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ORIGINAL ARTICLE

Causal inference of the effect of adiposity on bone mineral density in adults

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Summary

Objective The causal effect of adipose tissue on bone mass and the direction of its net influence have not been directly assessed in adult humans. Using the Mendelian randomization analysis, we assessed the causality of adiposity in measurements of bone mass in adult males and females.

Design and Methods Subjects consisted of 2154 adults aged 25–54 years from a cross-sectional cohort of the employees of the Electricity Generating Authority of Thailand. Body composition was determined after at least 3 h of fasting using multifrequency bioelectrical impedance analysis. Bone mineral density (BMD) was assessed by dual energy X-ray absorptiometry. A polymorphism in the fat mass and obesity-associated gene (*FTO* rs9939609) was used as an instrument in the Mendelian randomization analysis.

Results The genotype distribution of the *FTO* rs9939609 polymorphism was 61·1% TT, 33·9% AT and 5·0% AA. The average body mass index (BMI), body fat mass and percentage body fat were 23·9 kg/m^2 (SD = 3·6), 17·9 kg (SD = 6·6) and 26·8% (SD = 7·2), respectively. The *FTO* rs9939609 polymorphism was significantly correlated with BMI (coefficient = 0·673 kg/m^2, $P < 0·001$), body fat mass (coefficient = 0·948 kg, $P < 0·001$) and percentage body fat (coefficient = 0·759%, $P < 0·01$). An instrumental variable (IV) regression model, using BMI as the intermediate phenotype, suggested that *FTO* was a strong IV. Also, the *FTO*-BMI polymorphism was significantly associated with total hip and femoral neck BMD but was not correlated with total spine BMD, with estimated correlation coefficients of 0·0189 (95% CI: 0·0046, 0·0332), 0·0149 (95% CI: 0·0030, 0·0268) and 0·0025 (95% CI: −0·0131, 0·0136) g/cm^2, respectively. The variances of BMDs explained by the *FTO*-BMI were 19·0%, 21·3% and 1·1%, respectively. Similar trends were also observed for the *FTO*-body fat mass and *FTO*-percentage body fat correlations.

Conclusions Mendelian randomization analysis suggests that adiposity might be causally related to BMD at the femur but not at the spine.

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Introduction

Osteoporosis is a health problem worldwide. A number of risk factors are related to susceptibility to osteoporosis, including oestrogen deficiency, sedentary lifestyle and reduced adiposity. Adipose tissue is now considered an endocrine organ. In addition to its conventional role in energy storage, adipose tissue secretes a number of bioactive proteins, which influence a variety of biological processes, including energy homoeostasis and inflammation. As far as bone is concerned, leptin, an adipokine secreted from adipose tissue, has been shown to diminish bone formation through a central nervous system delay in animal models. In humans, a number of adipokines, including leptin,[1,2] adiponectin[3–5] and omentin-1,[6] have been shown in observational studies to be variably related to bone mineral density (BMD).

Although results from animal models and observational studies in humans suggest that adiposity influences bone mass, the effects of adipokines on bone mass are different. Results from observational studies can be confounded by factors, which influence both adiposity and bone mass, such as body size and weight. Moreover, the causality of adipose tissue on bone mass and the direction of net influence have not been directly assessed in adult humans.

The fat mass and obesity-associated (*FTO*) locus on chromosome 16 (16q12.2) has been identified from genome-wide association studies as a major candidate gene for obesity in children and adults.[7–9] The *FTO* rs9939609 polymorphism is of particular interest, as it was found to be associated with obesity in different

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ethnic groups.[8,10–13] The finding of common genetic variants of *FTO,* which have been consistently associated with adiposity, provided an opportunity to conduct a Mendelian randomization study of obesity and bone outcomes. Therefore, utilizing the Mendelian randomization analysis, we assessed the causality of adiposity in the attainment of BMD in adults.

Materials and methods

The study design was a cross-sectional cohort of the employees of the Electricity Generating Authority of Thailand (EGAT). Prior to commencement, the study was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine, Ramathibodi Hospital, Mahidol University. All subjects gave written informed consent. Survey data were collected using a self-administered questionnaire, physical examination, electrocardiography, chest radiography and blood analysis.

BMD measurements

After changing into lightweight clothing without dense objects, each participant underwent dual-emission X-ray absorptiometry (DXA, Hologic QDR 4500W; Hologic, Bedford, MA, USA) to obtain BMD values of the lumbar spine (L1–L4) and left proximal femur (femoral neck and total hip). The DXA procedure complied with the ISCD Position Statement.[14] For lumbar spine DXA, each participant lay flat on the midline of the imaging table, with legs elevated by a supporting pillow so that the spine was straight. The DXA scan included T12 and L5 vertebrae and both iliac crests. For the proximal femur DXA, the participant lay supine with the left foot fixed to a positioning device to keep the hip internally rotated and adducted so that the femoral shaft was straight, with minimal visualization of the lesser trochanter while the ischium and the greater trochanter were included on the scan. The scans were then analysed according to the manufacturer's recommendations. Each morning prior to the scheduled DXA scans, a quality-control procedure was performed as recommended by the manufacturer to ensure machine precision of more than 98·5%. The BMD coefficients of variation were 0·82%, 2·52% and 1·51% for the lumbar spine, femoral neck and total hip, respectively.

Adiposity measurements

Anthropometric variables including weight, height and waist circumferences were measured using standard techniques in all studies. Body mass index (BMI) was derived by weight (kg)/height $(m)^2$. Body composition was determined after at least 3 h of fasting using multifrequency bioelectrical impedance analysis with eight-point tactile electrodes (InBody 720; Biospace, Seoul, Korea).

FTO genetic analysis

DNA was extracted by phenol–chloroform method.[15] Individual genotyping of all subjects was performed using real-time PCR (TaqMan® MGB probes, Applied Biosystems, Foster City, CA, USA): 10 ng of DNA was added into the PCR reaction, consisting of TaqMan Universal Master Mix (1×) and TaqMan MGB probes for *FTO* rs9939609 SNP (1×) in a total volume of 10 μl. The real-time PCR reaction protocol was 10 min at 95 °C, 40 cycles of 15 s at 92 °C and 1 min at 60 °C using a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Data were expressed as mean (or median, where appropriate) and frequency for continuous and categorical data, respectively. Hardy–Weinberg equilibrium was assessed using an exact test.[16] Relationships between the *FTO* polymorphism and variables were assessed for both males and females using linear regression and a chi-square test (or exact test, where appropriate) for continuous and categorical data, respectively.

Mendelian randomization analysis[17] was applied to assess causal relationships between *FTO*, adiposity (i.e. BMI and body fat mass) and BMD. Instrumental variable (IV) regression with two-stage least squares method was applied to explore these causal relationships, using the *FTO* polymorphism with additive effect as the IV and BMI/body fat mass as the endogenous variables.[18,19] These models were also adjusted for confounding variables (i.e. alcohol, age and gender), as univariate analysis suggested that they were associated with intermediate phenotype and/or BMD. In the first-stage regression, the *F*-statistic (hereafter called *F*-First) was used to assess whether the *FTO* polymorphism was sufficiently strong to be an IV. A value of *F*-First greater than 10 indicated that the *FTO* was a strong IV, and thus the estimated causal relationships should be valid. In addition, linear regression with ordinary least squares (OLS) method was also applied to directly assess the association between *FTO*, adiposity and BMD. The Durbin–Wu–Hausman statistic was applied to compare the results between the IV and OLS regression approaches. All analyses were performed using STATA version 12·0 (StataCorp LP, College Stattion, TX, USA). A *P*-value of less than 0·05 was considered statistically significant.

Results

Table 1 describes the clinical characteristics, adiposity and BMD of the study subjects (n = 2154). The mean age of the subjects was 40·2 years (SD = 6·9). The average BMI, body fat mass and percentage body fat were 23·9 kg/m^2 (SD = 3·6), 17·9 kg (SD = 6·6) and 26·8% (SD = 7·2), respectively. The correlation matrix between parameters of adiposity and BMD is shown in Table 2. Measures of adiposity, including BMI, body fat mass and percentage body fat, were all significantly related to BMD at all skeletal sites.

The association of measures of adiposity and BMD can be confounded by variables such as age, gender, alcohol consumption and cigarette smoking. For being an IV, the *FTO* polymorphism should not be associated with these potential confounders. Therefore, associations between the subject characteristics and the *FTO* genotypes were assessed. As described in Table 3, none of these potential confounders were associated

Table 1. Clinical characteristics of cohort

Characteristic	Mean (SD)
Age (years)	40·0 (7·4)
Body weight (kg)	66·1 (12·5)
Height (cm)	166·2 (7·8)
BMI (kg/m^2)	23·9 (3·6)
Body fat mass (kg)	17·9 (6·6)
Percentage body fat (%)	26·8 (7·2)
Cigarettes smoking/day, median (range)	0 (0–50)
Alcohol consumption, *n* (%)	982/2325 (42·2)
Lumbar spine BMD (g/cm^2)	0·975 (0·118)
Femoral neck BMD (g/cm^2)	0·801 (0·121)
Total femur BMD (g/cm^2)	0·925 (0·129)

BMD, bone mineral density, BMI, body mass index.

with the *FTO* polymorphism. The *FTO* genotype frequencies complied with Hardy–Weinberg equilibrium rules ($P = 0{\cdot}510$).

Relationships between adiposity (i.e. BMI, body fat mass and percentage body fat) and the *FTO* polymorphism were explored (Table 4). The mean BMI was significantly higher in minor homozygous and heterozygous genotypes compared with the major homozygous genotype ($P < 0{\cdot}001$). Applying linear regression analysis by fitting *FTO* as an additive effect suggests that the *FTO* polymorphism was significantly correlated with BMI (coefficient = 0·637 kg/m^2, $P < 0{\cdot}001$), indicating that carrying an A allele would increase BMI of 0·637 kg/m^2. This trend was also observed with body fat mass and percentage body fat, that is, mean body fat mass or percentage body fat in minor homozygous and heterozygous genotypes was significantly higher than in the major homozygous genotype ($P < 0{\cdot}001$).

Table 5 describes the results from OLS and IV regression analyses by measures of adiposity and skeletal sites. In the IV regression model using BMI as the intermediate phenotype, *FTO* was a strong IV for total hip, femoral neck and total spine BMD, with *F*-statistics of 25·7, 21·9 and 21·8, respectively. The *FTO*-BMI was also significantly associated with total hip and femoral neck BMDs but not with total spine BMD, with estimated correlation coefficients of 0·0189 (95% CI: 0·0046, 0·0332), 0·0149 (95% CI: 0·0030, 0·0268) and 0·0025 (95% CI: −0·0131, 0·0136) g/cm^2, respectively. The variances of BMDs explained by the *FTO*-BMI were 19·0%, 21·3% and 1·1%, respectively. Similar trends were also observed for *FTO*-body fat mass, that is, the *FTO*-body fat mass was significantly associated with total hip and femoral neck BMDs but not with total spine BMD, with correlation coefficients of 0·0122 (95% CI: 0·0023, 0·0221), 0·0086 (95% CI: 0·0005, 0·0167) and 0·0012 kg (95% CI: −0·0074, 0·0098), respectively. Similar results for percentage body fat were obtained. The *F*-First statistics showed that *FTO* was a strong IV for all BMDs.

The results of OLS regression suggest that both BMI and body fat mass correlate significantly with all BMD sites after adjusting for *FTO* and other covariables. Although the magnitude of adiposity effects from IV estimates was higher than the OLS estimates for all BMDs except total spine BMD, Durbin–Wu–Hausman tests did not reach statistical significance (Table 5).

Table 3. Description and comparison of characteristics of subjects, according to *FTO* genotypes

	FTO genotype			
Characteristic	TT ($n = 1315$)	AT ($n = 731$)	AA ($n = 108$)	*P*-value
Age, mean (SD)	40·1 (6·9)	40·0 (6·9)	40·3 (6·8)	0·500
Gender				
Male	949 (72·2%)	532 (72·8%)	79 (73·2%)	0·955
Female	366 (27·8%)	199 (27·2%)	29 (26·8%)	
Alcohol consumption				
Yes	565 (43·0%)	305 (41·7%)	46 (42·6%)	0·941
No	749 (57·0%)	426 (58·3%)	62 (57·4%)	
Cigarette smoking, median (range)	0 (0, 50)	0 (0, 30)	0 (0, 15)	0·526

FTO, fat mass and obesity-associated gene.

Discussion

In the present study, using a Mendelian randomization analysis, we have demonstrated that adiposity is likely to play a causal role in the determination of bone mass. The magnitude of the effect, however, is relatively small. Although causality is suggested by these analyses, the underlying mechanism(s) still require further investigation. In addition to being the source of adipokines, adipose tissue is able to convert androgens to estrogens through the *CYP19* aromatase enzyme,[20] which may

Table 2. Correlation matrix between measures of adiposity and BMD

	BMI	Body fat mass (kg)	Body fat mass (%)	Lumbar BMD	Femoral neck BMD	Total femur BMD
BMI	1·0000					
Body fat mass (kg)	0·85 (<0·001)	1·0000				
Body fat mass (%)	0·51 (<0·001)	0·82 (<0·001)				
Lumbar BMD	0·19 (<0·001)	0·14 (<0·001)	0·03 (0·13)	1·0000		
Femoral neck BMD	0·39 (<0·001)	0·23 (<0·001)	−0·02 (0·39)	0·62 (<0·001)	1·0000	
Total femur BMD	0·39 (<0·001)	0·22 (<0·001)	−0·03 (0·162)	0·57 (<0·001)	0·81 (<0·001)	1·0000

BMD, bone mineral density.

Table 4. Relationship between adiposity and *FTO* genotypes

Adiposity	*FTO* rs9939609			*B* (linear regression*)	*P*-value
	TT	AT	AA		
BMI	23·7 (3·7)†	24·3 (3·6)	25·4 (4·2)	0·637	<0·001
Body fat mass (kg)	17·6 (6·6)	18·3 (6·9)	20·2 (7·9)	0·948	<0·001
Percentage body fat (%)	26·6 (7·2)	27·1 (7·1)	28·6 (8·2)	0·759	0·006

BMI, body mass index, *FTO*, fat mass and obesity-associated gene.
*Additive effect of *FTO* locus.
†Mean (SD).

Table 5. Linear and IV regression analysis of the relationships between BMD and BMI (A), body fat mass (B) and percentage body fat (C)

	Linear regression			IV regression				
	β	95% CI	*P*-value	β	95% CI	*P*-value	*F*-First*	WH *P*-value†
(A) BMI (kg/m^2)								
Total hip BMD (g/cm^2)	0·0138	0·0124, 0·0153	<0·001	0·0189	0·0046, 0·0332	0·010	25·734	0·486
Femoral neck BMD (g/cm^2)	0·0119	0·0107, 0·0132	<0·001	0·0149	0·0030, 0·0268	0·014	21·864	0·629
Total spine BMD (g/cm^2)	0·0069	0·0056, 0·0083	<0·001	0·0025	−0·0131, 0·0136	NS	21·826	0·313
(B) Body fat mass (kg)								
Total hip BMD (g/cm^2)	0·0052	0·0043, 0·0061	<0·001	0·0122	0·0023, 0·0221	0·016	15·378	0·142
Femoral neck BMD (g/cm^2)	0·0045	0·0041, 0·0055	<0·001	0·0086	0·0005, 0·0167	0·037	15·377	0·348
Total spine BMD (g/cm^2)	0·0026	0·0019, 0·0034	<0·001	0·0012	−0·0074, 0·0098	0·790	15·303	0·725
(C) Percentage body fat (%)								
Total hip BMD (g/cm^2)	0·0035	0·0026, 0·0044	<0·001	0·0134	0·0019, 0·0250	0·023	17·188	0·067
Femoral neck BMD (g/cm^2)	0·0032	0·0024, 0·0040	<0·001	0·0094	0·0002, 0·0187	0·046	17·188	0·168
Total spine BMD (g/cm^2)	0·0013	0·0004, 0·0021	0·002	0·0013	−0·0079, 0·0104	0·784	17·090	0·997

β, regression coefficient, BMD, bone mineral density, BMI, body mass index; IV, instrumental variable.
*First-stage regression *F*-statistic.
†Durbin–Wu–Hausman test comparing ordinary least square *vs* instrumental variable regression.

account for the observation that obese postmenopausal women have lower bone turnover, higher bone mass and fewer fractures.[21] Given the myriad of possible mechanisms affecting the influence of adiposity on bone mass, it remains unclear which one plays a predominant role; it is likely that additional underlying possibilities still remain to be discovered. On the other hand, it is well accepted that body size is also associated with higher areal BMD. It is therefore unclear whether adiposity directly leads to high bone mass or is simply related to BMD because of the confounding effect of body size and weight. Using the IV analysis, our results suggest that there is a net positive effect of adiposity on BMD, which is not likely to be confounded by body size and weight, a finding that is in agreement with a previous study using a different approach.[22]

It is noteworthy that in the present study the causal role of adiposity in the determination of bone mass is only apparent in the femur. Of the skeletal sites used for BMD measurement, the vertebra is comprised of more trabecular bone compared with the femur, which suggests that adiposity may predominantly influence cortical bone. The underlying reason for this phenomenon is not entirely clear. Metabolic differences exist between skeletal sites rich in trabecular bone and cortical bone. Trabecular BMD changes more (in either direction) in response to bone stimuli, including exercise[23] and antiresorptive[24] or bone-forming agents such as intermittent teriparatide.[24] However, unlike other bone-active stimuli, parathyroid hormone (PTH), particularly when continuously elevated, affects cortical bone to a greater extent than trabecular bone.[25,26] As a number of studies[27–29] have found that adiposity and serum PTH are correlated, it is conceivable that the influence of adiposity predominantly on cortical bone may be partly mediated through the effect of PTH. Such a hypothesis cannot be readily tested in the present study as serum PTH data are lacking. Further investigations involving the potential causal relationships among adiposity, PTH and bone mass are warranted.

The Mendelian randomization analysis has been increasingly utilized in health research to assess causality based on genetic observational studies. A proper Mendelian randomization study must comply with a number of assumptions.[24,30] In the present study, the *FTO* genotype was strongly associated with adiposity, which satisfies one of the assumptions for a proper IV. The associations between the *FTO* gene and osteoporosis phenotypes have recently been described.[31] The effect is likely to be mediated through the influence of adiposity. However, as the

function of the *FTO* gene is not entirely known, the possibility remains that there might be other *FTO* gene-determined confounders of adiposity and bone mass; this would render the use of the *FTO* gene as an IV in this case less valid.

In conclusion, the Mendelian randomization analysis suggested that adiposity might be causally related to BMD in the femur but not in the spine.

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Conflict of interest and financial disclosure

Nothing to declare.

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