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Gene-dietary fat interaction, bone mineral density and bone speed of sound in Children: a twin study in China

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Abstract

Scope—Dietary fat correlates with bone mineral density (BMD). We tested the association between fat intake and BMD, and tested if fat intake modified the degree of genetic influence on BMD and bone speed of sound (SOS).

Methods and results—We included 622 twins aged 7–15 y from South China. Data on anthropometry, dietary intake, BMD, and SOS were collected. Quantitative genetic analyses of structural equation models were fit using the Mx statistical package.

The within-pair intra-class correlations (ICC) for BMD in DZ twins were nearly half of that for MZ twins (ICC=0.39 vs 0.70). The heritability of BMD and SOS were 71% and 79%. Phenotypic correlation between fat intake and SOS was significant ($r=-0.19$, $p=0.04$). SOS was negatively correlated with fat intake in boys ($r=-0.11$, $p=0.05$), but not in girls. Full Cholesky decomposition models showed SOS has a strong genetic correlation with fat intake ($r_A=-0.88$, 95% CI=-0.94, 0.01); the environmental correlation between fat intake and SOS was weak ($r_E=-0.04$, 95% CI=-0.20, 0.13). Fat intake modified the additive genetic effects on BMD.

Conclusion—Genetic factors explained 71% and 79% of individual variance in BMD and SOS, respectively. Low fat intake counteracts genetic predisposition to low BMD.

Keywords

bone mineral density; speed of sound; heritability; gene-diet interaction; dietary fat; twin study

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YW designed research (project conception, development of overall research plan, study oversight, and secured funding support); TH conducted data analysis; TH and YW drafted the manuscript; YW has primary responsibility for final content. YW, JL, LH and WZ contributed to data collection. All coauthors contributed to reviewing and revising the study.

INTRODUCTION

Osteoporosis is a serious public health problem in many countries. Bone mineral density (BMD) has been widely regarded as a surrogate measure for osteoporosis [1]. Therefore, to better understand the mechanisms and determinants of bone density is important in the prevention and treatment of osteoporosis. Childhood is an important time for influencing prevention and this period might impact osteoporosis development later in life.

Lifestyle factors affect BMD are potentially modifiable risk factors for osteoporosis [2]. Evidence over the past several years showed that dietary fat has an important effect on bone health [3]. Animal studies indicated that high-fat diets adversely affect bone [4, 5]. The total amount of fat consumed was positively associated with fracture risk, and negatively associated with bone density [6, 7]. Saturated fatty acids (SFA) in particular, may impair bone health [8]. Study in a nationally representative large sample of American men and women reported that saturated fat intake was negatively associated with bone density. These results indicated that high diets in SFA might increase the risk of bone disease [3]. Several mechanisms from basic research may account for the effects of dietary fat intake on bone, including alterations in calcium absorption and osteoblast formation [8, 9]. Despite strong evidence from animal and in vitro studies implicating dietary fats in bone health, the associations of dietary fat with BMD in humans especially in children are still poorly understood.

Some genetic and environmental factors have been demonstrated to affect BMD [10, 11]. Genome wide association studies have identified several loci association with BMD in children and adults [12]. However, whether dietary intakes modulate the genetic effect on BMD is not fully understood. Genetic influence on BMD has been documented in both adults and children, but the estimated influence has varied greatly across studies [10, 11, 13]. Heritability is the ratio of genetic variance to the total phenotypic variance, and serves as a simple measure of the degree of possible genetic control of a phenotype. Heritability estimates, however, are affected not only by genetic variation but also environmental factors and will vary across populations [11].

Furthermore, BMD and speed of sound (SOS) measured by Qualitative ultrasound (QUS) were reported being correlated [14]. Quantitative ultrasonic measurement of bone is an alternative to BMD measurements. The SOS is affected not only by the amount of bone, but also by qualitative properties of bone [15]. The soft tissue also slows the SOS transmitted across bones [16, 17]. On the contrary, SOS measured in vivo along bone was corrected for soft tissue thickness [18], and is affected by body mass index (BMI) [19].

The present study aimed to: 1) quantify the relative contributions of genetic and environmental factors to BMD and SOS which reflect the bone quality in children; 2) explore the associations of dietary fat with BMD and SOS; and 3) examine the gene-dietary fat interaction in determining BMD and SOS. Data collected from children in a population-based twin study in China were used.

MATERIALS AND METHODS

Study Sample and Data Collection

The study was conducted in Jiaxing Maternity and Child Health Care Hospital (JMCHCH), Jiaxing, China. The study protocol was approved by the Institutional Review Boards of The Johns Hopkins University Bloomberg School of Public Health (JHBSPH) and Jiaxing Maternity and Child Health Care Hospital (JMCHCH), Jiaxing, China. Twins were eligible for recruitment if (1) both twins participated in the BDSS-China, and (2) both twins and their mother consented or agreed to participate in the study. The twins and their mothers visited local clinics on weekend morning. Physical examination of twins was performed by trained research physicians. Their mothers completed a general questionnaire about their demographic information. Data were collected from 622 twins aged 7–15 years old ($n=311$ pairs, monozygotic (MZ):dizygotic (DZ) $\approx 1:1$, male: female $\approx 1:1$) at Jiaxing, Zhejiang Province, China in 2009–2010.

Anthropometric Measures

The twins were measured by trained research physicians following standard protocols. Barefoot height (in cm to nearest 0.1) was measured with two readings using a wall-mounted stadiometer. Subjects were weighed to the nearest 0.1 kilogram (kg) using a digital scale integrated into the bioelectric impedance analyzer (BIA) system. Waist circumference (WC) was measured to the nearest 0.1 cm at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest using a flexible tape measure. Body mass index (BMI) was calculated as weight (kg)/height (m)².

Dietary intake assessment

A 145-item self-administered food frequency questionnaire (FFQ) which showed good reliability and moderate validity [20], was used to measure children's food intakes during the previous 12 months. Standardized interviews were conducted to help children better understand the questionnaire. Information on the amount and type of food consumed was collected. Energy and nutrient intakes were calculated using the Nutrition Data Systems established by the Institute of Nutrition and Food Safety, China Centers for Disease Control and Prevention [21].

Assessment of bone quality

Bone quality was measured by trained research physicians following standard protocols. BMD (percentile) which reflect the bone quality was measured on wrist using quantitative ultrasonography (QUS) (Omnisense 7000P, Sunlight Medical Ltd.) which uses a child reference population. The scatter of the ultrasound wave by the bone was indexed by speed of sound (SOS) (m/second). The relevant Z-scores were calculated based on BMD and derived from the manufacturer's database. The measure was taken once at each visit.

Zygosity

MZ and DZ twins were ascertained by 19 questions covered in the maternal questionnaire. These questions were adapted from a validated Taiwan twin similarity questionnaire [22]

and based on other related research [23]. The accuracy rate of the validated questionnaire was over 95% for self-reports of adolescent twins and their mothers compared to results based on DNA diagnosis [22].

Statistical Analysis

Descriptive analysis included mean and standard deviation for the continuous phenotypes (age, weight, height, BMI, waist, dietary fat, SOS, Z-score, and BMD). Anthropometrics were presented as means and standard deviations. T-tests or analysis of variance (ANOVA) were used to compare the differences between groups. Intraclass correlations in MZ (rMZ) and DZ (rDZ), which were calculated from a random-effects one-way analysis of variance were used to assess the similarity. Shrout-Fleiss intra-class correlation coefficients (ICCs) were calculated for MZ and DZ twins separately using SAS 9.2 (SAS Institute, Cary, NC).

The data were analyzed using quantitative genetic modeling of twin data. Twin studies are based on the assumption that MZ twins are genetically identical, DZ twins have 50% of their segregating genes identical-by-descent. Thus, differences between MZ cotwins are due to environmental effects, while differences between DZ cotwins are due to genetic effects. Based on the above assumptions, four sources of variation interpreted as latent and standardized variance components in the structural equation model can be identified: additive genetic (A, additive effects of genes on multiple loci), non-additive genetic (D, interactions between alleles at the same locus [dominance] or on different loci [epistasis]), common environmental (C, environmental effects shared by twins reared in the same family) and unique environmental effects (E, environmental effects unique to the individual). MZ pairs are assumed to share the same A and D genetic variance; DZ pairs are assumed to share one-half of the A variance and one-quarter of the D variance. The C variance is assumed to be the same for both MZ and DZ twin pairs. Structural equation modeling (SEM) with maximum likelihood estimation was used to estimate heritability of SOS and BMD. The heritability, which estimated the extent to which variation of a trait in a population can be explained by genetic variation, was defined as the proportion of genetic variance to total phenotypic variance. The genetic models were carried out using the Mx statistical package [24].

We fit univariate models for SOS and BMD to estimate genetic and environmental influences and identified the best model for each trait used in further modeling. The model fit was based on χ^2 tests, where a small χ^2 and a high p value indicated a good fit. Parsimony was assessed by means of the Akaike information criterion. The model with the lowest Akaike information criterion was preferred. Estimates of variance components were derived from the best-fitting model and presented with 95% confidence intervals (CIs).

Next, we analyzed pair-wise genetic and environmental correlations between these indicators using full Cholesky decomposition. According to this method, the trait correlations between dietary fat and BMD and SOS are because of additive genetic correlation (r_A) indicating the same or closely linked genes and unique environmental correlation (r_E) indicating same or correlated environmental factors. A gene-diet interaction model (Figure 1) was also carried out. The moderator factor, dietary fat, is denoted as M. This factor can affect the mean trait value (β_M), but also modify the effects of genetic (β_X)

and environmental factors (β_Y and β_Z) on the trait. The model implies that dietary fat can affect both the mean BMD/SOS and variances of model components (Figure 1).

Gene-environment interaction analyses

The twin model enabled us to examine whether dietary fat modulate the genetic effect in persons who were genetically predisposed to osteoporosis (gene-environment interaction) by using methods described elsewhere [25]. The question to be examined is whether participants who are genetically predisposed to osteoporosis have a differential effect of dietary fat on BMD. Given the strong genetic influence on BMD in this twin study and in the absence of susceptibility genes, the phenotype of a twin's co-twin can be used as a marker of underlying genetic risk [26]. That is, if a twin's co-twin has low BMD (<25 percentile), it can be assumed that the twin also has a higher genetic risk for having osteoporosis. We presented the data from one twin from each pair. The twins had a low and higher genetic risks for having osteoporosis were identified. Dietary fat were dichotomized into high and low levels. We tested for an interaction between genetic risk category and dietary fat category by using a two-factor analysis of variance. This data analysis was conducted by using SAS 9.2 (SAS Institute, Cary, NC).

RESULTS

Characteristics of study population

There were no systematic differences in means and variances of anthropometric measures and dietary fat between the MZ and DZ twins (Table 1). Same sex DZ twins had significant lower BMD (55.9 ± 32.3 vs 59.1 ± 32.9 , BMD based Z-score (0.33 ± 1.41 vs 0.53 ± 1.41) and SOS (3698.6 ± 174.1 vs 3725.5 ± 389.6 , respectively) than opposite sex DZ twins.

Heritability of BMD and SOS

We started genetic modeling by estimating the best model for BMD and SOS. The additive genetic/specific environment (AE) model offered the best fit for all traits: dropping common environmental (C) effect from the model had virtually no effect on model fit either ($\chi^2=0.239$, $p=0.62$). When compared to the saturated model, the fit of the AE model was good for all traits. Table 2 summarized the proportions of the phenotypic variance of SOS and BMD explained by additive genetic and unique environmental factors in the best fitting AE model. The heritability estimates of BMD and SOS were 71% and 79% respectively.

Phenotypic correlation between dietary fat and BMD and SOS

In the whole sample, a phenotypic correlation between dietary fat and SOS was significant ($r=-0.185$, $p=0.035$) (Table 3). To examine possible gender difference, analyses of the trait relationships also were conducted separately by gender. SOS negatively correlated dietary fat in male twins ($r=0.111$, $p=0.048$). In female twins, we did not observe this association (Table 3).

Genetic and environmental correlations between dietary fat and BMD

Table 4 shows the model-based genetic and environmental correlations between these traits, which were estimated from Cholesky models. SOS and dietary fat was significantly correlated, with a strong genetic correlation between the two ($r_A = -0.88$, 95% CI = -0.94 , 0.01). The environmental correlation was somewhat weak ($r_E = -0.04$, 95% CI = -0.20 , 0.13). In addition, dietary fat also genetically associated with Z-score ($r_A = -0.49$, 95% CI = -0.70 , 0.07). The genetic and environmental correlations between BMD and dietary fat measures were relatively weak.

Gene and environment interactions on BMD

Finally, we tested dietary fat as a moderator of genetic and/or environmental effects on BMD. Our results showed that an interaction between dietary fat and additive genetics on BMD. Dietary fat modified additive genetic (A) effects on BMD, so that additive genetic variances decreased with increasing dietary fat (Table 4). This indicated that genetic factors play a less important role in determining the BMD of subjects with high dietary fat. The results indicated low dietary fat intake was beneficial in those genetically predisposed to osteoporosis.

Data from MZ and DZ twin pairs were examined for a contributory gene-diet interaction in participants who were predisposed to osteoporosis. The mean difference in z-score between high- and low-dietary fat groups in participants with high genetic risk was higher than that between high- and low dietary fat groups in participants with low genetic risk. Similarly, in the groups with high genetic risk for osteoporosis, high dietary fat was significantly associated with low BMD level, while, no significant difference of BMD between high- and low dietary fat groups was observed in the subjects with low genetic risk (Figure 2).

DISCUSSION

We found a strong association between dietary fat and SOS, in particular, in boys in these Chinese children twins. Heritable factors play an important role behind the variation of BMD and SOS. The strong relationship between dietary fat and SOS was due to both genetic and environmental correlations. Further, dietary fat significantly modifies the effect of genetic factors on the variation of BMD. Low dietary fat intake seems beneficial in those genetically predisposed to osteoporosis. However, prospective cohort and intervention studies are needed to confirm the effect and causality.

The reported heritability of different bone phenotypes is high [27, 28]. Previous study reported that BMD had a strong genetic component at all sites with estimates of heritability ranging from 46% to 84% [29]. Genetic effects on cross-sectional BMD are pronounced with heritability estimates of up to 90% depending on sites [29, 30]. Postmenopausal Caucasian female twins showed an estimate of 47% for bone loss at femoral neck, 44% for lumbar spine, and 56% for forearm [11]. Recent Framingham study showed DXA-derived BMD and bone geometry is under strong genetic control, 50% to 70% heritability [31]. The heritability of BMD in the present study is 71%. These apparent inconsistent results of heritability for the BMD are probably in part explained by the larger measurement error. The

effect of measurement error on heritability estimates is usually negligible in cross-sectional BMD studies because the error variance of the DXA measurement is small relative to the total variance in the population [32]. As the twin model does not allow the separation of unique environment and measurement error, these heritability estimates are likely to be conservative as environmental effects may be inflated by the inherent errors in DXA measures over time.

Nutrition also plays an important role in affecting bone mass and has been demonstrated as a significant tool for the prevention of fractures in individuals with osteoporosis [33]. Body weight, physical activity, calcium intake, vitamin D intake, and smoking have been documented to associate with BMD [2]. Any factors that can affect bone density are worthy of attention, especially those modifiable lifestyle. Dietary fat has been suggested to be important to bone health [34]. Associations between dietary fat intake and BMD in several regions of the hip were examined in a nationally representative large sample of American men and women, which showed that saturated fat intake was negatively associated with bone density [3]. The fact that dietary fat was negatively associated with bone density in the femoral neck region of the hip is important because hip fractures induce serious pain, reduced mobility, reduced quality of life, premature mortality, and increased health care costs [35]. However, our study did not confirm the relationship between dietary fat and BMD reported by previous results [3, 36].

Our study found a sex specific association which showed that dietary fat was significantly associated with SOS, but not BMD in male twins. Previous results indicated that associations between dietary fat and bone health are particularly strong in men [3]. One reason that dietary fat has a stronger inverse association with bone density in men may be that, in general, men consumed more fat or saturated fatty acids than women [3]. Furthermore, 1-y post-hip fracture mortality is higher in men than in women [37]. Sex-based differences in the absolute amount of fat in the diet were reported previously across NHANES surveys which indicated that men consume more grams of fat than do women [38]. In addition, the total amount of saturated fat consumed was greater in the younger men than in the older men. This provided the explanations for the finding that 28–47% of American men are estimated to have osteopenia [39]. The fact that effects were strongest in the younger cohort of men is of particular concern and may reflect reduced dietary quality in that segment of the population [3].

A study demonstrated that a brief combined nutritional-physical activity intervention that leads to a controlled decrease in BMI and body fat is associated with improved bone strength [40]. Bone strength determined by QUS measurements of bone SOS was reduced in obese children, but was not affected by the degree of obesity [19]. This means that a long term increased mechanical strain is needed in order to achieve the “protective” effect of body weight on bone mineralization. These results implied that lifestyle play an important role in bone health. In the present study, we also found that dietary fat was significantly associated with SOS in male. It was shown that the model-based genetic and environmental correlations between dietary fat and SOS. SOS and dietary fat was genetically correlated.

We further found dietary fat modified additive genetic (A) effects on BMD, so that additive genetic variances decreased with increasing dietary fat. This indicates that genetic factors play a less important role in determining the BMD of subjects with high dietary fat intake. Interestingly, recent study in the Young Finns Cohort study also investigated the gene-environment interactions on bone traits with longitudinal saturated fatty acids (SAFA) intake [41]. Their results showed dietary saturated fat intake modified the effect of apolipoprotein E promoter polymorphisms on bone structural traits. In the highest SAFA tertile, women with the -219T/T genotype had the smallest total area and SSI at the radial shaft. Therefore, high SAFA intake seems to be more detrimental to bone in women with the -219T/T and +133C/C genotypes than others [41]. Since osteoporosis is considered to have multiple genetic backgrounds, in the future the need for genome wide association study will grow to gather the complete genetic profile of osteoporosis, and define all significant SNPs related to bone fragility. Furthermore, gene-lifestyle interactions contributing directly to bone strength are an important research area in the future, especially for such diseases that are partly preventable.

The mechanism by which dietary fat correlate with BMD is not fully understood. The intimate association between fat and bone has been extensively described. This link is based on the fact that cells which are important in obesity and osteoporosis share a common cell progenitor, the mesenchymal stromal cell (MSC) [42]. The demonstration of the reciprocal relationship between bone and energy metabolism may also explain our results[43]. In addition, the adenosine monophosphate -activated protein kinase (AMPK) has indeed been shown to be a major regulator of food intake by modulating the hypothalamic fatty acid metabolic pathway [42]. The AMPK activation may be involved in the relationship between fat and bone in the marrow by affecting expression and/or activity of transcription factors essential for osteoblastic and adipocytic functions [42]. Finally, hyperlipidemia has been shown to inhibit osteoblastic differentiation in preosteoblast cell and to increase osteoclastic bone resorption [44].

Compared with previous research, our study has several important strengths, including its comprehensive measures of anthropometry and dietary assessment, and use of the twin study design to separate the genetic and environmental effects on BMD. However, our twin study has some shortcoming. For example, no biochemical bone markers, such as osteocalcin or bone-specific alkaline phosphatase, were measured, which if available could have yielded additional information on bone remodeling.

In conclusion, our study showed that approximately 71% and 79% of individual variance in BMD and SOS were due to genetic factors, providing a clear rationale for identifying susceptibility genes via linkage or association studies as well to identify individuals predisposed to higher risk of low BMD. Low dietary fat seemed counteract genetic predisposition to low BMD.

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Abbreviations

BMD	bone mineral density
MZ	monozygotic
DZ	dizygotic
SOS	speed of sound

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Figure 1.

Gene-diet interaction model for dietary fat and BMD

A=Additive genetic factors, E=Specific environmental factors, a=additive genetic path coefficient, e=specific environment path coefficient, μ =mean, β_M =mean modifier, β_X =additive genetic modifier, β_Z =specific environment modifier.

Figure 2.

The influence of genetic risk and dietary fat level on bone health in Chinese child twins*

Data were presented from one twin from each pair. The children had a low (low BMD, <25 percentile) and higher genetic risks for having osteoporosis were identified. Dietary fat were dichotomized into high and low levels.

*White bar represent high dietary fat; Gray bar represent low dietary fat. Error bar represent upper bound of SE.

Two-factor analysis of variance was used to test an interaction between genetic risk category and dietary fat category on BMD

Table 1

Characteristics of child twins in south China(aged 7–15) by zygosity

	All n=622	MZ n=324	DZ n=298	SS DZ n=162	OS DZ n=136
Age, year	11.3±2.5	11.6±2.5	11.0±2.5	11.1±2.6	11.0±2.4
Weight, kg	36.29±11.65	36.85±12.27	35.69±10.93	35.03±10.6	36.47±11.31
height, cm	145.81±14.44	146.33±14.52	145.26±14.35	144.8±14.19	145.8±14.58
BMI, kg/m ²	16.63±2.96	16.75±3.13	16.51±2.76	16.31±2.74	16.74±2.77
Waist, cm	59.96±8.75	60.09±8.98	59.81±8.51	59.52±8.15	60.16±8.94
Dietary fat, kg	78.25±32.82	76.27±43.34	80.40±32.12	82.73±56.99	77.63±32.52
SOS, m/second	3718.9±237.5 ^{ab}	3726.3±171.7 ^b	3710.8±292.6 ^a	3698.6±174.1 ^a	3725.5±389.6 ^b
Z-score	0.63±1.43 ^c	0.81±1.44 ^c	0.42±1.41 ^{ab}	0.33±1.41 ^a	0.53±1.41 ^b
BMD, percentile	61.3±32.5 ^{bc}	65.0±31.8 ^c	57.4±32.8 ^a	55.9±32.3 ^a	59.1±32.9 ^b

Data were expressed as Mean±SD.

^{a,b,c} superscript show the significant differences between groups (p<0.05).

Between-group differences were tested with t test or ANOVA.

The relevant Z-scores were calculated based on BMD and derived from the manufacturer’s database.

MZ, monozygotic; DZ, dizygotic; BMI, body mass index; BMD, bone mineral density; SS, same sex; OS, opposite sex; SOS, speed of sound

Table 2

Parameter estimates for covariates and heritability of BMI, BMD, and SOS from AE models, in child twins in south China (N=622)

	Standardized variance components	
	Additive genetic factor	Unique environmental factor
BMI, kg/m ²	0.23(0.03,0.46)	0.27(0.21, 0.35)
SOS, m/second	0.79(0.78,0.85)	0.20(0.15, 0.28)
Z-score	0.44(0.18, 0.72)	0.31(0.24, 0.38)
BMD, percentile	0.71(0.63, 0.77)	0.28(0.23, 0.36)

BMI, body mass index; BMD, bone mineral density; SOS, speed of sound

Data were presented as model coefficients and 95% confidence intervals.

Table 3

Phenotypic partial correlations between dietary fat and bone mineral density (BMD) in child twins in south China, by gender

	<u>All¹ (n=622)</u>		<u>Boys² (n=298)</u>		<u>Girls² (n=324)</u>	
	Coeff. ³	P	Coeff.	P	Coeff.	P
SOS, m/second	-0.185	0.035	-0.111	0.048	-0.076	0.172
Z-score	-0.071	0.077	-0.067	0.248	-0.072	0.193
BMD, percentile	-0.052	0.201	-0.053	0.364	-0.045	0.415

The phenotypic partial correlations between dietary fat and bone mineral density were tested using linear regression model.

¹ Adjusted for age and sex

² Adjusted for age

³ Pearson partial correlation coefficients

Table 4

Correlations and moderating effect of dietary fat intake on genetic and specific environmental variances of BMD and SOS in child twins in south China (n=622)

Dietary fat	Correlations ¹			Moderating effects ²		
	Additive genetic correlation		Specific environmental correlation	Additive genetic modifications		Specific environmental modifications
	r _A	95%CI		A	95% CI	95% CI
SOS, m/second	-0.88	-0.94, 0.01	-0.04	-0.02	-0.05, 0.04	0.01, 0.05
Z-score	-0.49	-0.70, 0.07	0.03	0.03	-0.11, 0.51	0.01, 0.36
BMD, percentile	-0.13	-1.00, 0.73	-0.03	-0.25	-0.43, 0.03	0.10, 0.28

Additive genetic and specific correlations are based on reparametrization of Cholesky decomposition model.

¹Correlations between additive genetic and specific environmental factors explaining trait correlations between BMD and SOS and dietary fat

²Moderating effect of dietary fat on genetic and specific environmental variances of BMD and SOS.