Leptin Receptor Polymorphisms Interact with Polyunsaturated Fatty Acids to Augment Risk of Insulin Resistance and Metabolic Syndrome in Adults

Catherine M. Phillips, Louisa Goumidi, Sandrine Bertrais, Martyn R. Field, Jose M. Or dovas, L. Adrienne Cupples, Catherine Defoort, Julie A. Lovegrove, Christian A. Drevon, Ellen E. Blaak, Michael J. Gibney, Beata Kiec-Wilk, Britta Karlstrom, Jose Lopez-Miranda, Ross McManus, Serge Hercberg, Denis Lairon, Richard Planells, and Helen M. Roche

Abstract

The leptin receptor (LEPR) is associated with insulin resistance, a key feature of metabolic syndrome (MetS). Gene-fatty acid interactions may affect MetS risk. The objective was to investigate the relationship among LEPR polymorphisms, insulin resistance, and MetS risk and whether plasma fatty acids, a biomarker of dietary fatty acids, modulate this. LEPR polymorphisms (rs10493380, rs1137100, rs12067936, rs1805096, rs2025805, rs3790419, rs3790433, rs6673324, and rs8179183), biochemical measurements, and plasma fatty acid profiles were determined in the LIPGENE-SU.VI.MAX study of MetS cases and matched controls (n = 1754). LEPR rs3790433 GG homozygotes had increased MetS risk compared with the minor A allele carriers [odds ratio (OR) = 1.65; 95% CI: 1.05–2.57; P = 0.028], which may be accounted for by their increased risk of elevated insulin concentrations (OR 2.40; 95% CI: 1.28–4.50; P = 0.006) and insulin resistance (OR = 2.15; 95% CI: 1.18–3.90; P = 0.012). Low (less than median) plasma (n-3) and high (n-6) PUFA status exacerbated the genetic risk conferred by GG homozygosity to hyperinsulinemia (OR 2.92–2.94) and insulin resistance (OR 3.40–3.47). Interestingly, these associations were abolished against a high (n-3) or low (n-6) PUFA background. Importantly, we replicated some of these findings in an independent cohort. Homozygosity for the LEPRrs3790433 G allele was associated with insulin resistance, which may predispose to increased MetS risk. Novel gene-nutrient interactions between LEPR rs3790433 and PUFA suggest that these genetic influences were more evident in individuals with low plasma (n-3) or high plasma (n-6) PUFA.

Introduction

Insulin resistance, a condition in which normal amounts of insulin fail to maintain normal blood glucose, is the core perturbation of metabolic syndrome (MetS), a clustering of dyslipidemia, abdominal obesity, and hypertension associated...
with increased risk of type 2 diabetes mellitus, cardiovascular disease, and atherosclerosis (1). Leptin, an adipocytokine, has been shown to stimulate glucose uptake and fatty acid oxidation (2). Furthermore, leptin modulates insulin secretion and action via leptin receptors (LEPR) that are present in pancreatic β cells, adipose tissue, and muscle (3,4). Several studies have shown that LEPR polymorphisms are associated with insulin and glucose metabolism (5), insulin resistance (6–8), and type 2 diabetes mellitus (9–11). More recently, an association was identified between the LEPR Gln223Arg (rs1137101) polymorphism and MetS in a small elderly population (12).

Inter-individual variation in measures of insulin resistance and MetS risk are probably due to interaction between environmental and genetic factors. The first indication that environmental factors could influence the relationship between LEPR polymorphisms and insulin sensitivity came from the HERITAGE family study, wherein LEPR Lys656Asn (rs8179183) modulated the effect of exercise, resulting in increased insulin sensitivity in certain genotypes (13). Furthermore, investigation of the influence of this single nucleotide polymorphism (SNP) in response to a lifestyle modification (Mediterranean hypocaloric diet and exercise) revealed that Lys656Lys individuals, but not Asn carriers, had significant improvements in their anthropometric profiles (14). Environmental factors, in particular plasma fatty acid composition, may also modulate MetS risk (15–18). Insulin-sensitizing effects of (n-3) PUFA and/or fish oil have been reported in animal studies (19,20). However, intervention trials to confirm functional effects of dietary PUFA are mixed (21–24), which may reflect interaction between an individual’s genetic background and dietary fat exposure to affect risk of insulin resistance and MetS (25,26). Animal, cell, and human studies have demonstrated that fatty acids also influence leptin expression and concentration. Dietary (n-3) PUFA intake negatively correlates with leptin concentrations (27,28), whereas reduced leptin expression and concentration have been reported following intervention with fish oil and/or long-chain (n-3) PUFA (19,20). Although the evidence suggests that the LEPR and fatty acids play a role in insulin resistance, it is unknown whether interactions between fatty acids and LEPR polymorphisms influence the risk of developing insulin resistance or MetS. Therefore, this prospective study investigated the potential relationship between LEPR polymorphisms and MetS and its insulin-related phenotypes, in particular insulin resistance, and whether they are modulated by plasma PUFA, a biomarker of habitual dietary PUFA intake. Furthermore, we sought to replicate our findings in a separate independent LIPGENE study of MetS cases participating in a 12-wk dietary intervention to alter dietary fatty acid composition.

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 from 1994 to 2002 (29). 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 (CPPPB of 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. 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 [increased waist circumference, increased fasting blood glucose, increased triacylglycerol (TAG), decreased HDL cholesterol (HDL-C), and increased systolic/diastolic blood pressure] 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 (30) and were defined as previously described (31); 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) and gender. For replication purposes, we analyzed data from a separate independent LIPGENE MetS case-only cohort of 464 individuals who participated in a 12-wk dietary intervention to alter dietary fatty acid composition (32).

Biochemical analysis. Fasting plasma glucose, TAG, HDL-C, and total cholesterol were measured as previously described (29). 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), a measure of insulin resistance, was calculated as: [(fasting plasma glucose × fasting plasma insulin)/22.5] (33). Quantitative insulin-sensitivity check index (QUICKI), a measure of insulin sensitivity, was calculated as: [1/log fasting insulin + log fasting glucose + log fasting FFA)] (34). For the replication study, the minimal model-derived insulin sensitivity index (Si) was used (35).

Fatty acid analysis. Baseline plasma fatty acid composition [14:0 to 22:6(n-3)] was determined as a biomarker of habitual dietary fat intake. FAME were analyzed by GC and fatty acid mass was determined as a relative percentage of the total quantified fatty acids as previously described (36).

DNA extraction and genotyping. DNA extraction from buffy coats and whole genome amplification of low yielding samples (<10 ng) was performed as previously described (31). LEPR genotype data from HapMap v1.1 and Perlegen (37,38) was uploaded into HITAGENE, a 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 SNP tagger (39). Including polymorphisms from the literature (40), 10 LEPR polymorphisms with minor allele frequencies > 5% [rs10493380, rs1137100 (Lys109Arg), rs1137101 (Gln223Arg), rs12067936, rs1805096, rs2025805, rs3790419, rs3790433, rs6673324, and rs8179183 (Lys656Asn)] were genotyped as part of the entire genotyping component of the LIPGENE study by Illumina using the Golden Gate Assay on a Beadstation 500G genotyping system. We achieved an average genotyping success rate of 99% and call rate of 99%. All polymorphisms were in Hardy-Weinberg equilibrium (P > 0.01). Linkage disequilibrium between polymorphisms was assessed using Golden Helix software. One pair of polymorphisms, rs12067936 and rs1805096, were in linkage disequilibrium (r² = 0.98; D’ = 0.99) (Supplemental Fig. 1).

Replication study. A separate independent LIPGENE MetS case-only cohort (n = 464) (32) was used for replication purposes of the findings from the main case-control study. In brief, individuals were randomized to 1 of 4 isonenergetic dietary treatments, differing both in quality and
quantity of dietary fatty acids, for 12 wk: high-fat (38% energy), SFA-rich diet; high-fat (38% energy), monounsaturated fatty acid (MUFA)-rich diet; and low-fat (28% energy), high-complex carbohydrate diet (LFHCC), with either 1 g/d high oleic sunflower oil supplement (LHFCC) or 1.24 g/d (n-3) LCPUFA [LHFCC (n-3) PUFA]. Further details of the dietary intervention procedure have been published previously (41).

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, TAG, QUICKI, and HOMA were normalized by logarithmic transformation. Genotype frequencies were compared between cases and controls in HITAGENE using Fisher’s exact test. Conditional logistic regression determined associations between genotypes and MetS and its risk phenotypes (high HOMA, low QUICKI, fasting hyperglycemia, high TAG, low HDL, abdominal obesity, and hypertension). Cutoff points for these MetS risk phenotypes were determined by the MetS criteria. HOMA and QUICKI values were dichotomized based on control participant medians. Three genotype groups were first considered to check different inherent models (additive, dominant, and recessive). Where a dominant or recessive effect existed, analysis was repeated comparing carriers compared with noncarriers of that particular allele.

To determine modulation by plasma fatty acids, association analyses were repeated using the median concentration of control participants to dichotomies fatty acids. The generalized estimating equation linear multivariate analysis included age, gender, smoking status, physical genetic effects. Haplotype analysis was conducted using the THESIAS program (42). Potential confounding factors used in the adjusted multivariate analysis included age, gender, smoking status, physical activity, medication use, and alcohol and energy intake. A P-value < 0.05 was considered significant. To account for multiple testing, false discovery rates (FDR) (43) were computed and FDR-adjusted P-values calculated separately for each test are reported. FDR ≤0.05 were considered significant. Paired t-tests were used to compare associations between genotype and insulin-related phenotypes in the LIPGENE MetS case-only replication study (41).

Results

Association between LEPR polymorphisms, MetS, and its phenotypes. Genotype frequencies of the LEPR polymorphisms did not significantly differ between cases and controls in the LIPGENE-SU.VI.MAX study (Table 1). However, when multivariate logistic regression analysis was conducted for each of the LEPR polymorphisms, a significant association was identified between 1 of the polymorphisms (rs3790433) and MetS risk, whereby GG homozygotes had greater MetS risk relative to the minor A allele carriers [odds ratio (OR) = 1.65; 95% CI: 1.05–2.57; P = 0.028; FDR 0.08). This association was gender specific, deriving primarily from the male participants (OR = 1.92; 95% CI: 1.10–3.36; P = 0.023; FDR 0.07). Although the association was in the same direction, it did not reach significance in the female participants (OR = 1.14; 95% CI: 0.53–2.45; P = 0.74; FDR 0.16). The increased MetS risk associated with GG homozygosity for rs3790433 may be accounted for by their increased risk of insulin resistance (OR = 2.40; 95% CI: 1.28–4.50; P = 0.006; FDR 0.04) contributed to by their increased risk of elevated insulin concentrations (OR = 2.15; 95% CI: 1.18–3.90; P = 0.012; FDR 0.08) relative to the minor A allele carriers (Table 2). No gender differences were noted for associations with these metabolic traits.

Clinical characteristics and plasma fatty acid profiles according to LEPR rs3790433 genotype. Age, gender distribution, alcohol intake, and medication use did not differ across genotypes (Table 2). In terms of their phenotype, the GG homozygotes had higher insulin concentrations and increased insulin resistance (P < 0.05) compared with the A allele carriers. BMI, waist circumference, blood pressure, lipid concentrations, and plasma fatty acid profiles were similar across genotypes.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Clinical characteristics and plasma fatty acid profiles of all individuals at study end according to LEPR rs3790433 genotype¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>999</td>
</tr>
<tr>
<td>Male/female, %</td>
<td>60/40</td>
</tr>
<tr>
<td>Age, y</td>
<td>58 ± 5.2</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>51.94 ± 11.2*</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.26 ± 1.03</td>
</tr>
<tr>
<td>HOMA</td>
<td>1.83 ± 0.80*</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.33 ± 0.07</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.1 ± 4.4</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>88 ± 13</td>
</tr>
<tr>
<td>SBP², mm Hg</td>
<td>131 ± 15</td>
</tr>
<tr>
<td>DBP², mm Hg</td>
<td>82 ± 9</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.74 ± 0.92</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.47 ± 0.40</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.55 ± 1.10</td>
</tr>
<tr>
<td>TAG, mmol/L</td>
<td>1.28 ± 0.66</td>
</tr>
<tr>
<td>Lipid-lowering medication, %</td>
<td>19.50</td>
</tr>
<tr>
<td>Antidiabetic medication, %</td>
<td>2.90</td>
</tr>
<tr>
<td>Hypertensive medication, %</td>
<td>22.10</td>
</tr>
<tr>
<td>Alcohol intake, % energy</td>
<td>6.69 ± 0.90</td>
</tr>
<tr>
<td>Total PUFAs, % total measured fatty acids</td>
<td>43.75 ± 6.00</td>
</tr>
<tr>
<td>[n-6] PUFAs, % total measured fatty acids</td>
<td>39.29 ± 5.62</td>
</tr>
<tr>
<td>[n-3] PUFAs, % total measured fatty acids</td>
<td>4.45 ± 1.88</td>
</tr>
<tr>
<td>[n-3] LCPUFAs, % total measured fatty acids</td>
<td>3.37 ± 1.56</td>
</tr>
<tr>
<td>SFA, % total measured fatty acids</td>
<td>34.31 ± 5.79</td>
</tr>
<tr>
<td>MUFA, % total measured fatty acids</td>
<td>21.95 ± 3.83</td>
</tr>
</tbody>
</table>

¹Values are means ± SD. All measurements were made in plasma. * indicates P < 0.05 for linear regression adjusted for potential confounding factors compared with A allele carriers.
²SBP, Systolic blood pressure; DBP, diastolic blood pressure.
Gene-nutrient interactions modulate the association with MetS. We investigated whether plasma fatty acid composition and insulin resistance and MetS risk. Among all participants in the bottom 50th percentile of plasma PUFA (<45.85% of total measured fatty acids), MetS risk was exacerbated among GG homozygotes compared with the minor A allele carriers (OR = 2.98; 95% CI: 1.20–7.39; P = 0.018; FDR 0.03) (Table 3). However, among all individuals with PUFA intake in the top 50th percentile, the MetS risk conferred by this SNP was abolished. Examination of individual PUFA constituents revealed a nonsignificant trend toward increased MetS risk against a background of low (n-3) PUFA (P = 0.19) and (n-3) LCPUFA (P = 0.23) status (<4.4 and 3.85% of total measured fatty acids, respectively). Among all individuals in the top 50th percentile of (n-6) PUFA (>41% of total measured fatty acids), MetS risk was significantly increased in the GG homozygotes relative to the minor A allele carriers.

Interaction with PUFA augments the association between LEPR rs3790433 with insulin resistance. Interestingly, the risks of elevated insulin concentrations and insulin resistance were modulated by plasma PUFA status (Table 3). Among all individuals in the lowest median of (n-3) PUFA, the risk of hyperinsulinemia and insulin resistance conferred by GG homozygosity was exacerbated (OR = 2.92; 95% CI: 1.35–5.28; P = 0.006; FDR 0.01 and OR = 3.47; 95% CI: 1.55–5.77; P = 0.002; FDR 0.03). Similar results were obtained when all participants in the lowest median of (n-3) LCPUFA were examined. Among participants in the highest median of (n-3) PUFA and (n-3) LCPUFA, the risk of hyperinsulinemia and insulin resistance associated with the GG homozygotes was effectively eliminated. In contrast, high (n-6) PUFA levels accentuated the genetic susceptibility to insulin resistance (OR = 3.40; 95% CI: 1.24–5.28; P = 0.017; FDR 0.06) and hyperinsulinemia (OR = 2.94; 95% CI: 1.12–4.37; P = 0.028; FDR 0.11) in the GG homozygotes relative to the minor A allele carriers, which was abolished against a low (n-6) PUFA background. Examination of actual HOMA levels confirmed these gene-nutrient interactions whereby GG homozygotes with the highest plasma PUFA (% values 1.35 ± 0.13 vs. 1.70 ± 0.08; P = 0.009) and lowest (n-6) PUFA (HOMA values 1.39 ± 0.20 vs. 1.74 ± 0.11; P = 0.01) displayed reduced insulin resistance compared with the A allele carriers. High-SFA status (>32% of total measured fatty acids) was associated with elevated insulin concentrations and insulin resistance in the GG homozygotes compared with the A allele carriers (OR = 2.39; 95% CI: 1.08–3.29; P = 0.032; FDR 0.09) and insulin resistance (OR = 3.05; 95% CI: 1.32–5.06; P = 0.009; FDR 0.03). In contrast, MUFA status did not modulate risk of either phenotype.

LEPR haplotypes and insulin resistance. Haplotype analysis investigated the combined effect of LEPR rs3790433 with 3 functional LEPR polymorphisms [Lys109Arg (rs1137100), Gln223Arg (rs1137101), and rs8179183 (Lys656Asn)], which have been associated with insulin-related phenotypes (40), on the risk of insulin resistance. The GAAG haplotype, representing over 20% of the observed haplotypes, was associated with increased risk of insulin resistance (OR = 1.22; 95% CI: 1.02–1.47; P = 0.037) (Table 4). This haplotype effect was modulated by plasma fatty acid status whereby low (n-3) PUFA levels exacerbated the risk (OR = 1.63; 95% CI: 1.10–2.59; P = 0.039). Of note when the rs3790433 G allele was replaced by the A allele in the AAAG haplotype, the association with insulin resistance was not observed.

Replication of gene-nutrient interactions in an independent cohort. We attempted to replicate our findings in a separate independent LIPGENE MetS case-only cohort (n = 464) (42). Preintervention, the “at risk” LEPR rs3790433 GG homozygotes displayed reduced insulin sensitivity as assessed by Si (Si values 2.70 ± 0.14 vs. 3.12 ± 0.18; P = 0.02) compared with the A allele carriers. Interestingly, we noted enhanced insulin sensitivity (13% improvement in Si values; P = 0.01) and reduced insulin resistance (13% reduction in HOMA; P = 0.04) following a 12-wk LFHCC dietary intervention supplemented with 1.24 g/d (n-3) LCPUFA [LFHCC (n-3) PUFA diet] in GG homozygotes compared with AA allele carriers (Fig. 1). Interestingly, no changes were observed between genotypes when participants received the same low-fat diet supplemented with 1 g/d high oleic acid (LFHCC control diet).

Discussion

In this case-control study, we examined the association between LEPR polymorphisms with insulin resistance and MetS risk and their potential modulation by plasma fatty acids. We demonstrated that a common genetic variant at the LEPR locus, rs3790433, was associated with increased MetS risk, which may be explained by increased risk of insulin resistance. Interestingly, plasma fatty acid composition and status modulated these genetic influences, with the deleterious effects more evident against a background of low (n-3) or high (n-6) PUFA and to a lesser extent in the A allele carriers (n = 755). Potential confounding factors included in the analyses were age, gender, smoking status, physical activity, use of medication, and alcohol and energy intake. P-values represent the uncorrected P-value generated by the logistic regression analyses.

**TABLE 3** OR and 95% CI for the associations between LEPR rs3790433 and hyperinsulinemia, insulin resistance, and MetS according to plasma fatty acid composition and status

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>OR (95% CI) for hyperinsulinemia</th>
<th>OR (95% CI) for insulin resistance</th>
<th>OR (95% CI) for MetS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PUFA</td>
<td>2.59 (1.14–4.86) 0.023</td>
<td>0.96 (0.60–1.62) 0.854</td>
<td>2.47 (1.04–5.88) 0.041</td>
</tr>
<tr>
<td>(n-6) PUFA</td>
<td>1.06 (0.71–1.58) 0.786</td>
<td>2.94 (1.12–4.37) 0.028</td>
<td>4.05 (1.68–1.60) 0.837</td>
</tr>
<tr>
<td>(n-3) PUFA</td>
<td>2.92 (1.35–5.28) 0.006</td>
<td>0.96 (0.60–1.55) 0.878</td>
<td>3.47 (1.55–5.77) 0.002</td>
</tr>
<tr>
<td>(n-3) LCPUFA</td>
<td>2.99 (1.37–4.54) 0.006</td>
<td>1.02 (0.64–1.61) 0.937</td>
<td>3.55 (1.55–4.80) 0.002</td>
</tr>
<tr>
<td>MUFA</td>
<td>0.75 (0.52–1.08) 0.121</td>
<td>0.94 (0.67–1.32) 0.720</td>
<td>0.79 (0.56–1.15) 0.223</td>
</tr>
<tr>
<td>SFA</td>
<td>0.99 (0.65–1.52) 0.986</td>
<td>2.39 (1.08–3.29) 0.032</td>
<td>0.96 (0.62–1.50) 0.872</td>
</tr>
</tbody>
</table>

1 OR and 95% CI for the associations between LEPR rs3790433 and hyperinsulinemia, insulin resistance, and MetS, stratified according to plasma fatty acid composition and status (median levels of fatty acids expressed as percent total measured fatty acids) were determined by logistic regression analyses comparing GG homozygotes (n = 999) to the A allele carriers (n = 755). Potential confounding factors included in the analyses were age, gender, smoking status, physical activity, use of medication, and alcohol and energy intake. P-values represent the uncorrected P-value generated by the logistic regression analyses.
TABLE 4  Haplotype frequencies and OR for insulin resistance among all participants

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>&lt;Median HOMA, n=590&gt;</th>
<th>&gt;Median HOMA, n=1164</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGGG</td>
<td>24.00%</td>
<td>24.60%</td>
<td>0.310</td>
<td></td>
</tr>
<tr>
<td>GAAG</td>
<td>22.12%</td>
<td>15.75%</td>
<td>1.22 (1.02–1.47)</td>
<td>0.037</td>
</tr>
<tr>
<td>GAGG</td>
<td>15.90%</td>
<td>16.72%</td>
<td>1.14 (0.92–1.42)</td>
<td>0.232</td>
</tr>
<tr>
<td>AAAG</td>
<td>14.32%</td>
<td>13.35%</td>
<td>1.02 (0.79–1.29)</td>
<td>0.893</td>
</tr>
<tr>
<td>GAAC</td>
<td>11.00%</td>
<td>11.50%</td>
<td>1.15 (0.88–1.49)</td>
<td>0.310</td>
</tr>
</tbody>
</table>

1 OR, 95% CI, and P-values for the association between LEPR haplotypes, represented by rs3790433, rs1137100, rs1137101, and rs8179183, on the risk of insulin resistance. HOMA values were dichotomized based on control subject medians. GGGG represents the most common haplotype and is thus used as the reference haplotype.

lesser extent with high-SFA status. In a separate independent LIPGENE MetS case cohort (32), we replicated our findings with LEPR rs3790433 genotype and insulin responsiveness. Furthermore, we report significant improvements to indices of insulin sensitivity and insulin resistance by the GG homozygotes following a 12-wk, low-fat dietary intervention supplemented with (n-3) LCPUFA. These data suggest that genetic influences associated with this LEPR polymorphism may be selectively modulated by (n-3) LCPUFA. Notwithstanding the study design differences, these data strengthen our findings in the case-control cohort. Nevertheless, functional studies are needed to ascertain the potential biological mechanisms. While plasma fatty acid composition reflects the combination of dietary fat consumption and endogenous de novo fatty acid biosynthesis and metabolism, it is tempting to speculate that such gene-nutrient interactions suggest that fatty acids may have the potential to modify genetic predisposition to insulin resistance and developing MetS.

Environmental factors such as exercise have been shown to modulate the relationship between LEPR polymorphisms and insulin-related phenotypes (13). However, data on the interaction between diet and LEPR polymorphisms are limited (14,44). Animal, cell, and human studies have shown that fatty acids influence leptin expression and concentration (19,20,27,28). To our knowledge, no studies have investigated the relationship between plasma or dietary fatty acids and LEPR polymorphisms on MetS risk. Whereas beneficial effects of (n-3) PUFA and/or fish oil on insulin responsiveness have been reported in animal studies (19,20), replication of these functional effects in humans has been contradictory (21–24). In contrast, experiments in rodents and epidemiological studies in human populations indicate that SFA decrease insulin responsiveness (45–48). In the current study, the risk conferred by rs3790433 GG homozygosity to hyperinsulinemia, insulin resistance, and MetS was subject to considerable modification by plasma fatty acid status. GG homozygotes with the lowest plasma (n-3) PUFA and (n-3) LCPUFA and highest (n-6) PUFA levels had the greatest risk of these insulin-related traits. High-plasma SFA status also accentuated these deleterious associations but to a lesser extent than observed with PUFA. Interestingly, when all individuals with the highest plasma (n-3) PUFA and (n-3) LCPUFA and lowest (n-6) PUFA and SFA levels were examined, the genetic risks conferred by rs3790433 GG homozygosity were abolished. One interpretation could be that individuals who are genetically predisposed to insulin resistance, and thus MetS (57% of this population), are most sensitive to PUFA, such that either low (n-3) or high (n-6) PUFA status exacerbates their genetic predisposition. It is somewhat perplexing why our observations with total PUFA (Table 3) are mirrored by the (n-3) but not the (n-6) PUFA, which represent their major constituent. In the absence of functional studies, we are unable to unravel the molecular basis of this finding.

In contrast to dietary fat measurement, plasma fatty acid composition reflects the combination of dietary fat consumption and endogenous de novo fatty acid biosynthesis and metabolism, thus making direct comparisons between some plasma and dietary fatty acid measurements difficult. However, the relationship between dietary PUFA intake and their plasma concentrations has previously been examined in the SU.VI.MAX cohort. Linoleic acid, arachidonic acid, and the (n-3) LCPUFA eicosapentaenoic and docosahexaenoic acid were acceptable markers of their dietary intake (49). Our data suggests that LEPR rs3790433 GG homozygotes are sensitive to plasma fatty acid composition and that these individuals may derive the most benefit from dietary manipulation and current guidelines to reduce dietary SFA and increase (n-3) PUFA intake. (n-3) PUFA may reduce insulin resistance through a number of different mechanisms. (n-3) PUFA are ligands of PPARγ, a nuclear receptor that regulates leptin expression (50). Recently, toll-like receptors (TLR) have been identified as the molecular link among fatty acids, inflammation, and insulin resistance whereby (n-3) PUFA inhibit and SFA activates TLR2 and TLR4 (51,52).

There are no functional data on LEPR rs3790433; thus, we can only speculate about mechanisms underlying our findings. The intronic location of LEPR rs3790433 may potentially affect mRNA stability or modulate LEPR gene transcriptional activity. Furthermore, rs3790433 may be a surrogate marker for other functional LEPR polymorphisms in the region. Haplotype analysis identified a risk haplotype associated with 20% greater susceptibility of being insulin resistant. However, it should be noted that estimated haplotype clusters do not always correlate with true biological groups and thus should be considered with caution. It is also possible that LEPR rs3790433 may be a marker for functional polymorphisms of other genes on chromosome 1p31, an area previously linked to an acute insulin response (53). Cell studies have demonstrated that LC (n-3) PUFA reduce leptin promoter activity (27). The influence of genetic variation on this is unknown but would be worthwhile to
address in an attempt to understand the molecular mechanisms underlying how fatty acids and LEPR polymorphisms influence insulin resistance. One possible explanation for our findings is that altered leptin expression via either dietary or plasma fatty acid modulation of PPARγ and/or TLR combined with unknown effects of LEPR polymorphisms alter leptin-mediated signaling pathways, resulting in downstream changes to insulin-related phenotypes. Additional work and functional studies are needed to ascertain the biological importance of the gene-nutrient interactions identified in the current work.

We noted gender differences for some of our associations; this is consistent with data from a smaller study in nondiabetic obese participants (8) and may reflect lack of statistical power. Previous studies have also noted a profound divergence in leptin levels between males and females, with higher circulating leptin concentrations reported in women (54), perhaps due to gender-specific differences in adipose tissue mass distribution. Unfortunately, insufficient plasma precluded the measurement of leptin levels in this study. Recently described gender-dependent associations between leptin concentration and MetS-related measures provide further evidence of gender differences mediated by leptin in MetS-related metabolic pathways (55).

In conclusion, this study provides new data on LEPR genotype and plasma fatty acids, especially PUFA, in MetS. Furthermore, we replicated some of these results in an independent MetS cohort. These novel gene-nutrient interactions suggest that genetic predisposition to insulin resistance and MetS, according to LEPR genotype, is more evident in individuals with low plasma (n-3) PUFA, which may partly be due to low dietary PUFA intake. Clinical application of such data may help to identify individuals at greater risk of insulin resistance and MetS who respond to (n-3) LCPUFA intervention with improvements in insulin sensitivity and insulin resistance following (n-3) LCPUFA supplementation. Such an approach may also be useful in developing personalized dietary recommendations wherein genetic profile may determine choice of dietary therapy to aid responsiveness to dietary fatty acid interventions and reduce MetS risk.

Acknowledgments

Literature Cited


