Original Contribution

Association of the FTO Obesity Risk Variant rs8050136 With Percentage of Energy Intake From Fat in Multiple Racial/Ethnic Populations

The PAGE Study


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Initially submitted July 6, 2012; accepted for publication February 7, 2013.

Common obesity risk variants have been associated with macronutrient intake; however, these associations' generalizability across populations has not been demonstrated. We investigated the associations between 6 obesity risk variants in (or near) the NEGR1, TMEM18, BDNF, FTO, MC4R, and KCTD15 genes and macronutrient intake (carbohydrate, protein, ethanol, and fat) in 3 Population Architecture using Genomics and Epidemiology (PAGE) studies: the Multiethnic Cohort Study (1993–2006), the Atherosclerosis Risk in Communities Study (1987–1989), and the Epidemiologic Architecture for Genes Linked to Environment (EAGLE) Study, which accesses data from the Third National Health and Nutrition Examination Survey (1991–1994). We used linear regression, with adjustment for age, sex, and ethnicity, to estimate the associations between obesity risk genotypes and macronutrient intake. A fixed-effects meta-analysis model showed that the FTO rs8050136 A allele was positively associated with percentage of calories derived from fat (βmeta = 0.2244 (standard error, 0.0548); P = 4 × 10−5) and inversely associated with percentage of calories derived from carbohydrate (βmeta = −0.2796 (standard error, 0.0709); P = 8 × 10−5). In the Multiethnic Cohort Study, percentage of calories from fat assessed at baseline was a partial mediator of the rs8050136 effect on body mass index (weight (kg)/height (m)2) obtained at 10 years of follow-up (mediation of effect = 0.0823 kg/m2, 95% confidence interval: 0.0559, 0.1128). Our data provide additional evidence that the association of FTO with obesity is partially mediated by dietary intake.

Abbreviations: ARIC, Atherosclerosis Risk in Communities; BMI, body mass index; CALiCo, Causal Variants Across the Life Course; EAGLE, Epidemiologic Architecture for Genes Linked to Environment; FFQ, food frequency questionnaire; MEC, Multiethnic Cohort; NHANES III, Third National Health and Nutrition Examination Survey; PAGE, Population Architecture using Genomics and Epidemiology; SE, standard error; SNP, single-nucleotide polymorphism.

The prevalence of obesity has been increasing globally, resulting in increased rates of type 2 diabetes, cardiovascular disease, and certain cancers (1). Risk factors for obesity include a sedentary lifestyle, overconsumption of energy, and genetic susceptibility (2, 3). Recent genome-wide association studies have identified several common genetic variants associated with body mass index (BMI; weight (kg)/height (m)2), a measure of adiposity (4, 5). However, the mechanisms...
underlying these associations have yet to be characterized. Given that macronutrient consumption plays a key role in obesity (6), understanding the biological links between inherited genetic variation and components of energy intake may suggest strategies for reducing the increasing prevalence of obesity (7).

To date, at least 32 independent loci have been found to be associated with BMI (8). Many of the genes involved, such as the brain-derived neurotrophic factor gene (BDNF), the melanocortin 4 receptor gene (MC4R), and the fat mass and obesity-associated gene (FTO), are expressed in the central nervous system (9–11), suggesting that they may act through the neuroendocrine control of various aspects of energy balance. Indeed, studies have found that obesity risk variants in intron 1 of the FTO gene are associated with greater energy consumption (12–17) or frequency of energy consumption (18) and a reduction in satiety (19–21) and intake control (12, 22, 23). In a recent study of approximately 2,100 adults of mostly European descent (77%), McCaffery et al. (18) found that FTO rs1421085 was associated with percentage of calories derived from fat. Additionally, this and another study found that obesity risk variants in the potassium channel tetramerization domain-containing 15 gene (KCTD15) and BDNF were associated with macronutrient intake (24) or increased meat and dairy intake (18). However, many of these previous studies were conducted in populations of primarily European descent.

To further understand the relationship between obesity single-nucleotide polymorphisms (SNPs) and energy consumption, we investigated, as part of the Population Architecture using Genomics and Epidemiology (PAGE) Study (25), the relationship between 6 obesity risk variants and intake of macronutrients (carbohydrate, protein, ethanol, and fat and its subtypes) among type 2 diabetes-free adults from 5 racial/ethnic groups.

**MATERIALS AND METHODS**

**Study descriptions**

The PAGE Study (25) was initiated in 2008 by the National Human Genome Research Institute and is a collaboration between a coordinating center (25) and multiple studies carried out at several different sites. PAGE is comprised of the Epidemiologic Architecture for Genes Linked to Environment (EAGLE) Study, which accesses data from the Third National Health and Nutrition Examination Survey (NHANES III) (1991–1994) (26, 27); the Causal Variants Across the Life Course (CALiCo) Consortium (28); the Atherosclerosis Risk in Communities (ARIC) Study (1987–1989), a substudy of CALiCo (28); and the Multiethnic Cohort (MEC) Study (1993–2006) (29).

Information on individual study designs and population characteristics for MEC (29), ARIC (28), and EAGLE-NHANES III (26, 27, 30) has been previously published. Summary data describing the PAGE studies used in this analysis are provided in the Web Appendix (available at http://aje.oxfordjournals.org/). All study consents and protocols were approved by each study’s respective institutional review board.

We excluded persons who reported at baseline that a physician had diagnosed them with diabetes, because obesity is a strong risk factor for type 2 diabetes and diet modification is recommended for these patients. We note, however, that in a sensitivity analysis, the observed associations were unchanged when participants were not excluded for diabetes (data not shown). The numbers of participants who reported being diabetes-free at baseline and were not missing information on covariates or macronutrients were 19,529 in MEC, 11,114 in ARIC, and 6,347 in EAGLE-NHANES III (totaling 36,990 participants).

**Data collection**

Measures of dietary intake were obtained with study-specific dietary assessment instruments. MEC used a study-specific quantitative food frequency questionnaire (FFQ) (≥180 food items), which asked participants questions regarding the portion sizes and average frequencies of consumption for commonly eaten foods and mixed dishes over the past year. ARIC used a semiquantitative FFQ (66 food items), which was modified from the 61-item Willett FFQ (31) and assessed the average frequency of commonly consumed foods over the past year. In EAGLE-NHANES III, a single 24-hour dietary recall was administered by a trained interviewer during the participant’s physical examination (32).

Investigators calculated macronutrient intakes similarly at all of the study sites. The daily nutrient amount for a given food was calculated by multiplying the usual portion size (in grams) by the number of times the food was eaten per day and its nutrient content, determined from study-specific food composition values that were mostly derived from US Department of Agriculture data (33). A participant’s total daily consumption (g/day) of fat, protein, carbohydrate, and ethanol was calculated by summing data for the respective macronutrient across all food items. These values were then converted into calories by multiplying the total grams of the macronutrient by the number of calories per gram (1 g of fat = 9 calories, 1 g of protein or carbohydrate = 4 calories, and 1 g of ethanol = 7 calories). To account for differential completeness in the dietary intake assessment methods across studies, our primary strategy was to express each macronutrient as nutrient densities, where the percentage of energy derived from a macronutrient was computed by dividing the calories from that macronutrient by the sum of calories from all 4 macronutrients (i.e., carbohydrate, protein, fat, and alcohol).

**SNP selection**

We investigated 6 obesity risk variants in (or near) genes expressed in the central nervous system that could be related to energy balance (9–11); neuronal growth regulator 1 gene (NEGR1) variant rs2815752, transmembrane protein 18 gene (TMEM18) variant rs6548238, BDNF rs6265, FTO rs8050136, MC4R rs17782313, and KCTD15 rs11084753. These SNPs were selected from the National Human Genome Research Institute’s genome-wide association studies catalog (34) as having been associated with BMI as of December 31, 2008, and were genotyped in all 3 participating studies. Details regarding
genotyping and quality control have been previously published (35) and can be found in the Web Appendix.

Statistical analysis

The relationship of the obesity SNPs with BMI was initially examined in each study population. Subsequently, the relationship of these risk variants with macronutrient intakes in each study was investigated. The linear models regressing BMI or the nutrient value on genotype assumed an additive genetic model and adjusted for age at blood draw, sex, and race/ethnicity. All parameter estimates were modeled for the obesity risk allele (associated with increased BMI). The distributions for total energy intake, percentage of energy derived from ethanol, and BMI did not meet the model assumptions. Therefore, the variables were log-transformed (as log(x + 1)). Statistical analyses were performed using SAS, version 9.2 (SAS Institute, Inc., Cary, North Carolina).

To test for heterogeneity by race/ethnicity and sex within each study, we used an F test in which we compared a model that included the main effects and cross-product terms of the SNP and race/ethnicity or sex with a model including only the main-effect terms.

A meta-analysis of results across studies was performed using STATA, version 10 (StataCorp LP, College Station, Texas). Both the fixed-effects and DerSimonian and Laird random-effects models were considered. Heterogeneity of the regression coefficients was tested using Cochran’s Q statistic. Sex- and race-specific meta-analyses were also conducted.

Associations that remained significant after Bonferroni correction (P < 0.05/[6 SNPs × 8 macronutrients × 3 studies] = 3.5 × 10−3) were further examined. Specifically, we then examined the associations of the SNP with macronutrient intake, adjusting for energy intake using the method of residuals (36). Sensitivity analyses were also conducted by additionally adjusting the gene-macronutrient associations for energy intake, baseline BMI, and energy expenditure. In order to identify the potential temporal stability of our findings, we tested these associations using a repeated measure of macronutrient intake and BMI collected after approximately 10 years of follow-up (available in MEC only). With these prospective data, we also performed a mediation analysis using a program created by Preacher and Hayes (37), where the mediation of effect was estimated by bootstrapping (1,000 times) the indirect effect of baseline diet on the relationship between the SNP and BMI obtained at 10 years of follow-up.

RESULTS

The MEC participants (n = 19,529 men and women) were the oldest (median age, 68 years) and most ethnically diverse (only 17.8% European Americans) study population (Table 1). The ARIC participants (n = 11,114 men and women) included African Americans (22.1%) and European Americans (77.9%) and had a median age of 54 years. The EAGLE-NHANES III participants (n = 6,347 men and women) included African Americans (30.0%), Latinos (28.2%), European Americans (36.9%), and persons of other races (4.9%) and were the youngest study population (median age, 36 years). Using a median test, we found that the median values for percentage of calories derived from fat and protein differed across the 3 PAGE study populations (P < 0.001). The race/ethnicity-specific frequencies of the obesity risk allele for all 6 SNPs were similar across the 3 PAGE studies (Web Table 1).

The associations between these obesity risk variants (with the exception of BDNF rs6265) and BMI were examined in the PAGE populations in a previous study (38). Similar to these prior findings, when using the 3 study populations for the present analysis, the strongest association we observed was between FTO rs8050136 and log-BMI (MEC: P = 7.2 × 10−10; ARIC: P = 7.2 × 10−10; EAGLE-NHANES III: P = 0.046). Among 3 of the other 5 SNPs—TMEM18 rs6548238, BDNF rs6265, and MC4R rs17782313—weaker or null associations were observed in individual studies but statistically significant associations were found when the data were meta-analyzed across studies (P’s < 0.0005). Meta-analysis of NEGR1 rs2815752 and KCTD15 rs11084753 showed suggestive and null associations (P = 0.07 and P = 0.21, respectively).

When investigating the relationships of these obesity risk variants with macronutrient intake in the 3 PAGE studies, we detected associations for FTO rs8050136 with percentages of calories from carbohydrate, protein, and fat in MEC and ARIC (MEC: P’s = 5 × 10−3, 0.02, and 3 × 10−3, respectively; ARIC: P’s = 3 × 10−3, 5 × 10−6, and 0.02, respectively). In EAGLE-NHANES III, only a nominal association with percentage of calories derived from fat (P = 0.07) was detected. For the other genes, we found no associations that passed multiple test correction (Web Table 2).

In the meta-analysis, FTO rs8050136 was positively associated with percentage of calories derived from fat (βmeta = 0.2244 (standard error (SE), 0.0548); Pmeta = 4 × 10−5) and inversely associated with percentage of calories derived from carbohydrate (βmeta = −0.2796 (SE, 0.0709); Pmeta = 8 × 10−5) (Table 2). The positive association with percentage of calories from fat was observed for all major fat subtypes—that is, polyunsaturated, monounsaturated, and saturated fat (Pmeta’s > 0.05) (Table 2), although the strongest association was found with monounsaturated fat (βmeta = 0.0843 (SE, 0.0229); Pmeta = 2 × 10−4). Heterogeneity across studies was detected for the association with percentage of calories derived from protein (P heterogeneity = 0.004). Therefore, for that meta-regression, the random-effects model was used. In the meta-analysis, no associations were detected for rs8050136 with percentages of calories from protein and alcohol or with total energy intake (Pmeta’s > 0.05) (Table 2), although an inverse association with total energy intake was observed in ARIC (β = −0.0103 (SE, 0.0049); P = 0.03). No associations with macronutrient intake were observed for the other 5 obesity risk variants in the meta-analysis (P’s > 0.003) (Web Table 2).

We found that the meta-analyzed associations between rs8050136 and percentages of calories from carbohydrate and fat were consistent across the sexes (P heterogeneity = 0.42 and P heterogeneity = 0.70, respectively) (Web Table 3). Within-study, heterogeneity by sex was detected only in MEC, where the association between rs8050136 and percentage of calories derived from fat appeared to be stronger in men than in women (P heterogeneity = 0.03).

The associations of rs8050136 with percentages of calories from fat and carbohydrate were consistent across the 5
racial/ethnic groups ($P_{heterogeneity} \geq 0.10$) (Web Table 4). Rs8050136 was positively associated with percentage of calories from fat and inversely associated with percentage of calories from carbohydrate among both African Americans ($\beta_{meta} = 0.2549$ (SE, $0.1150$; $P_{meta} = 0.03$) for fat and $\beta_{meta} = -0.3013$ (SE, $0.1540$; $P_{meta} = 0.05$) for carbohydrate) and European Americans ($\beta_{meta} = 0.1624$ (SE, $0.0844$; $P_{meta} = 0.05$) for fat and $\beta_{meta} = -0.2248$ (SE, $0.1119$; $P_{meta} = 0.04$) for carbohydrate). The relationships for Latinos and Asian Americans pointed in the same directions as those in EAGLE-NHANES III and MEC in the overall data (Web Table 4).

Table 3 presents the study-specific and meta-analyzed results for the association between rs8050136 and percentage of calories from fat or from carbohydrate, after additional adjustment for total energy intake, physical activity, and BMI. Meta-analyzed results showed, per allele change, a 0.1800-percentage-point increase in consumption of percentage of calories from fat ($P_{meta} = 1 \times 10^{-3}$) and a 0.2172-percentage-point decrease in consumption of percentage of calories from carbohydrate ($P_{meta} = 3 \times 10^{-3}$). Additionally, similar results were obtained when using macronutrient intakes in grams per day, adjusted for energy intake by the residual method (36), with further adjustment for total energy intake in the model. We found that each “A” allele change was associated with a 0.4422-g/day increase in fat intake ($P_{meta} = 4 \times 10^{-5}$) and a 1.2735-g/day decrease in carbohydrate intake ($P_{meta} = 9 \times 10^{-5}$) (Web Table 5).

In the MEC Study, similar associations were observed with diet assessed after approximately 10 years of follow-up (median follow-up time, 10.5 years; $n = 10,766$ participants with such data). For a per-allele change, we found a 0.3397-percentage-point increase in percentage of calories from fat ($P = 1 \times 10^{-3}$) and a 0.3054-percentage-point decrease in percentage of calories from carbohydrate ($P = 0.02$). The geometric mean values are presented by genotype in Table 4. The association with percentage of calories from fat after 10 years remained after further adjustment for BMI at baseline or BMI after 10 years ($P = 0.01$) and was suggested after exclusion of persons who may have altered their diet due to a diabetes diagnosis during follow-up ($n = 5,350$) ($P = 0.12$).
Table 2. Associations Between FTO rs8050136 (Per-Allele Change) and Total Energy Intake and Percentages of Calories Derived From Carbohydrate, Protein, and Fat Among 36,973 Adults, PAGE Study, 2008–2012

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<tbody>
<tr>
<td></td>
<td>βb</td>
<td>SE</td>
<td>P Value</td>
<td>βb</td>
<td>SE</td>
</tr>
<tr>
<td>Total energy intake, kcal/day</td>
<td>−0.0006</td>
<td>0.0048</td>
<td>0.90</td>
<td>−0.0103</td>
<td>0.0049</td>
</tr>
<tr>
<td>% of calories from carbohydrate</td>
<td>−0.2624</td>
<td>0.0940</td>
<td>5 × 10⁻³</td>
<td>−0.3716</td>
<td>0.1259</td>
</tr>
<tr>
<td>% of calories from proteinc</td>
<td>0.0730</td>
<td>0.0307</td>
<td>0.02</td>
<td>0.2511</td>
<td>0.0548</td>
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<tr>
<td>% of calories from fat</td>
<td>0.2206</td>
<td>0.0750</td>
<td>3 × 10⁻³</td>
<td>0.2071</td>
<td>0.0909</td>
</tr>
<tr>
<td>% of calories from alcohol</td>
<td>0.0004</td>
<td>0.0102</td>
<td>0.97</td>
<td>−0.0006</td>
<td>0.0130</td>
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<tr>
<td>% of calories from specific types of fat</td>
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<tr>
<td>Polyunsaturated fat</td>
<td>0.0562</td>
<td>0.0211</td>
<td>8 × 10⁻³</td>
<td>0.0147</td>
<td>0.0197</td>
</tr>
<tr>
<td>Monounsaturated fat</td>
<td>0.0724</td>
<td>0.0302</td>
<td>0.02</td>
<td>0.0951</td>
<td>0.0400</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>0.0725</td>
<td>0.0267</td>
<td>7 × 10⁻³</td>
<td>0.0632</td>
<td>0.0404</td>
</tr>
</tbody>
</table>

Abbreviations: ARIC, Atherosclerosis Risk in Communities; CALiCo, Causal Variants Across the Life Course; EAGLE, Epidemiologic Architecture for Genes Linked to Environment; MEC, Multiethnic Cohort; NHANES III, Third National Health and Nutrition Examination Survey; PAGE, Population Architecture using Genomics and Epidemiology; SE, standard error.

a Heterogeneity by study.
b Slope from regression of macronutrient intake on FTO rs8050136, using an additive genetic model, adjusted for age at blood draw, sex, and race/ethnicity. This represents the change in the level of the nutrient, such as percentage of energy derived from fat, associated with a change in 1 variant allele.
c Because of heterogeneity by study, the meta-analyzed results from the random-effects model are presented.
Table 3. Associations Between FTO rs8050136 and Percentages of Calories Derived From Carbohydrate and Fat in Multivariable Models, by Study Site, PAGE Study, 2008–2012

<table>
<thead>
<tr>
<th>Macronutrient</th>
<th>Study Population</th>
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<tbody>
<tr>
<td>% of calories from carbohydrate</td>
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<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Model 1b</td>
<td>-0.1996</td>
<td>0.0940</td>
<td>0.03</td>
<td>-0.3784</td>
<td>0.1259</td>
<td>3 × 10^{-3}</td>
<td>-0.0759</td>
<td>0.2473</td>
<td>0.76</td>
<td>-0.2477</td>
</tr>
<tr>
<td>Model 2c</td>
<td>-0.2365</td>
<td>0.0972</td>
<td>0.01</td>
<td>-0.3224</td>
<td>0.1259</td>
<td>0.01</td>
<td>-0.0924</td>
<td>0.2104</td>
<td>0.66</td>
<td>-0.2478</td>
</tr>
<tr>
<td>Model 3d</td>
<td>-0.1767</td>
<td>0.0972</td>
<td>0.07</td>
<td>-0.3287</td>
<td>0.1260</td>
<td>9 × 10^{-3}</td>
<td>-0.0497</td>
<td>0.2472</td>
<td>0.84</td>
<td>-0.2172</td>
</tr>
<tr>
<td>% of calories from fat</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Model 1</td>
<td>0.1647</td>
<td>0.0748</td>
<td>0.03</td>
<td>0.2360</td>
<td>0.0901</td>
<td>9 × 10^{-3}</td>
<td>0.3254</td>
<td>0.2028</td>
<td>0.11</td>
<td>0.2036</td>
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<tr>
<td>Model 2</td>
<td>0.2075</td>
<td>0.0775</td>
<td>7 × 10^{-3}</td>
<td>0.1612</td>
<td>0.0908</td>
<td>0.07</td>
<td>0.3029</td>
<td>0.1719</td>
<td>0.08</td>
<td>0.2000</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.1512</td>
<td>0.0773</td>
<td>0.05</td>
<td>0.1962</td>
<td>0.0912</td>
<td>0.03</td>
<td>0.2987</td>
<td>0.2026</td>
<td>0.14</td>
<td>0.1800</td>
</tr>
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</table>

Abbreviations: ARIC, Atherosclerosis Risk in Communities; CALiCo, Causal Variants Across the Life Course; EAGLE, Epidemiologic Architecture for Genes Linked to Environment; MEC, Multiethnic Cohort; NHANES III, Third National Health and Nutrition Examination Survey; PAGE, Population Architecture using Genomics and Epidemiology; SE, standard error.

a Heterogeneity by study.
b Slope from regression of the exposure variable on FTO rs8050136, using an additive genetic model, adjusted for age at blood draw, sex, race/ethnicity, and baseline body mass index.
c Slope from regression of the exposure variable on FTO rs8050136, using an additive genetic model, adjusted for age at blood draw, sex, race/ethnicity, total energy intake, and energy expenditure.
d Model 2 with additional adjustment for baseline body mass index.
Using the same data, we confirmed the association between rs8050136 and the BMI value at 10 years of follow-up \((P<0.001)\) (data not shown). In a mediation analysis \((37)\), we found that percentage of calories from fat at baseline was a partial mediator in the relationship between rs8050136 and BMI obtained 10 years later (mediation of effect \(=0.0823\) kg/m\(^2\), 95% confidence interval: 0.0559, 0.1128).

**DISCUSSION**

This study investigated the association of 6 obesity risk variants with total energy intake and percentage of calories derived from protein, carbohydrate, fat, and ethanol in multiply ethnically diverse adult populations. We found that the obesity-risk allele for \(rs8050136\) was positively associated with percentage of calories from fat and inversely associated with percentage of calories from carbohydrate. There was no heterogeneity in these findings across studies or across meta-regressed strata of sex or race/ethnicity.

To date, more than 30 genetic loci have been found to be associated with BMI, many in or near genes expressed in the central nervous system \((9)\). Among the 6 loci examined in this study, polymorphisms in intron 1 of the \(FTO\) gene have been associated with percentage of BMI and genetic variants in \(FTO\) (12, 13, 22, 48). The study that did not detect an association was conducted in a multiethnic youth population (median age, 15 years) \((48)\). Four studies conducted in adults \((15, 16, 18, 24)\), with sample sizes less than 2,100, found either a positive \((18, 24)\) or a null \((15, 16)\) relationship between measures of relative or absolute fat intake and genetic variants in \(FTO\); however, a \(P\) value less than 0.05 was observed in only 1 study \((18)\).

We also found an inverse association between \(FTO\) rs8050136 and carbohydrate intake (percentage of calories or g/day). A similar inverse relationship has been reported in 3 past studies, although all had \(P\) values greater than 0.05 \((16, 18, 22)\). In contrast, a positive relationship was observed in 3 other studies \((12, 13, 15)\), with only 1 study detecting an association \((P<0.05)\) \((12)\).

### Table 4. Geometric Mean Values for Percentages of Calories Derived From Carbohydrate and Fat Measured 10 Years After Baseline, by \(FTO\) rs8050136 Genotype, Among Multiethnic Cohort Study Participants (1993–2006) With Available 10-Year Follow-up Diet Data \((n=10,766)\) and Those Who Remained Diabetes-Free \((n=5,350)\)

<table>
<thead>
<tr>
<th>(FTO) rs8050136 Genotype</th>
<th>% of calories from carbohydrate</th>
<th>% of calories from fat</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Geometric Mean</td>
<td>SE</td>
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<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
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<tr>
<td>CC</td>
<td>50.43</td>
<td>0.12</td>
</tr>
<tr>
<td>CA</td>
<td>50.52</td>
<td>0.12</td>
</tr>
<tr>
<td>AA</td>
<td>50.47</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>31.32</td>
<td>0.10</td>
</tr>
<tr>
<td>CA</td>
<td>31.21</td>
<td>0.10</td>
</tr>
<tr>
<td>AA</td>
<td>31.26</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Model 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>30.73</td>
<td>0.14</td>
</tr>
<tr>
<td>CA</td>
<td>30.73</td>
<td>0.14</td>
</tr>
</tbody>
</table>

**Participants who were diabetes-free during the 10 years postbaseline**

<table>
<thead>
<tr>
<th>% of calories from carbohydrate</th>
<th>% of calories from fat</th>
</tr>
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<tbody>
<tr>
<td>Model 1a</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>50.52</td>
</tr>
<tr>
<td>CA</td>
<td>50.06</td>
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<tr>
<td>AA</td>
<td>50.33</td>
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**Abbreviation: SE, standard error.**

\(a\) Results were adjusted for age at blood draw, sex, race/ethnicity, and follow-up time.

\(b\) Model 1 with additional adjustment for baseline body mass index.

\(c\) Model 1 with additional adjustment for body mass index at the time of diet measurement.
Past studies of FTO and diet were largely conducted in European Americans. While 2 studies included European Americans and African Americans (18, 48), race-specific findings were not reported. The largest nonwhite study included only 502 African-American youths (median age, 15 years) (48). Our study appears to be the largest and most ethnically diverse to date. No heterogeneity in magnitude or direction was found for the positive association between rs8050136 and percentage of calories derived from fat across racial/ethnic groups. The race-specific association was statistically significant in the 7,953 African Americans and 1,872 Native Hawaiians included in our analysis and showed a positive direction of association in the 6,864 Latinos and 5,800 Asian Americans.

Three past studies investigated whether dietary fat modified the association between the FTO obesity risk genotype and BMI (the SNPs studied were in high linkage disequilibrium with rs8050136 in US residents of European ancestry \((r^2 > 0.84)\) (17, 49, 50). Two of these studies found that saturated fat in grams per day \((P = 0.03)\) (50) or percentage of calories derived from fat \((P = 0.02)\) (17) positively modified the association of FTO with BMI. However, we did not detect such an interaction in our study for percentage of calories from fat or from specific types of fat.

Unlike some previous studies, we found no evidence for an association between the obesity risk allele for rs8050136 and total energy intake in MEC or EAGLE-NHANES III or in our meta-analysis. In ARIC, an association was detected \((P = 0.03)\); however, it was in the direction opposite that reported in the past. Eight previous studies that investigated the association between the FTO locus and dietary intake were conducted in children and mostly middle-aged adults (median ages, 38–57 years), primarily in European Americans. In 5 of these 8 studies, an increase in the number of obesity-risk alleles in FTO rs9939609 was associated with greater consumption of energy or energy-dense foods (12, 13, 15, 16) or number of eating occasions (18). Multiple food diaries (12, 15, 16) or a single test meal (13) was used in the 4 studies that found an association with energy or energy-dense foods. The 4 other studies that did not observe such an association measured dietary intake with FFQs (18, 24), two 24-hour recalls (48), or a test meal (22).

Additionally, in contrast to some of the previous reports, we found no association between 5 other obesity SNPs (NEGR1 rs2815752, TMEM18 rs6548238, BDNF rs6265, MC4R rs17782313, and KCTD15 rs11084753) and macronutrient intake (18, 24). One past study found that SNPs in or near the SH2B2 adapter protein 1 gene (SH2B1), KCTD15, the mitochondrial carrier homolog 2 gene (MTCH2), NEGR1, and BDNF were associated with consumption of macronutrients \((P<0.05)\) (24). In addition to the previously mentioned association between FTO rs1421085 and percentage of calories from fat, McCaffrey et al. (18) found associations of BDNF SNPs with decreased total energy intake and increased servings for specific dairy foods, meat, eggs, nuts, and beans; an association of SH2B1 rs4788099 with increased servings of dairy products; and an association of the troponin I, cardiac muscle (TNNT3) interacting kinase gene (TNNT3K) with a lower percentage of calories from protein. The SH2B1 and TNNT3K SNPs were not genotyped in all 3 of our PAGE study populations. We did not observe any associations between BDNF rs6265 and macronutrient intake or specific foods in our study (data not shown).

The inconsistencies in results across studies may reflect differences in populations. The associations between the 6 obesity SNPs and BMI were identified in populations of primarily younger adults and persons of European ancestry (51, 52). In addition, discovery of the relevant functional variant(s) at each obesity risk locus for each ethnic group would help to increase understanding of the underlying biology and possibly facilitate the identification of other potential dietary relationships.

Although we found that rs8050136 was associated with fat intake, as opposed to total energy intake, it is possible that for carriers of the FTO variant this association reflects a preference for fat intake, thereby resulting in greater consumption of energy. Previous studies have shown that energy-dense diets are associated with a higher percentage of calories from fat and a lower percentage of calories from carbohydrate (53, 54). Furthermore, our failure to detect an association with energy intake may have been due to the measurement error associated with FFQs and a single 24-hour recall and the resulting loss of power (36, 55). The differential underreporting of energy intake with these dietary assessment methods in subjects with an elevated BMI (56–58) could have attenuated a positive association by leading carriers of the obesity risk allele, who tend to have higher BMIs, to underreport their energy intake. We observed such an inverse relationship between rs8050136 and total energy intake in the ARIC data but not in the other 2 studies.

It remains possible that the relationship between rs8050136 and BMI may have influenced our findings due to reverse causation, given that heavier persons consume more calories and energy-dense foods. Indeed, when additionally adjusting for BMI, we observed an attenuation of estimates for both percentage of calories from fat and percentage of calories from carbohydrate. However, despite this attenuation, the association of the FTO variant with percentage of fat calories remained \((P \leq 1 \times 10^{-3})\). The association also remained when using dietary fat measured after 10 years of follow-up. Furthermore, we found in the MEC that percentage of calories from fat assessed at baseline was a partial mediator of the association between rs8050136 and BMI obtained 10 years later. Thus, these results suggest that the association between rs8050136 and dietary fat is robust.

To our knowledge, this is the largest and most ethnically diverse study to have investigated the relationship of obesity risk variants with energy and macronutrient consumption. Additional strengths include our ability to adjust for a variety of potential confounders and to examine the stability of the relationship during follow-up in the MEC.

In summary, the consistency of our findings across ethnic groups, study populations, and dietary assessment methods provides substantial support for an association between the FTO intron 1 obesity locus and a modestly increased intake of energy from fat. Our data provide additional evidence for the notion that the association of FTO with obesity is mediated by dietary intake.
ACKNOWLEDGMENTS

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The Population Architecture using Genomics and Epidemiology (PAGE) Study is funded by the National Human Genome Research Institute (NHGRI), supported by grants U1HG1004803 (Genetic Epidemiology of Causal Variants Across the Life Course (CALiCo) Consortium), U01HG004798 (Epidemiologic Architecture for Genes Linked to Environment (EAGLE) Study), U01HG004802 (Multiethnic Cohort (MEC) Study), U01HG004790 (Women’s Health Initiative), and U1HG004801 (PAGE Coordinating Center), and their respective NHGRI American Recovery and Reinvestment Act (ARRA) supplements. The data and materials included in this report resulted from a collaboration between the following studies: The EAGLE Study is funded through the NHGRI PAGE Study (grant U01HG004798-01 and its NHGRI ARRA supplement). The study participants were derived from the Third National Health and Nutrition Examination Survey, which is supported by the Centers for Disease Control and Prevention. The MEC characterization of epidemiologic architecture is funded through the NHGRI PAGE Study (grant U01HG004802 and its NHGRI ARRA supplement). The MEC Study is funded by the National Cancer Institute (grants R37CA54281, R01 CA63, P01CA33619, U01CA136792, and U01CA98758). Funding for CALiCo was provided through the NHGRI PAGE Study (grant U01HG004803). The Atherosclerosis Risk in Communities (ARIC) Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts HHSN268201100005C, HHSN26820 1100006C, HSN268201100007C, HHSN268201100008C, HSN268201100009C, HSN268201100010C, HHSN26820 1100011C, and HHSN268201100012C; National Heart, Lung, and Blood Institute grants R01HL086741, R01HL59367, and R01HL086694; NHGRI contract U01HG004402; and National Institutes of Health (NIH) contract HHSN268200625226C. The infrastructure of the ARIC Study was partly supported by grant U1RR025005, a component of the NIH and the NIH Roadmap for Medical Research. Assistance with phenotype harmonization, single-nucleotide polymorphism selection and annotation, data-cleaning, data management, integration, and dissemination, and general study coordination was provided by the PAGE Coordinating Center (grant U01HG 004801). The National Institute of Mental Health also contributes support for the Coordinating Center.

We thank all study staff for their important contributions.

The complete list of PAGE members can be found at http://www.pagesudy.org.

The contents of this paper are solely the responsibility of the authors. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the NIH or the Centers for Disease Control and Prevention. Conflict of interest: none declared.

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