1.002 (1.000, 1.003)	0.07	892.7 (891.4, 894.1)	893.7 (891.9, 895.4)	897.7 (893.1, 902.3)	1.002 (1.000, 1.004)	0.07

Means (95% CI) and effects derived from regression of DXA scan data on FTO (rs9939609) and MC4R (rs17782313) genotypes. Regression results are adjusted for sex (centered). Variables are log-transformed for analysis. Means are back-transformed, presented as geometric means, and effects are shown as ratios of geometric means.

TABLE 8. RELATIONSHIP BETWEEN SPINE AND UPPER AND LOWER LIMB BONE VARIABLES AND FTO AND MC4R GENOTYPES WITHIN THE ALSPAC COHORT

	FTO genotype (n= 2921 spine, 5282 limb)					*MC4R genotype (n= 2978 spine, 5387 limb)*				
Bone measure (n)	*0*	*1*	*2*	*Effect (linear regression)*	*p*	*0*	*1*	*2*	*Effect (linear regression)*	*p*
Spine BMC (g)	75.84 (74.944, 76.743)	77.20 (76.384, 78.018)	77.18 (75.801, 78.578)	1.011 (1.000, 1.021)	0.05	75.70 (74.982, 76.425)	77.78 (76.886, 78.694)	79.32 (76.892, 81.813)	1.026 (1.014, 1.038)	0.00003
Spine BA (cm^2)	99.01 (98.31, 99.72)	99.88 (99.25, 100.5)	99.91 (98.83, 101.0)	1.005 (0.999, 1.012)	0.09	98.93 (98.37, 99.50)	100.3 (99.56, 101.0)	100.7 (98.81, 102.6)	1.011 (1.004, 1.019)	0.002
Spine BMD (g/cm^2)	0.766 (0.761, 0.771)	0.773 (0.769, 0.777)	0.772 (0.765, 0.780)	1.005 (1.000, 1.011)	0.06	0.765 (0.761, 0.769)	0.776 (0.771, 0.781)	0.788 (0.775, 0.801)	1.014 (1.008, 1.021)	$9.2x10^{-6}$
Spine ABMC (cm^2)	77.54 (77.13, 77.96)	77.92 (77.55, 78.29)	77.78 (77.16, 78.41)	1.002 (0.998, 1.007)	0.4	77.51 (77.18, 77.84)	78.04 (77.64, 78.45)	79.11 (78.02, 80.22)	1.008 (1.003, 1.014)	0.002
Upper limb BMC (g)	115.4 (114.4, 116.5)	116.7 (115.7, 117.6)	118.4 (116.8, 120.1)	1.013 (1.004, 1.021)	0.003	115.8 (115.0, 116.7)	117.2 (116.1, 118.3)	119.4 (116.6, 122.3)	1.013 (1.004, 1.023)	0.005
Lower limb BMC (g)	433.6 (429.5, 437.7)	439.5 (435.9, 443.2)	446.0 (439.6, 452.4)	1.014 (1.006, 1.022)	0.0009	436.5 (433.2, 439.7)	439.9 (435.8, 444.0)	449.1 (438.2, 460.3)	1.011 (1.001, 1.020)	0.03

Means (95% CI) and effects derived from regression of DXA scan data on FTO (rs9939609) and MC4R (rs17782313) genotypes. Regression results are adjusted for sex (centered). Variables are log-transformed for analysis. Means are back-transformed, presented as geometric means, and effects are shown as ratios of geometric means.

TABLE 9. RELATIONSHIP BETWEEN TOTAL BODY BONE VARIABLES AND *FTO* AND *MC4R* GENOTYPES ADJUSTED FOR TOTAL FAT MASS WITHIN THE ALSPAC COHORT

	FTO genotype (n = 5282)					*MC4R genotype (n = 5387)*				
Bone measure (n)	*0*	*1*	*2*	*Effect (linear regression)*	*p*	*0*	*1*	*2*	*Effect (linear regression)*	*p*
BMC (g)	879.7 (873.6, 885.8)	877.7 (872.4, 883.1)	874.0 (864.8, 883.3)	0.997 (0.991, 1.003)	0.3	878.3 (873.5, 883.1)	876.1 (870.2, 882.1)	883.1 (867.2, 899.2)	1.000 (0.993, 1.007)	0.97
BA (cm^2)	1133.4 (1128.0, 1138.9)	1129.2 (1124.4, 1134.0)	1125.7 (1117.4, 1134.0)	0.997 (0.992, 1.001)	0.1	1131.3 (1127.0, 1135.6)	1128.3 (1123.0, 1133.7)	1131.3 (1117.1, 1145.7)	0.999 (0.994, 1.003)	0.6
BMD (g/cm^2)	0.776 (0.774, 0.778)	0.777 (0.776, 0.779)	0.776 (0.773, 0.780)	1.000 (0.998, 1.003)	0.7	0.776 (0.775, 0.778)	0.777 (0.774, 0.779)	0.781 (0.775, 0.786)	1.001 (0.999, 1.004)	0.3
ABMC (g)	891.9 (890.1, 893.6)	894.3 (892.7, 895.8)	894.5 (891.9, 897.2)	1.002 (1.000, 1.003)	0.05	892.7 (891.3, 894.1)	893.7 (892.0, 895.4)	897.8 (893.3, 902.5)	1.002 (1.000, 1.004)	0.06

Means (95% CI) and effects derived from regression of DXA scan data on *FTO* (rs9939609) and *MC4R* (rs17782313) genotypes. Regression results are adjusted for sex and total fat mass (centered). Variables are log-transformed for analysis. Means are back-transformed, presented as geometric means, and effects are shown as ratios of geometric means.

TABLE 10. RELATIONSHIP BETWEEN SPINE AND UPPER AND LOWER LIMB BONE VARIABLES AND *FTO* AND *MC4R* GENOTYPES ADJUSTED FOR TOTAL FAT MASS WITHIN THE ALSPAC COHORT

	FTO genotype (n = 2921 spine, 5282 limb)					*MC4R genotype (n = 2978 spine, 5387 limb)*				
Bone measure (n)	*0*	*1*	*2*	*Effect (linear regression)*	*p*	*0*	*1*	*2*	*Effect (linear regression)*	*p*
Spine BMC (g)	76.62 (75.90, 77.34)	76.69 (76.05, 77.34)	75.29 (74.23, 76.38)	0.993 (0.985, 1.001)	0.1	76.13 (75.56, 76.71)	76.79 (76.09, 77.51)	77.30 (75.41, 79.23)	1.008 (0.999, 1.018)	0.09
Spine BA (cm^2)	99.58 (99.00, 100.2)	99.52 (99.00, 100.0)	98.54 (97.66, 99.42)	0.996 (0.991, 1.001)	0.1	99.25 (98.78, 99.72)	99.54 (98.97, 100.1)	99.23 (97.71, 100.8)	1.002 (0.996, 1.008)	0.6
Spine BMD (g/cm^2)	0.769 (0.765, 0.773)	0.771 (0.767, 0.774)	0.764 (0.758, 0.770)	0.998 (0.993, 1.002)	0.3	0.767 (0.764, 0.770)	0.771 (0.767, 0.775)	0.779 (0.768, 0.790)	1.007 (1.001, 1.012)	0.02
Spine ABMC (cm^2)	77.67 (77.27, 78.07)	77.84 (77.48, 78.20)	77.49 (76.88, 78.10)	1.000 (0.995, 1.004)	0.8	77.58 (77.26, 77.90)	77.89 (77.49, 78.28)	78.80 (77.74, 79.88)	1.006 (1.000, 1.011)	0.03
Upper limb BMC (g)	116.8 (116.0, 117.7)	116.5 (115.8, 117.3)	116.4 (115.0, 117.8)	0.998 (0.991, 1.005)	0.5	116.5 (115.8, 117.2)	116.7 (115.8, 117.6)	117.5 (115.2, 119.8)	1.003 (0.995, 1.010)	0.5
Lower limb BMC (g)	440.1 (437.0, 443.2)	438.9 (436.2, 441.6)	436.8 (432.1, 441.5)	0.996 (0.990, 1.003)	0.3	439.5 (437.1, 442.0)	437.6 (434.5, 440.6)	440.3 (432.3, 448.5)	0.998 (0.991, 1.005)	0.6

Means (95% CI) and effects derived from regression of DXA scan data on *FTO* (rs9939609) and *MC4R* (rs17782313) genotypes. Regression results are adjusted for sex and total fat mass (centered). Variables are log-transformed for analysis. Means are back-transformed, presented as geometric means, and effects are shown as ratios of geometric means.

strategies to counteract any negative effect on skeletal development, for example, through increased exposure to weight-bearing exercise.

Individually, *FTO* and *MC4R* polymorphisms also showed broadly similar associations with bone mass to that observed for fat and bone mass. There were nominal indications of fat mass difference between those with and without genotype data, but because of the essentially random allocation of alleles with respect to this, any systematic effect this may have had will be uniformly distributed. We are therefore confident in our observations that, like fat mass, *FTO* and *MC4R* polymorphisms seem positively related to BMC at equivalent regions (i.e., total body, spine, and upper and lower limbs).

As seen for fat mass, these genetic markers exerted their effects on bone mass largely by influencing bone size, as reflected by associations with BA, which were broadly similar to those with bone mass. Although neither *FTO* or *MC4R* polymorphisms were found to affect height in this study, a small positive association between the *MC4R* locus studied here and height was reported in 90,000 individuals.[20] Nevertheless, this association with height was considerably weaker than that with bone size, suggesting these genetic markers predominantly affect periosteal rather than longitudinal bone growth, leading to an increase in bone cross-section rather than length, equivalent to effects of fat mass as reported previously.[9] The fact that this study provides further support for the hypothesis that fat mass is a positive influence on periosteal growth is significant, in light of the fact that bone cross sectional area is a major determinant of bone strength and hence fracture risk in later life.[32]

Although size effects accounted for the major part of the relationship between fat and bone mass, both IV analyses and observational analysis showed positive relationships with total body and spinal ABMC, suggesting that the positive influence of fat mass on bone mass also involves effects on volumetric BMD. A tendency for fat mass to enhance volumetric density could result from an increase in cortical/trabecular ratio secondary to suppression of endosteal expansion or by a gain in trabecular bone mass. Further studies are planned to examine these possibilities, based on analysis of pQCT scan data that we are in the process of acquiring in this cohort.

Although the findings from IV analyses largely supported those of sex- and anthropometrically adjusted observational analysis, there were trends that may offer insight into the interpretation of previous studies. For example, for total and limb-based measures, effects of fat mass on BMC, BA, BMD, and ABMC seemed consistently lower in observational analyses. Although these differences were not robust, it seems likely that these may be the result of a combination of overadjustment (i.e., with sex, sitting height, and height in the model) and the greater ability of genotype effects to provide estimates of life-course fat mass effects.

Pleiotropism, whereby genetic markers affect the outcome of interest independently of the exposure variable (in this instance fat mass), is one of several factors that can complicate interpretation of MR analyses.[18] For example,

TABLE 11. INSTRUMENTAL VARIABLE ANALYSIS OF THE EFFECT OF FAT MASS TERTILE ON TOTAL BODY BONE VARIABLES WITHIN THE ALSPAC COHORT (UNADJUSTED)

	Tertile of fat mass								
Bone measure (n)	*0*	*1*	*2*	*Effect (linear regression)*	*IV effect (IV regression)*	*ffirst*	*p*	*p′*	*WH_p″*
BMC (g) (n_reg = 6845) (n_IV =5192)	766.1 (760.6, 771.5)	858.6 (852.7, 864.6)	1017.5 (1010.4, 1024.6)	1.260 (1.251, 1.268)	1.223 (1.134, 1.319)	26.92	$<10^{-10}$	$<10^{-10}$	0.5
BA (cm^2) (n_reg = 6845) (n_IV = 5192)	1026.1 (1021.0, 1031.2)	1110.9 (1105.5, 1116.3)	1257.5 (1251.4, 1263.7)	1.181 (1.176, 1.187)	1.132 (1.073, 1.194)	26.92	$<10^{-10}$	$<10^{-10}$	0.1
BMD (g/cm^2) (n_reg = 6845) (n_IV = 5192)	0.747 (0.745, 0.749)	0.773 (0.771, 0.775)	0.809 (0.807, 0.811)	1.067 (1.064, 1.069)	1.081 (1.050, 1.113)	26.92	$<10^{-10}$	$<10^{-10}$	0.3
ABMC (cm^2) (n_reg = 6845) (n_IV = 5192)	893.6 (892.0, 895.3)	893.9 (892.3, 895.5)	892.4 (890.8, 894.0)	0.999 (0.997, 1.000)	1.028 (1.005, 1.051)	26.92	0.1	0.02	0.006

Means (95% CI) and effects derived from regression of DXA scan data on tertiles of total fat mass. IV effect taken using both *MC4R* (rs17782313) and *FTO* (rs9939609) as instruments for truncal fat mass, adjusting for sex (centered). Means are back-transformed, presented as geometric means, and effects are shown as ratios of geometric means. p represents p value from basic linear regression of DXA scan variables on tertile of truncal fat mass, p' represents that of instrumental variable analysis using *FTO* and *MC4R* genotypes as instruments for truncal fat mass, and p'' represents the Wu/Hausmann test for difference between these estimates.

TABLE 12. INSTRUMENTAL VARIABLE ANALYSIS OF THE EFFECT OF FAT MASS TERTILE ON SPINE AND UPPER AND LOWER LIMB BONE VARIABLES WITHIN THE ALSPAC COHORT (UNADJUSTED)

	Tertile of fat mass								
Bone measure (n)	*0*	*1*	*2*	*Effect (linear regression)*	*IV effect (IV regression)*	*ffirst*	*p*	*p′*	*WH_p″*
Spine BMC (g) (n_reg = 3810) (n_IV = 2878)	67.69 (67.03, 68.36)	74.36 (73.67, 75.05)	88.40 (87.58, 89.23)	1.245 (1.234, 1.256)	1.249 (1.154, 1.352)	25.06	$<10^{-10}$	$<10^{-10}$	0.9
Spine BA (cm^2) (n_reg = 3810) (n_IV = 2878)	92.71 (92.15, 93.26)	98.05 (97.49, 98.61)	107.7 (107.1, 108.4)	1.130 (1.124, 1.136)	1.110 (1.057, 1.167)	25.06	$<10^{-10}$	$<10^{-10}$	0.5
Spine BMD (g/cm^2) (n_reg = 3810) (n_IV = 2878)	0.730 (0.726, 0.734)	0.758 (0.755, 0.762)	0.820 (0.816, 0.825)	1.102 (1.096, 1.108)	1.125 (1.075, 1.176)	25.06	$<10^{-10}$	$<10^{-10}$	0.3
Spine ABMC (g) (n_reg = 3810) (n_IV = 2878)	76.67 (76.29, 77.06)	76.97 (76.60, 77.33)	79.59 (79.22, 79.97)	1.035 (1.030, 1.040)	1.063 (1.017, 1.111)	25.06	$<10^{-10}$	0.007	0.2
Upper limb BMC (g) (n_reg = 6845) (n_IV = 5192)	101.9 (101.1, 102.7)	115.3 (114.4, 116.1)	133.4 (132.4, 134.4)	1.238 (1.229, 1.247)	1.232 (1.134, 1.338)	26.92	$<10^{-10}$	$<10^{-10}$	0.9
Lower limb BMC (g) (n_reg = 6845) (n_IV = 5192)	374.7 (372.0, 377.4)	431.2 (428.1, 434.2)	518.6 (514.9, 522.4)	1.297 (1.288, 1.306)	1.237 (1.145, 1.336)	26.92	$<10^{-10}$	$<10^{-10}$	0.3

Means (95% CI) and effects derived from regression of DXA scan data on tertiles of total fat mass. IV effect taken using both *MC4R* (rs17782313) and *FTO* (rs9939609) as instruments for truncal fat mass, adjusting for sex (centered). Means are back-transformed, presented as geometric means, and effects are shown as ratios of geometric means. p represents p value from basic linear regression of DXA scan variables on tertile of truncal fat mass, p' represents that of instrumental variable analysis using *FTO* and *MC4R* genotypes as instruments for truncal fat mass, and p'' represents the Wu/Hausmann test for difference between these estimates.

MC4R is known to be a major regulator of cocaine- and amphetamine-regulated transcript (CART) in the hypothalamus, the absence of which in mice leads to a low bone mass phenotype secondary to increased bone resorption.[33] Moreover, children heterozygous for an *MC4R* loss of function mutation were found to have increased levels of CART and reduced bone resorption compared with control subjects matched for weight and fat mass.[34] To the extent that the rs17782313 rare allele is associated with reduced function of *MC4R*, alterations in bone resorption could conceivably contribute to the association between this marker and skeletal phenotypes reported here. Nevertheless, the finding that associations between the *MC4R* marker and skeletal measures were largely attenuated by adjusting for fat mass suggests that any direct effect of this marker on skeletal phenotype independently of fat mass is likely to be relatively weak.

In summary, we used two independent genetic markers of obesity, related to the *FTO* and *MC4R* genes, as instrumental variables to explore the relationship between fat and bone mass in ~5000 9-yr-old children. We found that the relationship between these instrumental variables and bone mass mirrored that between bone mass and fat mass as measured by DXA, suggesting that fat mass is on the causal pathway for bone mass. This relationship between *FTO* and *MC4R* genetic markers, fat mass, and bone mass largely involved effects on bone size, which we assume represents a stimulatory effect of fat mass on periosteal bone formation. In addition, *FTO* and *MC4R* polymorphisms and fat mass were both associated with total body and spinal volumetric BMD, suggesting that fat mass may also act to influence bone remodeling.

ACKNOWLEDGMENTS

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer, and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses. The UK Medical Research Council, the Wellcome Trust, and the University of Bristol provide core support for ALSPAC. This publication is the work of the authors, who serve as guarantors for the contents of this paper.

REFERENCES

1. Goulding A, Taylor RW, Jones IE, McAuley KA, Manning PJ, Williams SM 2000 Overweight and obese children have low bone mass and area for their weight. Int J Obes Relat Metab Disord **24:**627–632.
2. Lazcano-Ponce E, Tamayo J, Cruz-Valdez A, Diaz R, Hernandez B, Del Cueto R, Hernandez-Avila M 2003 Peak bone mineral area density and determinants among females aged 9 to 24 years in Mexico. Osteoporos Int **14:**539–547.
3. Mobley SL, Ha E, Landoll JD, Badenhop-Stevens NE, Clairmont A, HGoel P, Matkovic V 2005 Children with bone fragility fractures have reduced bone mineral areal density at the forearm and hip and higher percent body fat. J Bone Miner Res **20:**S34.
4. Skaggs DL, Loro ML, Pitukcheewanont P, Tolo V, Gilsanz V 2001 Increased body weight and decreased radial cross-sectional dimensions in girls with forearm fractures. J Bone Miner Res **16:**1337–1342.
5. Weiler HA, Janzen L, Green K, Grabowski J, Seshia MM, Yuen KC 2000 Percent body fat and bone mass in healthy Canadian females 10 to 19 years of age. Bone **27:**203–207.
6. Petit MA, Beck TJ, Shults J, Zemel BS, Foster BJ, Leonard MB 2005 Proximal femur bone geometry is appropriately adapted to lean mass in overweight children and adolescents. Bone **36:**568–576.
7. Klein KO, Larmore KA, de Lancey E, Brown JM, Considine RV, Hassink SG 1998 Effect of obesity on estradiol level, and its relationship to leptin, bone maturation, and bone mineral density in children. J Clin Endocrinol Metab **83:**3469–3475.
8. Lorentzon M, Landin K, Mellstrom D, Ohlsson C 2006 Leptin is a negative independent predictor of areal BMD and cortical bone size in young adult Swedish men. J Bone Miner Res **21:**1871–1878.
9. Clark EM, Ness AR, Tobias JH 2006 Adipose tissue stimulate bone growth in prepubertal children. J Clin Endocrinol Metab **91:**2534–2541.
10. Janicka A, Wren TA, Sanchez MM, Dorey F, Kim PS, Mittelman SD, Gilsanz V 2007 Fat mass is not beneficial to bone in adolescents and young adults. J Clin Endocrinol Metab **92:**143–147.
11. Zhao LJ, Jiang H, Papasian CJ, Maulik D, Drees B, Hamilton J, Deng HW 2008 Correlation of obesity and osteoporosis: Effect of fat mass on the determination of osteoporosis. J Bone Miner Res **23:**17–29.
12. Bouillon R, Bex M, Vanderschueren D, Boonen S 2004 Estrogens are essential for male pubertal periosteal bone expansion. J Clin Endocrinol Metab **89:**6025–6029.
13. Lorentzon M, Swanson C, Andersson N, Mellstrom D, Ohlsson C 2005 Free testosterone is a positive, whereas free estradiol is a negative, predictor of cortical bone size in young Swedish men:The GOOD Study. J Bone Miner Res **20:** 1334–1341.
14. Turner CH, Forwood MR, Rho JY, Yoshikawa T 1994 Mechanical loading thresholds for lamellar and woven bone formation. J Bone Miner Res **9:**87–97.
15. Reid IR 2008 Relationships between fat and bone. Osteoporos Int **19:**595–606.
16. Fisch RC, Revelle R 1971 Height and weight at menarche and a hypothesis of menarche. Arch Dis Child **46:**695–701.
17. Davey Smith G, Ebrahim S 2003 'Mendelian randomization': Can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol **32:**1–22.
18. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G 2008 Mendelian randomization: Using genes as instruments for making causal inferences in epidemiology. Stat Med **27:**1133–1163.
19. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD, Hattersley AT, McCarthy MI 2007 A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science **316:**889–894.
20. Loos RJ, Lindgren CM, Li S, Wheeler E, Zhao JH, Prokopenko I, Inouye M, Freathy RM, Attwood AP, Beckmann JS, Berndt SI, Jacobs KB, Chanock SJ, Hayes RB, Bergmann S, Bennett AJ, Bingham SA, Bochud M, Brown M, Cauchi S, Connell JM, Cooper C, Smith GD, Day I, Dina C, De S, Dermitzakis ET, Doney AS, Elliott KS, Elliott P, Evans DM, Sadaf Farooqi I, Froguel P, Ghori J, Groves CJ, Gwilliam R, Hadley D, Hall AS, Hattersley AT, Hebebrand J, Heid IM, Lamina C, Gieger C, Illig T, Meitinger T, Wichmann HE,

Herrera B, Hinney A, Hunt SE, Jarvelin MR, Johnson T, Jolley JD, Karpe F, Keniry A, Khaw KT, Luben RN, Mangino M, Marchini J, McArdle WL, McGinnis R, Meyre D, Munroe PB, Morris AD, Ness AR, Neville MJ, Nica AC, Ong KK, O'Rahilly S, Owen KR, Palmer CN, Papadakis K, Potter S, Pouta A, Qi L, Randall JC, Rayner NW, Ring SM, Sandhu MS, Scherag A, Sims MA, Song K, Soranzo N, Speliotes EK, Syddall HE, Teichmann SA, Timpson NJ, Tobias JH, Uda M, Vogel CI, Wallace C, Waterworth DM, Weedon MN, Willer CJ, Wraight L, Yuan X, Zeggini E, Hirschhorn JN, Strachan DP, Ouwehand WH, Caulfield MJ, Samani NJ, Frayling TM, Vollenweider P, Waeber G, Mooser V, Deloukas P, McCarthy MI, Wareham NJ, Barroso I 2008 Common variants near MC4R are associated with fat mass, weight and risk of obesity. Nat Genet **40:**768–775.

21. Golding J, Pembrey M, Jones R 2001 ALSPAC: The Avon Longitudinal Study of Parents and Children 1. Study methodology. Paediatr Perinat Epidemiol **15:**74–87.
22. Jones RW, Ring S, Tyfield L, Hamvas R, Simmons H, Pembrey M, Golding J 2000 A new human genetic resource: A DNA bank established as part of the Avon longitudinal study of pregnancy and childhood (ALSPAC). Eur J Hum Genet **8:**653–660.
23. Tobias JH, Steer CD, Emmett PM, Tonkin R, Cooper C, Ness A 2005 Bone mass is related to maternal diet in pregnancy. Osteoporos Int **16:**1731–1741.
24. Tanner JM 1986 Normal growth and techniques of growth assessment. J Clin Endocrinol Metab **15:**411–451.
25. Clark EM, Ness A, Tobias JH 2005 Social position affects bone mass in childhood through opposing actions on height and weight. J Bone Miner Res **20:**2082–2089.
26. Greenland S 2000 An introduction to instrumental variables for epidemiologists. Int J Epidemiol **29:**722–729.
27. Timpson NJ, Lawlor DA, Harbord RM, Gaunt TR, Day IN, Palmer LJ, Hattersley AT, Ebrahim S, Lowe GD, Rumley A, Davey Smith G 2005 C-reactive protein and its role in metabolic syndrome: Mendelian randomisation study. Lancet **366:**1954–1959.
28. Staiger D, Stock JH 1997 Instrumental variables regression with weak instruments. Econometrica: J Econometric Soc **65:**557–586.
29. Stock JH, Wright JH, Yogo M 2002 A survey of weak instruments and weak identification in generalized method of moments. J Bus Econ Stat **20:**518–529.
30. Ferron M, Hinoi E, Karsenty G, Ducy P 2008 Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. Proc Natl Acad Sci USA **105:**5266–5270.
31. Cummings SR, Black DM, Nevitt MC, Browner W, Cauley J, Ensrud K, Genant HK, Palermo L, Scott J, Vogt TM 1993 Bone density at various sites for prediction of hip fractures. Lancet **341:**72–75.
32. Seeman E 2003 Periosteal bone formation - a neglected determinant of bone strength. N Engl J Med **349:**320–323.
33. Elefteriou F, Ahn JD, Takeda S, Starbuck M, Yang X, Liu X, Kondo H, Richards WG, Bannon TW, Noda M, Clement K, Vaisse C, Karsenty G 2005 Leptin regulation of bone resorption by the sympathetic nervous system and CART. Nature **434:**514–520.
34. Ahn JD, Dubern B, Lubrano-Berthelier C, Clement K, Karsenty G 2006 Cart overexpression is the only identifiable cause of high bone mass in melanocortin 4 receptor deficiency. Endocrinology **147:**3196–3202.

Address reprint requests to:
JH Tobias, MD, PhD
Academic Rheumatology
Avon Orthopaedic Centre
Bristol BS10 5NB, UK
E-mail: Jon.Tobias@bristol.ac.uk

Received in original form July 3, 2008; revised form September 8, 2008; accepted October 28, 2008.