Dietary Saturated Fat Modulates the Association between STAT3 Polymorphisms and Abdominal Obesity in Adults\textsuperscript{1,2}

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Abstract

Signal transducer and activator of transcription 3 (STAT3) plays a key role in body weight regulation and glucose homeostasis, 2 important determinants of metabolic syndrome (MetS). Dietary fat is a key environmental factor that may interact with genotype to affect MetS risk. In this study, we investigated the relationship between STAT3 polymorphisms and MetS phenotypes and determined potential interactions with dietary fatty acids. STAT3 polymorphisms rs8069645, rs744166, rs2306580, rs2293152, and rs10530050, biochemical measurements, and dietary fat composition were determined in the LIPGENE-SU.VI.MAX study of MetS cases and matched controls (\(n = 1754\)). STAT3 polymorphisms were not associated with MetS risk. However, minor G allele carriers for rs8069645, rs744166, and rs1053005 and major GG homozygotes for rs2293152 had increased risk of abdominal obesity compared with noncarriers [odds ratio (OR) = 2.22, \(P = 0.0005\); OR = 2.08, \(P = 0.0017\); OR = 2.00, \(P = 0.0033\); and OR = 1.95, \(P = 0.028\), respectively]. The number of risk alleles additively increased obesity risk (\(P = 0.0003\)). Dietary SFA intake exacerbated these effects; among all participants with the highest SFA intake (\(\geq 15.5\%\) of energy), individuals carrying \(\geq 2\) risk alleles had further increased risk of obesity (OR = 3.30; 95% CI = 1.50–7.28; \(P = 0.0079\)) compared with those carrying \(\leq 1\) risk allele. Interaction analysis confirmed this gene-nutrient interaction whereby increasing SFA intake was predictive of increased waist circumference (\(P = 0.038\)). In conclusion, STAT3 gene polymorphisms influenced the risk of abdominal obesity, which is modulated by dietary SFA intake, suggesting novel gene-nutrient interactions. J. Nutr. 139: 2011–2017, 2009.

Introduction

Metabolic syndrome (MetS)\textsuperscript{10} is a cluster of metabolic abnormalities, including abdominal obesity, insulin resistance, dyslipidemia, and hypertension, that predispose an individual to greater risk of type 2 diabetes (T2DM) and cardiovascular disease (1). Low-grade inflammation plays an important role in the pathogenesis of MetS and T2DM (2). High circulating levels of proinflammatory cytokines such as interleukin 6 (IL-6) are associated with greater risk of T2DM and several MetS phenotypes (2,3). Signal transducer and activator of transcription 3 (STAT3) is a transcription factor released during the acute-phase response wherein it is activated by cytokines, such as IL-6 (4). Evidence from knockout mouse studies showed that disruption of neural STAT3 causes obesity, diabetes, and thermal dysregulation (5). Loss- and gain-of-function experiments in a mouse model with liver-specific disruption of the STAT3 gene demonstrate the key role of STAT3 in the expression of hepatic gluconeogenic genes and highlight the physiological role of STAT3 in normal glucose homeostasis. Knockout mice displayed insulin resistance that was normalized by restoration of STAT3 (6). Furthermore, leptin, a hormone that regulates appetite and energy metabolism, induces STAT3 activation in the hypothalamus (7). Loss of STAT3 from the hypothalamus interferes with...
normal body weight and glucose homeostasis (8). Interestingly, in knockout mice in which STAT3 was disrupted in the insulin-producing cells, animals exhibited increased appetite and obesity as well as partial leptin resistance and glucose intolerance (9). More recently, mice with an adipocyte-specific disruption of the STAT3 gene displayed increased adiposity and adipocyte hypertrophy, demonstrating the essential role of STAT3 in regulating body weight homeostasis (10).

Although STAT3 may be a good candidate gene for investigating obesity and insulin resistance susceptibility alleles, few studies have been performed. In a large female twin cohort, an association was found between rs2293152 and insulin resistance (11). Subsequently, in a male cohort, no association was found between STAT3 polymorphisms (rs2293152, rs6503695, and rs9891119) and obesity or glucose-related traits (12). These inconsistencies may reflect gender differences, sample size, and genetic heterogeneity of the populations studied. Also, the impact of environmental factors in diet-related polygenic disorders cannot be underestimated in light of the global epidemic of the incidence of MetS and T2DM. Dietary fat composition represents an important environmental factor that may alter MetS risk (13–15). Epidemiological and cohort studies suggest detrimental effects of SFA on insulin sensitivity, promoting the development of diabetes (16–18). Increases in the amount of dietary fat, in particular SFA, have also been shown to increase the risk of obesity (19,20). However, not all studies have been consistent, perhaps reflecting gene-nutrient interactions.

Because STAT3 is involved in body weight regulation and glucose homeostasis, it is reasonable to speculate that STAT3 polymorphisms may contribute to susceptibility of MetS. Evidence of associations between genetic variants of STAT3 and those traits in a MetS cohort is currently unavailable. Therefore, this prospective study investigated the potential relationship between common genetic polymorphisms of STAT3 and MetS phenotypes in men and women and whether this is modulated by dietary fat intake.

**Methods**

*Participants, MetS classification, and study design.* This study is part of a prospective case control candidate gene study of LIPGENE, an EU Sixth Framework Program Integrated Project entitled “Diet, Genomics and the Metabolic Syndrome: an Integrated Nutrition, Agro-Food, Social and Economic Analysis.” Participants were selected from an existing French SU.VI.MAX cohort including 13,000 adults studied over 7.5 y beginning in 1994–2002 (21). The LIPGENE-SU.VI.MAX study is a nested case control study of MetS consisting of women aged 35–60 y and men aged 45–60 y recruited from SU.VI.MAX. Additional ethical approval from the ethical committee (Comité Consultatif de Protection des Personnes se prêtant à des Recherches Biomédicales de Paris-Cochin Hospital) included an additional clause (no. Am 2840–12–706) to perform the biochemical analysis and genetic analysis required for the LIPGENE study. LIPGENE participants were informed of the study objectives and signed a consent form. Participants were invited to provide a 24-h dietary record every 2 mo for a total of 6 records/y. Information was collected using computerized questionnaires that were transmitted during a brief telephone connection via the Minitel Telematic Network (France Télécom, Paris, France), a small terminal that was widely used in France as an adjunct to the telephone. Participants were guided by the software’s interactive facilities and by a previously validated instruction manual for coding food portions that included >250 foods presented in 3 different portion sizes. Two intermediate and extreme portions could also be chosen, yielding a total of 7 choices for estimating quantities consumed (22). Baseline daily dietary intake data were estimated using food composition tables validated for the French population (23).

Baseline and 7.5-y follow-up data, including plasma lipid profiles and full clinical examination records, were made available to LIPGENE. These data were used to identify cases, individuals who developed elements of MetS over the 7.5-y follow-up period, and controls. MetS cases were selected according to the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III [ATP III]) criteria for MetS. Participants were required to fulfill at least 3 of the following 5 criteria: increased waist circumference (>94 cm [men] or >80 cm [women]), increased fasting blood glucose (≥5.5 mmol/L or treatment for diabetes), increased triacylglycerol (TAG) (≥1.5 mmol/L or treatment for dyslipidemia), decreased HDL cholesterol (<1.04 mmol/L [men] or <1.29 mmol/L [women]), and increased systolic/diastolic blood pressure (>130/85 mm Hg or antihypertensive treatment). Cases were defined as both men and women with ≥3 abnormalities and controls were defined as men and women with no abnormalities or men with ≤1 abnormality. Cases and controls (n = 1754) were matched according to age (±5 y), gender, and number of dietary records available.

**Biochemical analysis.** Fasting plasma glucose, TAG, and HDL and total cholesterol were measured as previously described (21). Insulin was determined by electrochemiluminescence immunoassays (Roche Diagnostics). FFA and LDL cholesterol were measured by enzymatic colorimetric methods (Randox Laboratories and Roche Diagnostics). Homeostasis model assessment (HOMA-IR), a measure of insulin resistance, was calculated as: [(fasting plasma glucose × fasting plasma insulin)/22.5] (24). Quantitative insulin-sensitivity check index (QUICKI), a measure of insulin sensitivity, was calculated as = 1/[log fasting insulin + log fasting glucose + log fasting FFA]) (25).

**DNA extraction and genotyping.** DNA extraction from buffy coats was performed using the Puregene protocol for DNA extraction and samples were processed using the AutoPure LS automated system (Genetec Systems). Low-yielding samples (<10 ng) were subjected to whole genome amplification using the REPLI-g kit (Qiagen). STAT3 genotype data from HapMap v1.1 and Perlegen (26,27) were uploaded into HITAGENE, a Web-based combined database and genetic analysis software suite developed by Hitachi Dublin Laboratory. Haplotype frequencies were estimated by implementation of the expectation maximization algorithm. Using a 5% cutoff for individual haplotype frequency and >70% for the sum of all haplotype frequencies, haplotype-tagged polymorphisms were identified using single nucleotide polymorphism (SNP) tagger (28). Including a SNP from the literature (rs2293152) (11,12), 5 STAT3 polymorphisms with minor allele frequencies > 0.05% (rs8069645, rs744166, rs2306380, rs2293152, and rs10530050) were genotyped by Illumina as part of the entire genotyping component of the LIPGENE study, using the Golden Gate Assay on a BeadStation 500G genotyping system. Genotyping accuracy was ascertained by sample success rates and call rates of 99%. All polymorphisms were in Hardy-Weinberg equilibrium (P < 0.01).

**Statistical analysis.** Statistical analysis was performed using SAS for Windows, version 9.0 (SAS Institute). Data are expressed as means ± SEM. After checking for skewness and kurtosis, glucose, insulin, FFA, triglycerides, QUICKI, and HOMA-IR were normalized by logarithmic transformation. Departure of genotype distributions from Hardy-Weinberg equilibrium in all participants, cases, and controls was assessed using chi-square tests in HITAGENE. Logistic regression determined associations between genotypes and MetS and its risk phenotypes (abdominal obesity, fasting hyperglycemia, high HOMA-IR, low QUICKI, high TAG, low HDL, and high blood pressure). Cutoff points for these MetS risk phenotypes were determined by MetS criteria. HOMA-IR and QUICKI values were dichotomized based on control participants’ median levels. Generalized estimating equation linear regression investigated interactions with fatty acids on these MetS phenotypes. Predicted values were calculated from the regression model containing dietary fat, genotype or number of risk alleles, their interaction term, and potential confounders as described below. To further determine modulation by fatty acids, association analyses were
repeated using the median level of control participants to dichotomies dietary fat intake. Three genotype groups were first considered to check different inherent models; where a dominant or recessive effect existed, analysis was repeated comparing carriers and noncarriers of that particular allele. Analyses were performed on the whole study population. Case control status and gender were examined to ascertain the homogeneity of genetic effects. Potential confounding factors used in the adjusted multivariate analyses included age, smoking status, physical activity, energy, and use of medications, including lipid-lowering, hypertension, and diabetes treatments. P < 0.05 was considered significant. To account for multiple testing for associations and interactions, false discovery rates (FDR) (29) were computed and we report FDR-adjusted P-values that were calculated separately for each test. FDR = 0.05 was considered significant.

Results

Association between STAT3 polymorphisms and MetS and its phenotypes. Genotype distributions of the STAT3 polymorphisms were investigated. Genotype frequencies were not different between MetS cases and controls (Table 1). However, several STAT3 polymorphisms were associated with risk of abdominal obesity. Minor G allele carriers for rs8069645 [odds ratio (OR) = 2.22 (95% CI 1.43–3.57); P = 0.0005; FDR = 0.0025], rs744166 [OR = 2.08 (95% CI 1.32–3.33); P = 0.0017; FDR = 0.0043], and rs1053005 [OR = 2.00 (95% CI 1.25–3.12); P = 0.0033; FDR = 0.0055] had increased risk of abdominal obesity compared with their AA homozygotes and major GG homozygotes for rs2293152 [OR = 1.95 (95% CI 1.07–3.55); P = 0.028; FDR = 0.035] had increased risk of abdominal obesity compared with their A allele carriers. Genotype distributions did not deviate from Hardy-Weinberg equilibrium (P > 0.01) and none of the polymorphisms were in significant linkage disequilibrium (i.e. $r^2 < 0.80$ and $D' < 0.80$).

Combined effect of carrying multiple risk alleles. As individual STAT3 genetic variants conferred risk of abdominal obesity, we investigated the cumulative risk of carrying multiple risk alleles. Risk alleles for each SNP were identified by the logistic regression analyses as the G allele for rs8069645, rs744166, rs1053005, and rs2293152. The majority of individuals carried 2 risk alleles therefore the entire population was split into 3 groups: those carrying ≤1 risk allele, 2 risk alleles, and >2 risk alleles. We examined the effect of carrying increasing numbers of the STAT3 rs8069645, rs744166, rs1053005, and rs2293152 risk alleles on the risk of abdominal obesity (Fig. 1). There was an association between the number of risk alleles and risk of abdominal obesity (P = 0.0012). In particular, individuals carrying 2 risk alleles had almost 2-fold higher risk of being abdominally obese [OR = 1.95 (95% CI 1.02–3.75); P = 0.04; FDR = 0.049] compared with those carrying 1 or fewer risk alleles. This risk was further amplified in individuals carrying >2 risk alleles [OR = 2.52 (95% CI 1.54–4.14); P = 0.01; FDR = 0.037], resulting in P = 0.0003 for the additive model.

Gene-nutrient interactions modulate the association with obesity. Dietary SFA intake (≥15.5% of energy) modulated the association with abdominal obesity (P = 0.01). Among all participants with the highest 50th percentile of SFA consumption, individuals carrying >2 risk alleles had further increased risk of abdominal obesity [OR = 3.30 (95% CI 1.50–7.28); P = 0.0079; FDR = 0.0158] compared with those carrying 1 or fewer risk alleles. Interaction analysis confirmed this gene-nutrient interaction, whereby increasing SFA intake was predictive of increased waist circumference in participants carrying >2 risk alleles (P = 0.038; FDR = 0.046) (Fig. 2). In contrast, participants carrying 1 or fewer risk alleles were not responsive to increasing dietary SFA intake, in that their waist circumference did not change significantly over a SFA intake of 10–30%. Dietary monounsaturated fatty acids (MUFA) and PUFA, including (n-3), long-chain (n-3) and (n-6) PUFA, were also examined, but the intake of these dietary fatty acids did not modulate the genetic associations with waist circumference.

![Figure 1](https://academic.oup.com/jn/article-abstract/139/11/2011/4751038/13912114174510438?batchId=13912114174510438)

**FIGURE 1** OR for abdominal obesity for all participants stratified by number of STAT3 risk alleles and dietary SFA. Individuals carrying ≥2 risk alleles had increased risk (OR 1.95–2.52) relative to the ≤1 risk allele carriers. High dietary SFA intake accentuated this risk (OR 3.30) in individuals carrying >2 risk alleles compared with those carrying ≤1 risk allele.

### TABLE 1 Genotype distributions of the STAT3 polymorphisms in MetS cases and controls

<table>
<thead>
<tr>
<th>SNP</th>
<th>Major allele homozygotes</th>
<th>Heterozygotes</th>
<th>Minor allele homozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case (n, %)</td>
<td>Control (n, %)</td>
<td>Case (n, %)</td>
</tr>
<tr>
<td>rs8069645</td>
<td>465 (53.0)</td>
<td>444 (50.7)</td>
<td>347 (39.6)</td>
</tr>
<tr>
<td>rs744166</td>
<td>285 (32.5)</td>
<td>292 (33.3)</td>
<td>447 (51.0)</td>
</tr>
<tr>
<td>rs1053005</td>
<td>542 (61.8)</td>
<td>554 (63.2)</td>
<td>303 (34.5)</td>
</tr>
<tr>
<td>rs2293152</td>
<td>340 (38.8)</td>
<td>314 (35.8)</td>
<td>406 (46.2)</td>
</tr>
<tr>
<td>rs2306580</td>
<td>752 (85.7)</td>
<td>747 (85.2)</td>
<td>121 (13.8)</td>
</tr>
</tbody>
</table>

Gene-nutrient interactions, dietary saturated fat, and obesity 2013
Clinical characteristics and dietary fat intakes according to number of STAT3 risk alleles. Age, gender, and the distribution of MetS cases and controls did not differ according to the number of STAT3 risk alleles (Table 2). In terms of their phenotype, increasing numbers of risk alleles was associated with increased waist circumference (P = 0.0078) and BMI (P = 0.0172). Plasma glucose and insulin concentrations did not differ among the groups, whereas insulin sensitivity, as assessed by QUICKI, was reduced with increasing number of risk alleles (P = 0.023). Plasma cholesterol concentrations (total, HDL, and LDL) did not differ between groups, but higher TAG concentrations were noted in the ≥2 risk allele carriers compared with individuals carrying <2 risk alleles (P = 0.042). Dietary SFA, PUFA, and MUFA intake did not differ between groups.

Homogeneity of the genetic effects according to gender. The association with obesity risk was gender specific wherein the deleterious genetic effect was primarily in the male participants. Male minor G allele carriers for rs8069645, rs744166, and rs1053005 and major GG homozygotes for rs2293152 had increased risk of abdominal obesity compared with noncarriers [OR = 1.68 (95% CI 1.02–2.87), P = 0.048, FDR = 0.06; OR = 2.38 (95% CI 1.35–4.17), P = 0.0027, FDR = 0.0067; OR = 2.58 (95% CI 1.45–4.59), P = 0.0012, FDR = 0.006; and OR = 2.02 (95% CI 1.17–3.50), P = 0.0123, FDR = 0.02, respectively]. In addition, male GG homozygotes for rs2293152 also had increased risk of fasting hyperglycemia [OR = 2.40 (95% CI 1.30–4.42); P = 0.0051; FDR = 0.001]. Interestingly, when the female participants were analyzed separately, rs2293152 major GG homozygotes and G allele carriers of rs1053005 and rs744166 were associated with reduced insulin sensitivity as assessed by QUICKI [OR = 3.30 (95% CI 1.58–6.88), P = 0.0015, FDR = 0.0075; OR = 1.72 (95% CI 1.04–2.22), P = 0.024, FDR = 0.046; and OR = 1.79 (95% CI 1.07–2.94), P = 0.0329, FDR = 0.05, respectively] compared with noncarriers.

Table 2 Clinical characteristics and dietary fat intakes of all participants according to number of STAT3 risk alleles

<table>
<thead>
<tr>
<th>Number of risk alleles</th>
<th>≤1</th>
<th>≥2</th>
<th>&gt;2</th>
<th>P-value additive model</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>518</td>
<td>289</td>
<td>947</td>
<td></td>
</tr>
<tr>
<td>Case/control, %</td>
<td>54/55</td>
<td>18/15</td>
<td>28/30</td>
<td>0.334</td>
</tr>
<tr>
<td>Male/female, %</td>
<td>54/56</td>
<td>18/15</td>
<td>28/29</td>
<td>0.421</td>
</tr>
<tr>
<td>Age, y</td>
<td>58 ± 0.2</td>
<td>58 ± 0.2</td>
<td>58 ± 0.3</td>
<td>0.892</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>88 ± 0.6</td>
<td>89 ± 0.6</td>
<td>91 ± 0.4</td>
<td>0.008</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.9 ± 0.2</td>
<td>26.1 ± 0.2</td>
<td>28.1 ± 0.3</td>
<td>0.017</td>
</tr>
<tr>
<td>Plasma glucose, mmol/L</td>
<td>5.21 ± 0.04</td>
<td>5.29 ± 0.06</td>
<td>5.27 ± 0.04</td>
<td>0.382</td>
</tr>
<tr>
<td>Plasma insulin, pmol/L</td>
<td>48.40 ± 1.67</td>
<td>52.92 ± 2.43</td>
<td>52.50 ± 1.48</td>
<td>0.075</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.35 ± 0.01</td>
<td>0.34 ± 0.01</td>
<td>0.31 ± 0.01</td>
<td>0.023</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.67 ± 0.07</td>
<td>1.88 ± 0.11</td>
<td>1.89 ± 0.07</td>
<td>0.153</td>
</tr>
<tr>
<td>Plasma total cholesterol, mmol/L</td>
<td>5.72 ± 0.04</td>
<td>5.74 ± 0.06</td>
<td>5.71 ± 0.03</td>
<td>0.579</td>
</tr>
<tr>
<td>Plasma LDL, mmol/L</td>
<td>3.53 ± 0.05</td>
<td>3.56 ± 0.09</td>
<td>3.59 ± 0.03</td>
<td>0.381</td>
</tr>
<tr>
<td>Plasma HDL, mmol/L</td>
<td>1.48 ± 0.02</td>
<td>1.45 ± 0.02</td>
<td>1.48 ± 0.01</td>
<td>0.697</td>
</tr>
<tr>
<td>Plasma TAG, mmol/L</td>
<td>1.25 ± 0.04</td>
<td>1.25 ± 0.02</td>
<td>1.34 ± 0.04</td>
<td>0.042</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>131 ± 0.8</td>
<td>131 ± 0.5</td>
<td>132 ± 0.9</td>
<td>0.362</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>82 ± 0.4</td>
<td>82 ± 0.3</td>
<td>83 ± 0.6</td>
<td>0.545</td>
</tr>
<tr>
<td>Dietary SFA, % energy</td>
<td>15.12 ± 0.31</td>
<td>15.59 ± 0.39</td>
<td>15.73 ± 0.24</td>
<td>0.107</td>
</tr>
<tr>
<td>Dietary PUFA, % energy</td>
<td>13.98 ± 0.28</td>
<td>14.22 ± 0.34</td>
<td>14.47 ± 0.21</td>
<td>0.888</td>
</tr>
<tr>
<td>Dietary MUFA, % energy</td>
<td>5.61 ± 0.14</td>
<td>5.55 ± 0.16</td>
<td>5.67 ± 0.09</td>
<td>0.237</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM.
2 SBP, Systolic blood pressure; DBP, diastolic blood pressure.

Discussion
In this case-control study, we examined the association between STAT3 polymorphisms with MetS risk. Although no associations were found between STAT3 polymorphisms and MetS, we...
demonstrated that common genetic variants at the STAT3 locus were associated with increased risk of abdominal obesity, the key causal factor of MetS. As individual genetic variants generally confer only a moderate risk to a trait, analyzing multiple risk alleles simultaneously can be more informative and enhance predictive power (30). Therefore, we assessed the cumulative risk of possessing several of the STAT3 alleles associated with increased obesity. We noted a significant genotype effect between the number of risk alleles and risk of abdominal obesity, with an ~2.5-fold increased risk of abdominal obesity in individuals carrying >2 risk alleles compared with individuals carrying 1 or fewer risk alleles. Interestingly, the association with obesity derived primarily from the male participants, whereas in female participants, STAT3 polymorphisms were associated with insulin sensitivity.

Examination of the impact of STAT3 polymorphisms on obesity and glucose-related traits to date is limited and conflicting, which may reflect gender differences, insufficient sample size, genetic heterogeneity, and gene-nutrient interactions within the populations studied. To our knowledge, this is also the first study of STAT3 polymorphisms in relation to such phenotypes in both male and female participants and, interestingly, we noted some gender differences with regard to associations with MetS phenotypes. The association with obesity reported in this study was driven mainly by the male participants, whereas the effects in the same direction in the female participants were not significant ($P = 0.10–0.31$), which may reflect lack of statistical power or difference in age range between male and female participants. However, when we compared male and female participants of comparable age (data not shown), the gender difference associated with the relationship between STAT3 polymorphisms and obesity risk remained. Associations between rs2293152 and insulin resistance have been previously reported in females (11). In keeping with this, when female participants were analyzed separately, we reported a 3-fold increased risk of insulin resistance and also insulin sensitivity in the rs2293152 major GG homozygotes. Conversely, a previous study in male participants did not detect any association between rs2293152 with glucose-related traits or obesity (12). In the current study, male rs2293152 GG homozygotes had increased risk of abdominal obesity and fasting hyperglycemia.

An individual’s phenotype represents a complex interaction between the genetic background and environmental factors over the course of an individual’s lifetime. Fatty acids are key environmental factors in the pathogenesis and progression of MetS (13–15,31,32). Population-based studies have demonstrated a positive correlation between SFA concentrations and MetS risk, with serum SFA concentrations predictive of MetS development over a 20-y follow-up (13,15). This may be in part due to the detrimental effect of SFA on insulin sensitivity. Cell culture studies have demonstrated that SFA induces insulin resistance at the level of both insulin signaling and gene expression through inhibition of insulin receptor, insulin receptor substrate 1 and 2 tyrosine phosphorylation, and activation of phosphoinositide-3 kinase and Akt (33,34). Furthermore, increasing dietary SFA intake has been shown to increase the risk of obesity (19,20). In this study, high dietary SFA intake ($\geq 15.5\%$ of energy) modulated the genetic association with obesity; carriers of $>2$ risk alleles with the highest SFA consumption further increased their risk of abdominal obesity by 32% compared with those carrying 1 or fewer risk alleles. Interaction analysis confirmed that increasing SFA intake in these individuals ($>2$ risk allele carriers) was predictive of increased waist circumference. The finding that a high dietary SFA intake magnified the genetic predisposition to abdominal obesity suggests that individuals with certain STAT3 genotypes are more sensitive to SFA and that these individuals may derive the most benefit from dietary manipulation and current guidelines to reduce dietary SFA intake.

Animal studies in mice with targeted disruption of the STAT3 gene initially highlighted the importance of STAT3 in body weight regulation and normal glucose homeostasis (5–10). STAT3 activation is induced by cytokines, such as IL-6 (4) and also by leptin (7). The binding of leptin to its receptors, most importantly LRb, activates the JAK-STAT3 pathway, which subsequently mediates a number of transcriptional events including the transcription of pro-opiomelanocortin and the suppressor of cytokine signaling 3 (35). The long form of the leptin receptor-STAT3 signal is central to regulation of food intake and energy expenditure by leptin (36). Unfortunately, we did not have sufficient plasma to measure plasma concentrations of IL-6 or leptin in the current study. Using a model of diet-induced obesity, Townsend et al. (37) reported altered hypothalamic expression of genes whose products regulate the nuclear translocation or activity of STAT3. Furthermore, they showed that the type of fat (saturated vs. unsaturated) did not influence weight gain when total fat content of the diet is high but that saturated fats induced weight gain in animals receiving the low-fat diet. More recently, in vitro studies have demonstrated that treatment with the MUFA oleic acid induced STAT3 phosphorylation and subsequent STAT3 activation and increased DNA binding of STAT3 (38). Further studies are required to understand the mechanisms whereby fatty acids modulate STAT3 activation and function. Interestingly, we did not find any evidence that dietary MUFA or PUFA composition, including (n-3), (n-6), and long-chain (n-3) PUFA subclasses, modulate the genetic susceptibility to abdominal obesity. This suggests a specific effect of SFA. Recently, toll-like receptor-4 (TLR4), a key player in innate immunity, was identified as the molecular link between fatty acids, obesity, inflammation, and insulin resistance (39). SFA activate TLR4 and recent data suggest that SFA serve as a naturally occurring TLR4 ligand (40). Of note, TLR4 deficiency selectively protects against high-SFA diet-induced obesity and alters obesity-related inflammatory responses in adipose tissue (41). Crosstalk among TLR4 and JAK-STAT3 pathways is regulated by complex interactions at the level of signal transduction (42). Taken together, these findings suggest that TLR4 may be one gateway by which SFA and STAT3 potentially affect insulin resistance/insulin sensitivity.

In terms of potential functional effects of these STAT3 polymorphisms, rs1053995 and rs744166 are located in the 3’UTR and 5’UTR regions, respectively, and therefore may have the potential to disrupt regulation of translation efficiency or mRNA stability. Although rs8069645 and rs2293152 are intronic region polymorphisms, their potential impact on STAT3 expression or function is less clear, but it is possible that they could also affect mRNA stability or modulate STAT3 gene transcriptional activity. Functional studies are needed to ascertain their biological importance. We corrected for multiple testing in this study and almost all results remained significant; however, replication of these results in an independent cohort is required to validate these findings.

In conclusion, this study provides new data on STAT3 genetic variants, abdominal obesity, and modulation by dietary fatty acids in MetS. These associations and novel gene-nutrient interactions suggest that dietary SFA intake may have the
potential to modify the genetic susceptibility of obesity, which is highly relevant in diet-related polygenic disorders, such as MetS. Understanding the molecular mechanisms whereby STAT3 and STAT3 genetic variants are involved in obesity development and how dietary fatty acids can modulate these processes may be useful in identifying potential therapeutic targets in obesity.

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Literature Cited

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