FTO variant rs9939609 is associated with adiposity but not with energy intake or expenditure in European- and African-American youth

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Abstract

**Background:** Genome-wide association studies found common variants in the fat mass and obesity-associated (FTO) gene associated with obesity in Caucasians and Asians but the association was not confirmed in African populations. Association between FTO variants and insulin resistance, energy intake or energy expenditure showed inconsistent results in previous studies. This study aimed to assess the influence of FTO variant rs9939609 on adiposity, insulin resistance and energy intake and expenditure in European- (EA) and African-American (AA) youths.

**Methods:** We conducted a cross-sectional study in EA and AA youths. One thousand, nine hundred and seventy-eight youth (48.2% EAs, 47.1% male, mean age 16.5 years) had measures of anthropometry. Percent body fat (%BF) was measured by dual-energy X-ray absorptiometry, visceral adipose tissue (VAT) and subcutaneous abdominal adipose tissue (SAAT) by magnetic resonance imaging. Physical activity, energy expenditure and dietary intake were based on self report from up to 7 24-hour recalls.

**Results:** Rs9939609 was significantly associated with BMI ($P=0.01$), weight ($P=0.03$) and waist circumference ($P=0.04$), with per-allele effects of 0.4 kg/m$^2$, 1.3 kg and 0.8 cm, respectively. No significant association was found between rs9939609 and %BF, VAT, SAAT or insulin resistance ($P>0.05$), or between rs9939609 and vigorous physical activity, energy expenditure or energy intake ($P>0.05$). No significant interactions of the FTO variant with ethnicity, gender, vigorous physical activity, energy expenditure or energy intake were observed ($P>0.05$).

**Conclusions:** The FTO variant rs9939609 is associated with general and central obesity, but not with energy intake or energy expenditure. Improved understanding of the effect of the FTO variant on adiposity will offer new insights into obesity.

**Keywords:** FTO variant, general and central adiposity, energy intake, energy expenditure, youth, European- and African- American
Background

Obesity is becoming an increasingly important clinical and public health challenge worldwide and is associated with several comorbidities such as type 2 diabetes, cardiovascular diseases, metabolic syndrome and certain forms of cancer\cite{1-3}. Obesity results from the combined effects of genes, environment and lifestyle\cite{4}. In this context, an understanding of the effects of lifestyle and genes on obesity and also their interactions is important to provide a basis for determining the role they could have on the development and prevention of obesity.

Lifestyle factors, including diet and physical inactivity, are important contributors to weight gain and obesity. However, previous studies showed inconsistent results regarding the association of obesity with physical activity\cite{5, 6} or energy intake\cite{7, 8}.

Because genetic factors play an important role in the development of obesity, the identification of susceptibility genes for obesity, especially common genetic variants in the general population, are of great importance to improve prediction and preventive efforts. Several independent genome-wide association (GWA) studies reported significant associations of common genetic variants (rs9939609) in the fat mass and obesity-associated (\textit{FTO}) gene\cite{9-12} with body mass index (BMI) as a measure of general obesity. The associations of \textit{FTO} and obesity-related phenotypes were further replicated in various populations including Caucasians and Asians\cite{10, 11, 13, 14}, but could not be confirmed in an African population\cite{15}. The \textit{FTO} gene was also reported to be associated with fasting glucose and insulin, but additional adjustment for BMI abolished the association in some studies\cite{16, 17}, but not in others\cite{18, 19}.

The function of \textit{FTO} remains incompletely understood. Because this gene is expressed particularly in the brain, skeletal muscle and adipose tissue, \textit{FTO} may be associated with fatness through effects on regulation of energy homeostasis in the hypothalamus\cite{20}. Two recent studies suggested that the polymorphisms of the \textit{FTO} gene were associated with energy intake rather than energy expenditure\cite{21, 22}. However, other studies suggested there is no association of \textit{FTO} with either energy intake or energy expenditure (e.g., physical activity)\cite{23, 24}.

The main objectives of this study were, first, to assess whether the previously identified common variant rs9939609 in the \textit{FTO} gene is associated with adiposity and insulin resistance in African-
American (AA) and European-American (EA) youth available from the Georgia Cardiovascular Twin study[25], the Lifestyle, Adiposity and Cardiovascular Health in Youths (LACHY) study[8] and the Adiposity Prevention through Exercise (APEX) study[26]; second, to investigate the potential interaction of rs9939609 with ethnicity, gender or lifestyle behaviors (diet and physical activity); third, to investigate whether rs9939609 has a direct influence on energy intake and energy expenditure. In addition to anthropometric measures, more accurate indices for adiposity such as visceral adipose tissue (VAT) and subcutaneous abdominal adipose tissue (SAAT) measured by magnetic resonance imaging (MRI), and percentage of body fat (%BF) based on dual-energy X-ray absorptiometry (DXA) were used in our study.

Subjects and Methods

Subjects

The present study included 1978 subjects from 3 cohorts, the Georgia Cardiovascular Twin study [(n=1210 twins with 588 MZ (291 pairs and 6 singletons) and 622 DZ twins (285 pairs and 52 singletons)], the LACHY study (n=525, including 38 sib-pairs) and the APEX study (n=243, including 29 sib-pairs). All twins in the Georgia Cardiovascular Twin study[25] were recruited from public middle and high schools in the Augusta, Georgia area and the cohort consisted of roughly equal numbers of AAs and EAs (56.1% EA, 47.2% male, mean age [SD]: 18.1[3.8] years). All twin pairs were reared together and zygosity was determined by genotyping 5 standard microsatellite markers using buccal swabs or buffy coat DNA[27]. The LACHY study consisted of approximately equal numbers of EA and AA boys and girls (52.8% EA, 43.8% male) aged 14-18 years recruited from high schools in the Augusta, Georgia area[8]. In the APEX study, subjects were AA boys and girls only (53.6% male), aged 8 to 12 years recruited from local elementary schools. Subjects eligible for the study were only those that weighted <136.1kg[26]. The criteria for classifying subjects as AAs or EAs using self-identification of ethnicity have been described previously[28]. Subjects in all the 3 studies were apparently healthy, free of any acute or chronic illness on the basis of parental reports and were taking no medication that could influence the results. The Institutional Review Board at the Medical College of Georgia approved the studies. Informed consent was obtained from all subjects and by parents if subjects were <18 years of age.
**Anthropometrics and body composition assessment**

Height and weight were measured by standard methods using a wall-mounted stadiometer and a digital scale, respectively. BMI was calculated as weight/height$^2$ (kg/m$^2$). Waist circumference (in cm) was measured twice at the center of the umbilicus over the T-shirt and the values were averaged. Skinfold thicknesses (i.e. triceps, subscapular, and suprailiac) were measured on the right side of the body with Lange calipers according to established protocols[29]. Three sets of measurements for each skinfold were recorded and averaged. The inter-correlations were >99%. Measurements of skinfold thickness were available in 1976 subjects. BMI and the sum of the 3 skinfold thicknesses were used as measures of general adiposity, while waist circumference was used as a measure of central adiposity.

**Biochemical assays**

Fasting glucose and insulin concentrations were measured at the NIDDK supported Clinical Nutrition Research Unit Core Laboratory at the University of Alabama. Glucose was measured in 10 µL of sera using an Ektachem DT II system (Johnson and Johnson Clinical Diagnostic, Rochester, NY). Insulin was assayed in duplicate 100-µL aliquots of serum by specific radioimmunoassay (Linco Research, Inc, St Charles, Mo). Cross-reactivity with proinsulin is <0.2%. Assay sensitivity was 3.41mU/mL. The intra-assay coefficient of variation was 3.7%. Fasting glucose and insulin were only available in a subsample of twins as twins coming on afternoon visits were not required to fast.

Based on fasting glucose and insulin we used the homeostasis model assessment (HOMA) 2 to calculate insulin resistance (HOMA2-IR) and beta-cell function (HOMA2-%B) using a nonlinear computer model as specified in the HOMA2 software (http://dtu.ox.ac.uk/homa).

**Dual-energy X-ray absorptiometry**

In the LACHY study, %BF was measured using DXA (Hologic QDR-4500W, software version 6.0, Waltham, MA, USA). DXA provides reliable values for %BF[8]. Repeat measurements were performed using the QDR-4500W machine with 219 adolescents and the intraclass correlation coefficient (ICC) for %BF was found to be 0.99. For some subjects, DXA values were only available from the Hologic QDR-1000W, but not from the Hologic QDR-4500W model. For these individuals, %BF values were derived from prediction equations based on 284 youths who
were assessed on both instruments, using linear regression; ethnicity, gender and the QDR-1000W measurement were the predictor variables. The multiple $R^2$ value for %BF was 0.99[30]. In the APEX study, all %BF measurements were obtained using a Hologic QDR-1000 (Waltham, MA) as previously described, the ICC for %BF was > 0.998 between two scans[31]. DXA calibration was done each day, as specified by the Hologic Company. DXA scans were not performed in the Georgia Cardiovascular Twin study.

**Magnetic resonance imaging**

In both the LACHY and APEX studies, VAT and SAAT was determined using MRI (1.5 T General Electric Medical Systems, Milwaukee, WI) as described previously[32]. Briefly, with subjects in the supine position, a series of five, 1-cm-thick, transverse images was acquired beginning at the inferior border of the fifth lumbar vertebra and proceeding toward the head. A gap is left between the slices to avoid cross-talk. Values for VAT and SAAT from a single image were calculated in terms of surface area ($cm^2$) and the volume ($cm^3$) estimated by multiplying the surface area by the image width (1 cm) and then summing the five images. VAT and SAAT were measured in the Department of Radiology on equipment dedicated to patient care. VAT and SAAT measures were obtained in those subjects who underwent testing on days when the MRI equipment was available for the research study. Eventually, VAT and SAAT measurements were available for 394 subjects. Measurements of VAT and SAAT were not available in the Georgia Cardiovascular Twin study and in the males of the APEX study.

**Environmental variables**

Diet was measured with individual, non-consecutive, 24-h recalls that covered the period from midnight to midnight of the previous day. In the LACHY study, we sought to obtain seven recalls from each participant, one of each day of the week and only those subjects that provided at least four recalls were included in the analysis. The diet assessment has been previously described in detail[8]. In the APEX study, two 24-h diet recalls were obtained from each participant. PA was self-reported and quantified using our modified version of the previous day PA recall[26], which recorded activities in 30-min time blocks for 24-h period (midnight to midnight). Subjects were asked to recall the activities concurrently with each 24-h diet recall and report at which level of effort (light, moderate, hard, very hard) they engaged in each activity. Total energy expenditure
(without sleep) (TEE) was estimated from weight and metabolic equivalents (METs, 1MET=4.184KJ/kg.hr) assigned to the four activity categories as follows: light (1.5 METs), moderate (4.0 METs), hard (6.0 METs), and very hard (10.0 METs). For example, energy expenditure from light physical activity (KJ/d) was calculated as light (hrs/d)*1.5*4.184(KJ/kg.hr)*weight. Vigorous energy expenditure[33] was defined as energy expenditure from vigorous physical activity (VPA), i.e., the combined hours of hard and very hard physical activity per day. Average TEE, VEE and VPA were estimated from available 24-hr recalls. The daily mean number of hours spent in VPA rather than those spent in moderate PA was included in the present study, since only VPA was previously shown to be negatively associated with %BF[8]. No measurements of energy intake, VPA or energy expenditure were available in the Georgia Cardiovascular Twin study.

Genotyping
DNA was extracted from buffy coats by using the QiaAmp DNA Blood Mini Kit (Qiagen, Valencia, CA) or from buccal swabs by using QuickExtract DNA Extraction Kit (Epicentre, Madison, WI). The FTO rs9939609 was genotyped by allelic discrimination Taqman assays (Applied Biosystems, Foster City, CA). PCR were performed in a 96-well format in a total of 5 µl reaction volume using 10ng of genomic DNA and FAM/VIC dye labeled allelic probes with the Taqman Universal Fast Master mix and subjected to 95°C for 15 min, and 40 cycles of 95°C for 15 sec and 60°C for 1 min on an ABI 9800 Fast Thermocycler (Applied Biosystems, Foster City, CA). The Taqman assay plates were transferred to an ABI 7500 Fast Real Time PCR system in which the fluorescence intensity in each well of the plate was recorded and genotypes were analyzed using Sequence Detection Software 1.3. Genotyping quality control procedures included genotyping 10% duplicates for accuracy checking and inclusion of both positive and non-template controls in each 96-well plate. Genotyping success rate was 99.5% for rs9939609. Genotyping accuracy as determined by concordance between duplicates, was 100%.

Statistical Analyses

The main effects of the SNP on obesity-related phenotypes were tested using structural equation modeling with the statistical software Mx[34]. In this approach a model is specified for both the
means and the variance-covariance matrices. We adapted the model described previously[35] to include MZ twin pairs, DZ twin pairs (or sib-pairs) and unpaired twins/singleton, which allows for non-independent observations in twin and family data. By modeling MZ and DZ (or sib-pair) covariances separately we accounted for their different degrees of relatedness. The SNP effect was analyzed in the combined data from all 3 cohorts. Effects of age, ethnicity, gender and cohort were regressed out for all variables before using the residuals in Mx models. Gender and ethnicity-specific effects of the SNP were modeled as interactions of the SNP with gender and/or ethnicity using regression analyses within a generalized estimating equations (GEE) framework, which takes the non-independency of twin and family data into account and yields unbiased $P$ values[36].

In the APEX study, subjects were randomized to a physical activity intervention or control group. Obesity-related phenotypes were measured three times, at the baseline, mid- and end-point of the study. The measurement at the baseline, prior to randomization, was used for the analysis.

Hardy-Weinberg equilibrium (HWE) and ethnic differences in allele and genotype frequencies were tested by a $\chi^2$ test in only one member per family (i.e, twins or sib pairs), which was chosen randomly to prevent inflated significance. All variables except %BF were log transformed to obtain better approximations of the normal distribution. Preliminary and GEE analyses were performed using Stata 10 software (StataCorp, College Station, TX). A $P$ value of $\leq0.05$ was considered to be statistically significant.

**Results**

**Participant characteristics**

Descriptive statistics for age, height, adiposity and insulin resistance related variables and environmental predictors of adiposity in combined data of the 3 studies are presented by ethnicity and gender in Table 1. Effects of ethnicity, gender and their interaction were tested using GEE with age and cohort identifier included as covariates. The mean age of the total sample was 16.5 years. Many significant gender differences were observed, although some of these were limited to one ethnic group. Similarly, many of the significant ethnic differences were limited to either males or females, as indicated in Table 1.
Environmental predictors of adiposity

Table 2 shows the regression analysis in LACHY and APEX studies combined (n=784) for BMI, waist circumference, sum of skinfolds, %BF, VAT and SAAT. After adjustment for age, ethnicity, gender, cohort and the interaction between ethnicity and gender, VPA was negatively related to sum of skinfolds ($P=0.01$), %BF ($P<0.001$) and SAAT ($P=0.05$) accounting for 0.43%, 2.35% and 1.11% of the variance respectively, but not to BMI ($P=0.96$), waist circumference ($P=0.83$) or VAT ($P=0.45$). EI was a negative determinant of BMI ($P=0.03$), sum of skinfolds ($P=0.02$), %BF ($P<0.001$), VAT ($P=0.02$) and SAAT ($P=0.01$) accounting for 0.61%, 0.74%, 1.82%, 1.16% and 1.21% of the variance respectively. %CARBO was negatively associated with BMI ($P=0.02$), sum of skinfolds ($P=0.008$) and %BF ($P=0.007$) (Table 2). Percentage of energy from protein (%PRO) was a positive predictor of BMI ($P<0.001$), waist circumference ($P<0.001$), sum of skinfolds ($P<0.001$) and %BF ($P<0.001$). The relationship of dietary %FAT with these dependent variables was not significant (Table 2).

Allele and genotype frequencies

Rs9939609 was common in both ethnic groups with minor allele frequency (MAF) of 44.6% in EA and 48.4% in AA. There was a significant difference in allele frequencies between EA and AA subjects ($P=0.03$), but no significant difference in genotype frequencies ($P=0.08$). Rs9939609 was in HWE in both ethnic groups ($P=0.34$ in EA and 0.92 in AA).

Associations between FTO rs9939609 and obesity-related phenotypes

We found that the rs9939609 A allele was positively associated with BMI (per-allele effect of 0.4kg/m², $P=0.01$), weight (per-allele effect of 1.3kg, $P=0.03$), and waist circumference (per-allele effect of 0.8cm, $P=0.04$) in additive models (Figure 1). The explained percentages of variance were 0.24%, 0.20% and 0.16%, respectively. In a recessive model, rs9939609 was also significantly associated with BMI ($P=0.03$), weight ($P=0.006$), and waist circumference ($P=0.02$), with explained percentages of variance of 0.17%, 0.22% and 0.17%, respectively. The A allele carriers showed higher sum of skinfold thicknesses, %BF, VAT and SAAT compared to the TT allele carriers, but the difference did not reach statistical significance (Table 3). No significant associations were found between rs9939609 and fasting glucose, fasting insulin, HOMA2-%B or
HOMA2-IR before or after additional adjustment for BMI (Table 3). No significant interactions between rs9939609 and ethnicity or gender were observed for any of these obesity-related phenotypes ($P>0.05$).

**Associations between FTO rs9939609 and energy intake, VPA and energy expenditure in LACHY and APEX studies combined**

No significant association was found between rs9939609 and the total energy intake, %CARBO, %PRO or %FAT. Neither did we find association of rs9939609 with VPA (irrespective of adjustment for BMI) or with VEE or TEE (Table 4).

No significant gene-environment interactions between rs9939609 and energy intake, VPA or energy expenditure were observed for any of the obesity or insulin resistance-related phenotypes ($P>0.05$) (data not shown).

**Discussion**

In the current study, the association between a common variant in the *FTO* gene and obesity and insulin resistance-related phenotypes was investigated in 1978 EA and AA youth, available from the Georgia Cardiovascular Twin, LACHY and APEX studies. We replicated the significant association between *FTO* variant rs9939609 and BMI, weight and waist circumference. Effects of *FTO* rs9939609 did not depend on ethnicity or gender. No significant associations of rs9939609 with fasting glucose, fasting insulin or insulin resistance were found, irrespective of correction for BMI; Associations of rs9939609 with energy intake, VPA or energy expenditure was not significant, nor were effects of rs9939609 on adiposity modified by energy intake or energy expenditure.

In the LACHY and APEX studies, VPA was a negative predictor of sum of skinfolds, %BF and SAAT, and energy intake was a strong negative determinant of all dependent variables except waist circumference. The inverse relation between energy intake and obesity could be explained by the fact that individuals performing more free-living physical activity tend to have less fat accumulation but at the same time higher energy intake. The dietary %PRO was a positive
predictor for BMI, waist circumference, sum of skinfolds and %BF, but the dietary %FAT was not found to be a predictor of any obesity-related phenotype. This finding is in line with a previous study on 6-14 year old children, which showed that higher %PRO was associated with overweight after adjusting for age, gender and total energy, whereas %FAT was not found to be a predictor of BMI[37]. We found an inverse association between %CARBO and BMI, sum of skinfolds and %BF, which is consistent with a previous finding[38]. However, it remains difficult to resolve cause and effect in cross-sectional studies, in which energy intake and adiposity are measured at the same time.

Since Frayling et al[12] first reported the significant associations between the FTO variant rs9939609 and obesity-related phenotypes such as BMI, weight, waist circumference, %BF and skinfolds in both children and adults, several studies have replicated these findings in Europeans[10, 11, 14, 16] and Asians[13]. In our sample of EA and AA youth, we replicated the significant associations of rs9939609 with BMI, weight and waist circumference using an additive model, although a recessive model was more appropriate for weight and waist circumference. Our per-A allele effect of 0.4kg/m² in BMI is similar to the effect that Frayling reported in UK children at the age of 11 years (0.4kg/m², \( P=7 \times 10^{-9} \)) and a little higher than that in Finnish children at the age of 14 years (0.1kg/m², \( P=0.04 \)). We found that the variance in BMI explained by rs9939609 was 0.24%, which is lower than previously reported ~1.0%[12]. The MAF in EAs (0.45) in our study was the same as that reported in HapMap, while the MAF in AAs (0.48) was slightly lower (0.52 in Hapmap). The MAF in EAs was a little higher than reported in other study[12]. In addition, overall per-A allele increases in weight (~1.3kg) and waist circumference (~0.8cm) observed in our study were similar to the effects reported by Frayling et al[12](~1.2kg and ~1.0cm, respectively). Similar to Frayling et al[12], we found that sum of skinfolds increased with the number of A allele carried; however, the difference did not reach significance. The lack of association between rs9939609 and %BF is not in agreement with Frayling et al’s finding in children at 9 years of age[12]. The discrepancy in the findings between the two studies could be partly due to differences in population characteristics, such as age, gender or ethnic composition, as well as environmental exposures. Although the A-allele carriers had slightly higher VAT and SAAT compared to TT carriers, we did not find significant
associations between rs9939609 and VAT or SAAT. This might due to the smaller number of subjects that had MRI examinations.

Previous studies reported associations of rs9939609 with fasting glucose, fasting insulin, or insulin resistance. In most of these studies adjustment for BMI abolished the associations[16, 17], but in some studies they remained significant[18, 19]. We did not find any significant associations before or after adjustment for BMI.

The FTO gene is significantly associated with weight, BMI and other obesity-related phenotypes. The mechanism underlying the association of FTO gene and adiposity may be related to a direct function of FTO[39] or linkage disequilibrium of the tested variant with other causative variants in a gene near the FTO locus. It is known that FTO is highly expressed in the hypothalamic region[40], an area that is known to be involved in the regulation of appetite; and studies on the expression of FTO suggest it might have a role in central control of energy homeostasis[20, 39].

Previous studies that have investigated the association of rs9939609 with energy intake and energy expenditure, all showed lack of association of rs9939609 with energy expenditure[21-23], but findings on the associations with energy intake were inconsistent[21, 22, 24, 41]. We also investigated the role of rs9939609 in the control of energy intake and energy expenditure in the LACHY and APEX studies in which accurate measures on VPA, energy intake and energy expenditure were available. No significant association was found between rs9939609 and VPA, energy intake or energy expenditure. A study in 2726 Scottish children found that rs9939609 does not appear to be involved in the regulation of energy expenditure, but may have a role in the control of food intake and food choice[41]. In 3337 UK children, Wardle et al found that the rs9939609 A allele is likely to exert its effects by influencing appetite[42] and the T allele might be protective against overeating by promoting responsiveness to internal signals of satiety[43]. Currently little is known about the function of the FTO gene. Functional tests in mice indicated that FTO may play a role in adipocyte function but not adipogenesis[44]. Wählen et al [45] studied FTO with regard to fat cell function and adipose tissue gene expression, their results suggested that FTO might be involved in body weight regulation through lipolysis.
A study reported that low physical activity accentuated the effect of rs9939609 on body fat accumulation[46]. However, we did not find any interaction of rs9939609 with physical activity on any phenotype related to obesity, which is in line with a number of other studies[47, 48].

Studying populations of different ancestry will help to globally identify and understand the genetic and environmental factors associated with obesity. As such, we included AAs as well as EAs in our study. We found significant association between rs9939609 and BMI, weight and waist circumference in the combined EA and AA sample. Although the MAF in EAs was significantly different from those in AAs, no interaction between rs9939609 and ethnicity was observed for weight, BMI or other obesity-related phenotypes. The association between rs9939609 and BMI has been widely established in Europeans[9-12], but could not be confirmed in an African population[15]. In a large sample of African Americans, no significant association was found between obesity and FTO variant rs8050136, which was in high LD with rs9939609 ($r^2=0.82$ in YRI population)[14]. However, our finding suggests that the effects of rs9939609 on obesity-related phenotype were similar for EAs and AAs. For example, the per-allele effect on BMI was 0.35kg/m$^2$ and 0.45kg/m$^2$ in EAs and AAs, respectively.

The major strengths of our study are (1) the inclusion of in-depth estimates based on multiple self reports of both dietary energy intake and total energy expenditure, which allowed us to investigate the direct association with rs9939609 and their potential interactions with the SNP in their effect on adiposity; and (2) the use of %BF by DXA and VAT and SAAT by MRI, which are more precise measures of general and/or central adiposity than BMI and waist circumference. Furthermore, the inclusion of AA as well as EA youth allowed us to investigate a potential interaction of rs9939609 with ethnicity. Several limitations of our study need to be acknowledged as well. In the Georgia Cardiovascular Twin study, no measurements of %BF, VAT and SAAT were available since DXA scans and MRI examinations were not performed. Meanwhile, in the twin study, fasting glucose and insulin were only available from part of the subjects since fasting was not requested for twins examined in the afternoon. Sexual maturation was not assessed in the twin cohort and could not be incorporated as a covariate. A final limitation is that our measure of energy intake was based on self report and not measured directly. However, our results were in
line with previous studies that did assess energy expenditure directly through more sophisticated means[23, 41].

**Conclusion**

In summary, we replicated in a large sample of EA and AA youth the significant association between the *FTO* variant rs9939609 and BMI, weight and waist circumference. Moreover, we did not find any significant association between rs9939609 and VPA, energy intake or energy expenditure. Furthermore, we did not observe any significant interactions between rs9939609 and ethnicity, gender, physical activity, energy expenditure or energy intake for any obesity-related phenotypes. These findings may be helpful in improving our understanding of the underlying mechanism and pathways whereby the variant influences the development of obesity.

**Abbreviations**

GWAS—genome-wide association study  
SNPs—single nucleotide polymorphisms  
FTO—fat mass and obesity-associated  
AA—African-American  
EA—Europe-American  
LACHY—the Lifestyle, Adiposity and Cardiovascular Health in Youths  
APEX—Adiposity Prevention through EXercise  
BMI—Body Mass Index  
%BF—percent body fat  
DXA—dual-energy X-ray absorptiometry  
VAT—visceral adipose tissue  
SAAT—subcutaneous abdominal adipose tissue  
MRI—magnetic resonance imaging  
MZ—monozygotic  
DZ—dizygotic  
HOMA—homeostasis model assessment  
ICC—intraclass correlation coefficient  
IR—insulin resistance
HOMA2-%B—homeostasis model assessment 2 β-cell function
GEE—generalized estimating equations
HWE—Hardy-Weinberg equilibrium
MAF—minor allele frequencies
CI—confidence interval
VPA—vigorouse physical activity
TEE—total energy expenditure
VEE—vigorouse energy expenditure

Competing interests
The authors have indicated they have no financial relationship to this article to disclose, and there is no conflict of interest associated with this work.

Author’s contributions
Gaifen Liu performed the statistical analysis and drafted the manuscript; Haidong Zhu did the genotyping and participated in the drafting of the manuscript; Vasiliki Lagou participated in the drafting of the manuscript; Bernard Gutin conducted the LACHY study and edited the manuscript; Inger S Stallmann-Jorgensen edited the manuscript; Frank A Treiber conducted the twin study and edited the manuscript; Yanbin Dong provided significant advice and participated in the drafting of the manuscript; Harold Snieder developed the original idea for the study and participated in the design of the study and in the drafting of the manuscript.

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References


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<td>Sum of skinfolds (mm)</td>
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<tr>
<td>Sum of skinfolds (mm)</td>
</tr>
<tr>
<td>%BF</td>
</tr>
<tr>
<td>%BF</td>
</tr>
<tr>
<td>VAT(cm³)</td>
</tr>
<tr>
<td>VAT(cm³)</td>
</tr>
<tr>
<td>SAAT (cm³)</td>
</tr>
<tr>
<td>%CARBO</td>
</tr>
<tr>
<td>%CARBO</td>
</tr>
<tr>
<td>%PRO</td>
</tr>
<tr>
<td>%PRO</td>
</tr>
<tr>
<td>%FAT</td>
</tr>
<tr>
<td>VPA (hours/d)</td>
</tr>
<tr>
<td>VEE(Kilojoules/d)</td>
</tr>
<tr>
<td>TEE((Kilojoules/d)</td>
</tr>
</tbody>
</table>

BMI=body mass index; SD=standard deviation; %BF=percentage of body fat; VAT=Visceral adipose tissue; SAAT=Subcutaneous abdominal adipose tissue; HOMA2-%B=homeostasis model assessment 2 beta-cell function; HOMA2-IR=homeostasis model assessment 2 insulin resistance; EI=energy intake; %CARBO= percentage of energy from carbohydrates; %PRO= percentage of energy from protein ;%FAT= percentage of energy from fat; VPA=Vigorous physical activity ; VEE=Energy expenditure from vigorous activity; TEE=Total energy expenditure(without sleep).

N: number of subjects wit phenotype and genotype data;  
a: adjusted for age and cohort identifier;  
b: adjusted for age, cohort identifier and BMI  

*significant only in males, **significant only in females, #significant only in EAs, ##significant only in AAs.
Table 2  Environmental predictors of BMI, waist circumference, sum of skinfolds, %BF, VAT and SAAT in the combined LACHY and APEX studies

<table>
<thead>
<tr>
<th>predictor</th>
<th>BMI</th>
<th>WC</th>
<th>Sum of skinfolds</th>
<th>%BF</th>
<th>VAT</th>
<th>SAAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>P</td>
<td>%V</td>
<td>β</td>
<td>P</td>
<td>%V</td>
</tr>
<tr>
<td>VPA (h/day)</td>
<td>-0.96</td>
<td>-</td>
<td>+0.83</td>
<td>-</td>
<td>0.01</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>0.01</td>
<td>2.35</td>
<td>-</td>
<td>0.45</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>&lt;0.001</td>
<td>0.05</td>
<td>1.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EI (Kilojoules/d)</td>
<td>0.03</td>
<td>0.61</td>
<td>-0.84</td>
<td>-</td>
<td>0.02</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>0.02</td>
<td>1.82</td>
<td>-</td>
<td>0.02</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>&lt;0.001</td>
<td>0.01</td>
<td>1.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%CARBO</td>
<td>-</td>
<td>0.02</td>
<td>0.48</td>
<td>-</td>
<td>0.008</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>0.007</td>
<td>0.73</td>
<td>-</td>
<td>0.96</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>0.21</td>
<td>-</td>
<td>1.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%PRO</td>
<td>+</td>
<td>&lt;0.001</td>
<td>1.84</td>
<td>+</td>
<td>&lt;0.001</td>
<td>1.19</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1.47</td>
<td>+</td>
<td>&lt;0.001</td>
<td>1.29</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>&lt;0.001</td>
<td>0.96</td>
<td>0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%FAT</td>
<td>+</td>
<td>0.44</td>
<td>-</td>
<td>0.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.20</td>
<td>-</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.84</td>
<td>-</td>
<td>0.40</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Based on the model with age, gender, ethnicity, cohort and the interaction of gender and ethnicity. Negative and positive indicate the direction of relation between the predictor and dependent variables. P<0.05 are indicated in bold. %V=explained variance.

Abbreviations: BMI= body mass index, WC=waist circumference, %BF= percentage of body fat, VAT=visceral adipose tissue, SAAT=subcutaneous abdominal adipose tissue, VPA=vigorous physical activity, EI=energy intake, %FAT= percentage of energy from fat, %CARBO= percentage of energy from carbohydrates, %PRO= percentage of energy from protein.
## Table 3 Association between rs9939609 and obesity-related phenotypes

<table>
<thead>
<tr>
<th>Variables</th>
<th>No.</th>
<th>Mean (95%CI)</th>
<th>$\chi^2$ (a/b)</th>
<th>$P$ (a/b)</th>
<th>Variance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT/AT/AA</td>
<td>TT/TA/AA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>599/962/417</td>
<td>61.5(60.4-62.6)</td>
<td>62.0(61.0-63.0)</td>
<td>64.1(62.6-65.7)</td>
<td>4.97</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>599/962/414</td>
<td>22.5(22.2-22.9)</td>
<td>22.8(22.5-23.1)</td>
<td>23.3(22.8-23.8)</td>
<td>6.38</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>599/960/416</td>
<td>75.1(74.3-76.0)</td>
<td>75.5(74.8-76.2)</td>
<td>76.7(75.6-77.9)</td>
<td>4.39</td>
</tr>
<tr>
<td>Suprailiac (mm)</td>
<td>599/962/417</td>
<td>14.4(13.7-15.0)</td>
<td>14.4(13.9-15.0)</td>
<td>14.7(13.7-15.6)</td>
<td>1.07</td>
</tr>
<tr>
<td>Subscapular (mm)</td>
<td>598/961/417</td>
<td>14.1(13.5-14.7)</td>
<td>14.1(13.6-14.6)</td>
<td>14.7(13.9-15.5)</td>
<td>2.28</td>
</tr>
<tr>
<td>Triceps (mm)</td>
<td>599/961/417</td>
<td>15.7(15.2-16.3)</td>
<td>15.6(15.1-16.1)</td>
<td>15.8(15.0-16.6)</td>
<td>0.37</td>
</tr>
<tr>
<td>Sum of skinfold (mm)</td>
<td>599/960/417</td>
<td>44.8(43.2-46.5)</td>
<td>44.9(43.4-46.4)</td>
<td>46.0(43.7-48.5)</td>
<td>1.24</td>
</tr>
<tr>
<td>%BF</td>
<td>183/377/208</td>
<td>24.9(23.7-26.1)</td>
<td>25.1(24.2-25.9)</td>
<td>25.2(24.0-26.4)</td>
<td>0.09</td>
</tr>
<tr>
<td>VAT (cm$^3$)</td>
<td>97/187/110</td>
<td>78.0(69.5-87.5)</td>
<td>82.1(75.2-89.8)</td>
<td>78.3(68.9-89.0)</td>
<td>0.00</td>
</tr>
<tr>
<td>SAAT (cm$^3$)</td>
<td>97/187/109</td>
<td>627.1(539.1-729.6)</td>
<td>661.6(587.2-745.4)</td>
<td>673.5(568.4-798.0)</td>
<td>0.69</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>213/391/211</td>
<td>5.07(5.02-5.13)</td>
<td>5.10(5.06-5.15)</td>
<td>5.10(5.05-5.16)</td>
<td>0.57/0.25</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>208/387/205</td>
<td>94.4(88.6-100.6)</td>
<td>99.7(94.8-104.9)</td>
<td>94.8(86.5-103.9)</td>
<td>0.66/0.19</td>
</tr>
<tr>
<td>HOMA2-%B</td>
<td>206/385/205</td>
<td>140.1(134.8-145.7)</td>
<td>142.4(138.1-146.8)</td>
<td>139.3(132.5-146.4)</td>
<td>0.01/1.09</td>
</tr>
<tr>
<td>HOMA2-IR</td>
<td>206/385/205</td>
<td>1.7(1.6-1.9)</td>
<td>1.8(1.8-1.9)</td>
<td>1.8(1.6-1.9)</td>
<td>0.62/0.18</td>
</tr>
</tbody>
</table>

BMI = body mass index; %BF = percentage of body fat; VAT = Visceral adipose tissue; SAAT = Subcutaneous abdominal adipose tissue; HOMA2-%B = homeostasis model assessment 2 β-cell function; HOMA2-IR = homeostasis model assessment 2 insulin resistance; $P$-values represent significance of the additive model (per-allele effect); Significant associations ($P \leq 0.05$) are indicated in bold.

All variables are presented as means and 95%CI adjusted for age, gender, ethnicity and cohort identifier.

%variance = 100* (the difference of R-square value of the regression model additional with SNP compared to the base model).

- a: adjusted for age, ethnicity, gender, cohort identifier;
- b: adjusted for age, ethnicity, gender, cohort identifier and BMI.
Table 4 Association between rs9939609 and energy intake, physical activity and energy expenditure in the combined LACHY and APEX studies

<table>
<thead>
<tr>
<th>Variables</th>
<th>No.</th>
<th>Mean (SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT/AT/AA</td>
<td>TT</td>
<td>TA</td>
</tr>
<tr>
<td>EI(Kilojoules/d)</td>
<td>187/379/209</td>
<td>7958.8(2772.2)</td>
<td>7706.8(2698.3)</td>
</tr>
<tr>
<td>%CARBO</td>
<td>187/379/210</td>
<td>53.3(7.3)</td>
<td>53.2(6.9)</td>
</tr>
<tr>
<td>%PRO</td>
<td>187/380/209</td>
<td>13.9(2.9)</td>
<td>13.7(2.9)</td>
</tr>
<tr>
<td>%FAT</td>
<td>186/380/210</td>
<td>33.6(5.3)</td>
<td>34.1(5.6)</td>
</tr>
<tr>
<td>VPA (hours/d)</td>
<td>181/359/204</td>
<td>0.5(0.6)</td>
<td>0.5(0.6)</td>
</tr>
<tr>
<td>VEE(Kilojoules/d)</td>
<td>181/359/204</td>
<td>1094.4(1499.4)</td>
<td>990.8(1299.9)</td>
</tr>
<tr>
<td>TEE(Kilojoules/d)</td>
<td>181/362/205</td>
<td>7829.8(3145.5)</td>
<td>7461.0(3217.7)</td>
</tr>
</tbody>
</table>

*P*-values represent significance of the additive model (per-allele effect); EI=energy intake; %CARBO=percentage of energy from carbohydrates; %PRO=percentage of energy from protein; %FAT=percentage of energy from fat; VPA=vigorous physical activity; VEE=energy expenditure from vigorous activity; TEE=total energy expenditure (without sleep).

a: adjusted for age, ethnicity, gender;
b: adjusted for age, ethnicity, gender and BMI;
Figure 1  Association between rs9939609 and weight, BMI and waist circumference

1. Weight, BMI and waist circumference were presented as geometric mean and 95% CI adjusted for age, ethnicity, gender and cohort;
2. $P$ values represent significance of the additive model, adjusted for age, gender, ethnicity and cohort identifier.