Gene-nutrient interactions with dietary fat modulate the association between genetic variation of the ACSL1 gene and metabolic syndrome

Catherine M. Phillips,* Louisa Goumidi,1 Sandrine Bertrais,2 Martyn R. Field,2
L. Adrienne Cupples,1† Jose M. Ordovas,2* Catherine Defoort,1 Julie A. Lovegrove,3
Christian A. Drevon,1†† Michael J. Gibney,4* Ellen E. Blaak,4*** Beata Kiec-Wilk,4††† Britta Karlstrom,4*** Jose Lopez-Miranda,5**** Ross McManus,5***** Serge Hercberg,5§
Denis Lairon,5† Richard Planells,1 and Helen M. Roche1,11

Nutrigenomics Research Group,* UCD School of Public Health and Population Science, UCD Conway Institute, and Institute of Food and Health, University College Dublin, Ireland; INSERM 476,1 Lipid nutrients and prevention of metabolic diseases, INRA, 1260, Université de la Méditerranée, Faculté de Médecine, 27 Bd Jean Moulin, Marseille, France; INSERM U557,5 INRA:CNAM; Université Paris 13, Bobigny, France; Hitachi Dublin Laboratory,** Dublin, Ireland; Boston University School of Public Health,17 Boston, MA; Nutrition and Genomics Laboratory,18 Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA; Hugh Sinclair Unit of Human Nutrition,*** Department of Food Biosciences, Institute for Cardiovascular and Metabolic Research, University of Reading, Reading, UK; Department of Nutrition,10 Institute of Basic Medical Sciences, University of Oslo, Norway; Institute of Food and Health,88 University College Dublin, Ireland; Department of Human Biology,**** Nutrition and Toxicology Research Institute Maastricht, Maastricht, The Netherlands; Department of Clinical Biochemistry,111 Jagiellonian University Medical College, Kopernika 15A, Krakow, Poland; Department of Public Health and Caring Sciences/Clinical Nutrition and Metabolism,88 Uppsala University, Uppsala Science Park, 751 85 Uppsala, Sweden; Lipid and Atherosclerosis Unit,**** Department of Medicine, Reina Sofia University Hospital, School of Medicine, University of Cordoba, Spain; and Institute of Molecular Medicine,1111 Trinity College Dublin, Ireland

Abstract Long-chain acyl CoA synthetase 1 (ACSL1) plays an important role in fatty acid metabolism and triacylglycerol (TAG) synthesis. Disturbance of these pathways may result in dyslipidemia and insulin resistance, hallmarks of the metabolic syndrome (MetS). Dietary fat is a key environmental factor that may interact with genetic determinants of lipid metabolism to affect MetS risk. We investigated the relationship between ACSL1 polymorphisms (rs4862417, rs6552828, rs13120078, rs9997745, and rs12503643) and MetS risk and determined potential interactions with dietary fat in the LIPGENE-SU.VI.MAX study of MetS cases and matched controls (n = 1,754). GG homozygotes for rs9997745 had increased MetS risk (odds ratio (OR) 1.90 [confidence interval (CI) 1.15, 3.13]; P = 0.01), displayed elevated fasting glucose (P = 0.001) and insulin concentrations (P = 0.002) and increased insulin resistance (P = 0.03) relative to the A allele carriers. MetS risk was modulated by dietary fat, whereby the risk conferred by GG homozygosity was abolished among individuals consuming either a low-fat (<35% energy) or a high-PUFA diet (>5.5% energy). In conclusion, ACSL1 rs9997745 influences MetS risk, most likely via disturbances in fatty acid metabolism, which was modulated by dietary fat consumption, particularly PUFA intake, suggesting novel gene-nutrient interactions.—Phillips, C. M., L. Goumidi, S. Bertrais, M. R. Field, L. A. Cupples, J. M. Ordovas, C. Defoort, J. A. Lovegrove, C. A. Drevon, M. J. Gibney, E. E. Blaak, B. Kiec-Wilk, B. Karlstrom, J. Lopez-Miranda, R. McManus, S. Hercberg, D. Lairon, R. Planells, and H. M. Roche. Gene-nutrient interactions with dietary fat modulate the association between genetic variation of the ACSL1 gene and metabolic syndrome. J. Lipid Res. 2010. 51: 1793–1800.

Supplementary key words genetic polymorphisms • insulin resistance • fatty acid metabolism • PUFA

Supported by the European Commission, Framework Programme 6 (LIPGENE); contract number FOOD-CT-2003-505944. Funds were also obtained from the Norwegian Foundation for Health and Rehabilitation, South-Eastern Norway Regional Health Authority, Johan Throne Holst Foundation for Nutrition Research, and Fries Medical Research Foundation. The SU.VI.MAX study is registered as NCT00272428 at ClinicalTrials.gov.

Manuscript received 3 November 2009 and in revised form 17 February 2010.

Published, JLR Papers in Press, February 17, 2010
DOI 10.1194/jlr.M003046

This article is available online at http://www.jlr.org

Abbreviations: ACSL1, long-chain acyl CoA synthetase 1; CI, confidence interval; HOMA, homeostasis model assessment; MetS, metabolic syndrome; OR, odds ratio; PPAR, peroxisome proliferator activated receptor; QUICKI, quantitative insulin-sensitivity check index; SNP, single nucleotide polymorphism; T2DM, type 2 diabetes mellitus; TAG, triacylglycerol.

1To whom correspondence should be addressed.
e-mail: helen.roche@ucd.ie
The metabolic syndrome (MetS) is a common, multi-component condition characterized by insulin resistance, dyslipidemia, abdominal obesity, and hypertension that is associated with an increased risk of type 2 diabetes mellitus (T2DM), cardiovascular diseases, and atherosclerosis (1). The pathway that links insulin resistance and obesity with the MetS and T2DM represents a progressive phenotype (2). Prolonged dietary excess (high-fat, obesogenic, insulin-desensitizing diets) and surplus adipose tissue initiate a state of cellular stress and secretion of proinflammatory cytokines leading to increased lipolysis, decreased triacylglycerol (TAG) synthesis, and increased nonesterified fatty acids (NEFA) availability. Subsequently, this results in accumulation of TAG and activated lipids in the form of long-chain fatty acyl-CoA esters, which disrupt normal metabolic functions in adipocytes, muscle, and liver (3). Ultimately, this series of events culminates in reduced insulin responsiveness, resulting in impaired insulin action, compensatory hyperinsulinemia, and glucose intolerance (2, 4–7).

Long-chain acyl CoA synthetase (ACSL) catalyses the first step in fatty acid activation for intracellular metabolism by converting long-chain fatty acids into acyl-CoA thioesters (8). These fatty acyl derivatives have been implicated in the pathogenesis of aspects of T2DM, including insulin resistance and pancreatic dysfunction (9, 10), and TAG biosynthetic enzymes, including ACSL, are upregulated in obesity (11). ACSL1 is the best studied and main ACSL isoforms, which is highly expressed in major energy-metabolizing tissues such as adipose, liver, and muscle (11, 12). In adipose tissue, ACSL1 is a target of peroxisome proliferator activated receptor (PPAR)-γ, whereas in the liver, ACSL1 is a target of PPAR-α, suggesting divergent functional roles of ACSL1 in different tissues (13, 14).

Inter-individual variation in MetS risk is likely due to interaction between environmental and genetic factors. Dietary fat is an important environmental factor, wherein excessive exposure plays a key role in the development of the MetS (15–20). In terms of compositional effects, evidence from epidemiological and cohort studies demonstrate detrimental effects of saturated fatty acids on insulin sensitivity, promoting the development of diabetes (19, 21–24). Conversely, diets rich in long chain n-3 PUFA and MUFA suggest improved insulin sensitivity in healthy and diabetic individuals (25–28). However, intervention trials to confirm these functional effects are mixed (29–33), perhaps reflecting genetic heterogeneity and interaction with dietary fat exposure (16, 17). Interestingly, dietary supplementation with PUFA and several PPAR-α and PPAR-γ agonists increases ACSL1 mRNA expression (13, 34, 35).

Although ACSL1 clearly plays an important role relevant to lipid metabolism, insulin resistance, and obesity, no studies to date have investigated the association between genetic variants of ACSL1 and those traits in the MetS. Therefore, this study investigated the potential relationship between common genetic polymorphisms of ACSL1 and the MetS and whether this is modulated by dietary fatty acid intake.

**METHODS AND MATERIALS**

**Subjects, 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 (36). 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 (37). Additional ethical approval from the ethical committee (CCPPRB 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. Participants were invited to provide a 24 h dietary record every 2 months, for a total of 6 records per year. Information was collected with the use of computerized questionnaires that were transmitted during a brief telephone connection via the Minitel Telematic Network (France Télécom, Paris, France). Participants were guided by the software’s interactive facilities and by a previously validated instruction manual for coding food portions that included more than 250 foods presented in three different portion sizes. Two intermediate and extreme portions could also be chosen, yielding a total of seven choices for estimating quantities consumed (38). Baseline daily dietary intake data was estimated by using food composition tables validated for the French population (39).

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 NCEP-ATP III criteria for MetS (40). Participants were required to fulfill at least three of the following five criteria: increased waist circumference [≥94cm (men) or ≥80cm (women)], elevated fasting blood glucose [≥5.5 mmol/L or treatment for diabetes], elevated TAG [≥1.5 mmol/L or treatment for dyslipidemia], low HDL cholesterol [<1.04 mmol/L (men) or <1.29 mmol/L (women)], and elevated systolic/diastolic blood pressure [≥130/85 mmHg or antihypertensive treatment]. MetS 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 = 1,754) were matched according to age (± 5 y), gender, and number of dietary records available. For replication purposes, we analyzed data from a separate independent LIPGENE MetS case-only cohort of 464 subjects who participated in a 12 week dietary intervention to alter dietary fatty acid composition and amount (41).

**Biochemical analysis**

Fasting glucose, TAG, HDL, and total cholesterol were measured as previously described (36). Insulin and C-peptide were determined by electrochemiluminescence immunoassays (Roche Diagnostics, France). NEFA and LDL cholesterol were measured by enzymatic colorimetric methods (Randox Laboratories, UK and Roche Diagnostics, France). Homeostasis model assessment (HOMA), a measure of insulin resistance, was calculated as: [(fasting plasma glucose × fasting serum insulin)/22.5] (42). Quantitative insulin-sensitivity check index (QUICKI), a measure of insulin sensitivity, was calculated as = [1/(log fasting insulin + log fasting glucose + log fasting NEFA)] (43).
DNA extraction and genotyping

DNA extraction from buffy coats and whole genome amplification of low yielding samples (<10 ng) was performed as previously described (44). ACSL1 genotype data from HapMap v1.1 (www.hapmap.org) was 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 single nucleotide polymorphisms (SNPs) (rs4862417, rs6552828, rs13120078, rs9997745, and rs12503643) were identified using SNP Tagger (45). SNPs were genotyped as part of the entire genotyping component of the LIPGENE study by Illumina Inc. (San Diego, CA) 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%. Linkage disequilibrium between SNPs and departure of genotype distributions from Hardy-Weinberg equilibrium were assessed in HITAGENE.

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, NEFA, 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 the MetS. 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 versus noncarriers of that particular allele. To determine modulation by dietary fatty acids, logistic analyses were repeated using the median concentration of control subjects to dichotomize fatty acids and associations were examined below and above the fatty acid median. The generalized estimating equation linear regression (46) investigated associations between genotypes and continuous MetS phenotypes. Potential confounding factors used in the adjusted multivariate analysis included age, gender, smoking status, physical activity, energy intake, and use of medications, including lipid-lowering, hypertension, and diabetes treatments. Haplotype analysis was conducted using the THESIAS program (http://ecgene.net/genecanvas) (47). A P-value of < 0.05 was considered as significant.

RESULTS

Association between ACSL1 polymorphisms and the MetS

Table 1 details the ACSL1 polymorphisms studied. All SNPs were in Hardy-Weinberg equilibrium (P > 0.01). Genotype frequencies were different between MetS cases and controls for rs9997745 (P = 0.019), whereby the major G allele was more frequent in the MetS cases in the recessive model (P = 0.0077). This association with MetS remained significant in the adjusted multivariate logistic regression model where risk of MetS conferred by GG homozygosity was almost 2-fold higher relative to the A allele carriers [odds ratio [OR] 1.90 [confidence interval (CI) 1.15, 3.13]; P = 0.01]. Thus, we focused our analyses on this polymorphism. Homogeneity of the genotype effect on MetS was assessed by stratifying according to gender. This analysis revealed that the association with MetS primarily derived from the male subjects [OR 2.24 (CI 1.21, 4.13); P = 0.01; GG homozygotes relative to the A allele carriers]. Although the effect was in the same direction in the female subjects [OR 1.65 (CI 0.59, 4.70)] it was not significant.

Clinical characteristics and dietary fat intake according to rs9997745 genotype

The clinical characteristics and dietary fatty acid intakes of the subjects according to rs9997745 genotype are presented in Table 2. In terms of their phenotype, the GG homozygotes had higher fasting glucose and insulin concentrations (P < 0.05) compared with the minor A allele carriers, with the result that the GG homozygotes were more insulin resistant as determined by HOMA (P < 0.05) relative to the A allele carriers. No differences were noted for any of the lipid parameters. Age, gender distribution, medication use, and alcohol and dietary fat intake were not different between groups. We also examined the clinical characteristics across genotypes according to gender and found no gender differences with between genotypes.

Gene-nutrient interactions modulate the association with the MetS

We examined the influence of dietary fat intake on MetS risk by stratifying according to the control median fat intake. Interestingly, MetS risk was modulated by dietary fat status and composition, whereby the risk conferred by GG homozygosity was abolished among individuals who consumed a low-fat diet (< 35% energy) but remained significant when dietary fat intake was high [OR 1.74 (CI 1.06, 2.84); P = 0.028]. We examined the individual fatty acid classes and identified a gene-nutrient interaction with PUFA, whereby GG homozygotes with low PUFA intake (<5.5% energy) had increased MetS risk [OR 1.70 (CI 1.04, 2.78); P = 0.034]. Conversely, among individuals within the top 50th percentile of PUFA intake, the MetS

| Table 1. Genotype distributions of the ACSL1 polymorphisms in MetS cases and controls |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| SNPs                            | Major Allele Homozygotes | Heterozygotes | Minor Allele Homozygotes |
|                                 | Case (n) % | Control (n) % | Case (n) % | Control (n) % | Case (n) % | Control (n) % |
| rs4862417                       | (506) 58   | (560) 57     | (318) 36   | (321) 36      | (53) 6     | (56) 6.5     |
| rs6552828                       | (307) 35.5 | (342) 39     | (424) 48   | (388) 44.5    | (146) 16.5 | (147) 16.5   |
| rs13120078                      | (346) 39.5 | (370) 42     | (435) 49   | (380) 43.5    | (96) 11.5  | (127) 14.5   |
| rs9997745                       | (660) 75.2 *| (606) 69.1   | (201) 23   | (250) 28.5    | (16) 1.8   | (21) 2.4     |
| rs12503645                      | (316) 36   | (302) 34.5   | (412) 47   | (435) 49.5    | (149) 17   | (140) 16     |

* Indicates P < 0.05 compared with control subjects within same genotype group.
risk conferred by GG homozygosity persisted \[\text{OR} = 1.79 \ (\text{CI} 1.10, 2.92); \ P = 0.019\]. Saturated and monounsaturated fat intake did not modulate MetS risk (data not shown).

**Dietary PUFA and rs9997745 genotype modulate markers of insulin resistance**

Given the associations between rs9997745 and insulin resistance and modulation of MetS risk by dietary PUFA, the clinical characteristics of the subjects were reexamined according to both rs9997745 genotype and PUFA status and composition. A habitual low dietary PUFA background magnified the genetic influence of *ACSL1* rs9997745 on insulin resistance \( (P = 0.02) \) (Fig. 1A), fasting insulin \( (P = 0.037) \) (Fig. 1B), and glucose concentrations \( (P = 0.01) \) (Fig. 1C), all of which were further exacerbated in the GG homozygotes \( (n = 848) \) compared with the A allele carriers \( (n = 327) \). These findings held true in the MetS cases and controls when both groups were analyzed separately. Furthermore, fasting glucose levels were significantly higher in the GG homozygotes consuming a low-PUFA diet compared with both GG homozygotes \( (n = 418, \ P = 0.01) \) and A allele carriers \( (n = 161, \ P = 0.03) \) with a high-PUFA intake. Interestingly, no differences were observed between genotypes with respect to insulin resistance and insulin concentrations against a high dietary PUFA intake \( (>5.5\% \text{ energy}) \).

**ACSL1 haplotypes are not associated with MetS**

Haplotype analysis investigated the combined effect of *ACSL1* rs9997745 with the other four *ACSL1* SNPs \( (\text{rs}4862417, \text{rs}6552828, \text{rs}13120078, \text{and rs}12503643) \) on MetS risk. Six haplotypes were identified after haplotypes with frequencies <5% were excluded from the analysis. However, no haplotype effect on MetS risk could be detected when each haplotype was compared with the most common reference haplotype (data not shown).

**Table 2.** Clinical characteristics and dietary fat intakes of all subjects according to *ACSL1* rs9997745 genotype

|                | GG                      | AG+AA                   |
|----------------|-------------------------|-------------------------|
| n              | 1266                    | 488                     |
| Male/female (%)| 60/40                   | 60/40                   |
| Age (y)        | 58 ± 0.15               | 58 ± 0.25               |
| Glucose (mmol/L)| 5.30 ± 0.03*           | 5.12 ± 0.03             |
| Insulin (mmol/L)| 7.49 ± 0.17*           | 7.12 ± 0.25             |
| HOMA           | 1.89 ± 0.06**           | 1.68 ± 0.07             |
| QUICKI         | 0.33 ± 0.00             | 0.34 ± 0.00             |
| BMI (kg/m²)    | 26.1 ± 0.12             | 25.8 ± 0.22             |
| Waist (cm)     | 88 ± 0.36               | 87 ± 0.60               |
| SBP (mm Hg)    | 131 ± 0.45              | 130 ± 0.72              |
| DBP (mm Hg)    | 82 ± 0.27               | 82 ± 0.41               |
| Total cholesterol (mmol/L)| 5.71 ± 0.02 | 5.71 ± 0.04           |
| HDL (mmol/L)   | 1.46 ± 0.01             | 1.50 ± 0.01             |
| LDL (mmol/L)   | 3.51 ± 0.03             | 3.56 ± 0.05             |
| TAG (mmol/L)   | 1.28 ± 0.02             | 1.26 ± 0.03             |
| NEFA (mmol/L)  | 0.89 ± 0.02             | 0.95 ± 0.04             |
| Lipid lowering medication (%) | 20.00       | 19.80                   |
| Anti-diabetic medication (%) | 3.30        | 2.70                   |
| Hypertensive medication (%) | 21.00       | 22.00                   |
| Alcohol intake (% energy) | 6.27 ± 0.22 | 6.45 ± 0.34          |
| Total PUFA intake (% energy) | 5.65 ± 0.06 | 5.67 ± 0.10         |
| n-6 PUFA intake (% energy) | 5.08 ± 0.05 | 5.09 ± 0.10         |
| n-3 PUFA intake (% energy) | 0.56 ± 0.01 | 0.57 ± 0.01         |
| Long-chain n-3 PUFA intake (% energy) | 0.17 ± 0.01 | 0.18 ± 0.01         |
| Saturated fatty acid intake (% energy) | 15.36 ± 0.11 | 15.44 ± 0.17   |
| MUFA intake (% energy) | 14.25 ± 0.10 | 14.19 ± 0.14 |

Values are means ± SEM. *Indicates \( P < 0.01 \) and ** indicates \( P < 0.05 \) for linear regression adjusted for potential confounding factors.

---

**Fig. 1.** Influence of *ACSL1* rs9997745 genotype and dietary PUFA status on insulin resistance as assessed by HOMA (A), fasting insulin (B), and glucose (C) concentrations. Values are means ± SEM. \( P \)-values for linear regression adjusted for potential confounding factors. When dietary PUFA intake was low \( (<5.5\% \text{ energy}) \), GG homozygotes with high n-6 PUFA intake \( (>5\% \text{ energy}) \) were no longer predisposed to increased MetS risk. When n-6 PUFA intake was in the bottom 50th percentile, the MetS risk conferred by GG homozygosity persisted \[ \text{OR} = 1.79 \ (\text{CI} 1.10, 2.92); \ P = 0.019 \]. Saturated and monounsaturated fat intake did not modulate MetS risk (data not shown).
Replication of gene-nutrient interaction in an independent cohort

We attempted to replicate our findings in a separate independent LIPGENE MetS case-only cohort (n = 464) (41). In this study, rs9997745 GG homozygotes displayed higher fasting glucose concentrations (P = 0.007) and greater insulin resistance (P = 0.04) compared with the A allele carriers (P = 0.007) (Table 3). Insulin levels were not significantly different between genotypes (10.79 ± 0.30 vs. 9.86 ± 0.54; P = 0.12). Consistent with our data from the case-control study, when stratified according to dietary fat intake, the deleterious influences of GG homozygosity on fasting glucose levels and insulin resistance were abolished among individuals consuming either a low-fat diet or a high-PUFA diet. In accordance with our original findings, a high-fat or low-PUFA diet accentuated the genotypic differences (Table 3). We observed that fasting glucose concentrations (P = 0.04 and P = 0.03) and insulin resistance score (P = 0.004 and P = 0.003) were higher for GG versus A allele carriers consuming high-fat and low-PUFA diets, respectively.

DISCUSSION

In this study, we demonstrated an association between a common genetic variant at the ACSL1 locus with increased MetS risk. This may be, in part, explained by higher fasting glucose and insulin concentrations and concomitant increased insulin resistance associated with ACSL1 rs9997745 GG homozygotes compared with the minor A allele carriers. Dietary fat composition and status modulated these genetic influences.

An individual’s phenotype represents a complex interaction between the genetic background and environmental factors over the course of a lifetime. In this study, MetS risk was subject to a significant modification by dietary fat intake, with the deleterious effect conveyed by rs9997745 GG homozygosity apparently abolished in individuals habitually consuming a low-fat diet (<35% energy). Dietary fat has previously been shown to influence ACSL1 mRNA levels in mice; indeed, adipose tissue stromal vascular cells from high-fat fed mice displayed increased ACSL1 expression compared with mice fed low-fat diets (48). Interestingly, when we examined the individual dietary fat components separately, we identified a gene-nutrient interaction with PUFA whereby GG homozygotes with low-PUFA intake (<5.5% energy) had increased MetS risk, which was effectively eliminated when dietary PUFA intake was in the top 50th percentile. One interpretation could be that individuals who are genetically predisposed to the MetS (72% of this population) are most sensitive to dietary fat, such that either low-fat intake or high-PUFA consumption abolishes their genetic risk conferred by ACSL1 rs9997745 to developing the MetS.

Examination of the HAPMAP data (49) indicates allele frequency differences between ethnic groups. Whereas the allele frequency in the current study is not far from that in the European HAPMAP population, the opposite is true in Africans where the G allele is the minor allele. What is quite interesting is that this SNP is not polymorphic in Asians. This implies that in an Asian population, everybody will carry the risk allele in the presence of a PUFA-poor diet poor (which has been the traditional diet in Asian countries) and may be particularly at risk of MetS. Because the mechanisms underlying interactions between PUFA and ACSL1 genotype are currently unknown, these results require further investigation. However, such gene-nutrient interactions suggest that dietary fatty acids may have the potential to modify the genetic susceptibility of developing the MetS. To our knowledge, this is the first case-control study to report an association between ACSL1 polymorphisms with MetS risk. We noted a gender difference for this association, and although the effect was in the same direction in the female subjects, it did not reach statistical significance, which may reflect lack of statistical power. Furthermore, we replicated the association between ACSL1 rs9997745 and insulin resistance and its modulation by dietary fat intake in a separate, independent, MetS-only study. Notwithstanding the study design differences, these data help strengthen our findings; however, functional studies are needed to ascertain their biological significance.

Interestingly, saturated and monounsaturated fat intake did not modulate MetS risk, suggesting a PUFA-specific effect. PPARs are members of a superfamily of nuclear hormone receptors that act as ligand-activated transcription factors. PPAR-γ is abundantly expressed in adipose tissue and is a key regulator of insulin and glucose metabolism (50), whereas PPAR-α is highly expressed in liver and muscle where it plays an important role in lipid metabolism (51). As such, it is possible that a genetic predisposition to the MetS is observed in individuals with a susceptible genotype and diet.

| Glucose (mmol/L) | Insulin Resistance (HOMA) |
|------------------|--------------------------|
|                  | GG | AG+AA | Pvalue | GG | AG+AA | Pvalue |
| Below dietary fat median | 6.13 ± 0.04 | 5.89 ± 0.08 | 0.007 | 2.96 ± 0.16 | 2.61 ± 0.09 | 0.04 |
| Above dietary fat median | 6.09 ± 0.10 | 6.09 ± 0.11 | 0.20 | 2.41 ± 0.14 | 2.86 ± 0.25 | 0.88 |
| Below dietary PUFA median | 6.14 ± 0.15 | 5.84 ± 0.04 | 0.03 | 3.52 ± 0.18 | 2.56 ± 0.11 | 0.003 |
| Above dietary PUFA median | 6.06 ± 0.09 | 6.14 ± 0.14 | 0.16 | 2.74 ± 0.18 | 2.81 ± 0.26 | 0.72 |

Values are means ± SEM. Pvalues obtained from linear regression analysis comparing GG homozygotes to the A allele carriers.
metabolism (51, 52). ACSL1 is a target for both PPAR-γ and PPAR-α (13, 14). Martin et al. (13) demonstrated that ACSL1 expression was altered by treatment with thiazolidinediones and fibrates, agents that affect fatty acid metabolism via the activation of PPAR-γ and PPAR-α. The authors also examined ACSL1 expression at the transcriptional level and reproduced their findings in hepatocytes and preadipocyte cell lines. More recently, Clemenz et al. (53) reported that treatment with telmisartan, a potent PPAR-γ modulator, induced ACSL1 stimulation in murine hepatocytes and increased hepatic ACSL1 expression in diet-induced obese mice displaying decreased TAG concentrations. Besides pharmacological agents, PUFA are natural ligands for both PPAR-γ and PPAR-α (54), and animal studies have shown that dietary supplementation with PUFA increases mRNA expression of ACSL1 (13, 34, 35). This raises the possibility that alteration of ACSL1 expression via modulation of PPAR-γ and/or PPAR-α is a potential mechanism by which gene-nutrient interaction of ACSL1 genotype and dietary PUFAs could influence ACSL1 levels and thus fatty acid metabolism, insulin resistance, and MetS risk. While this is speculative, it may be worthy of further investigation to help elucidate the molecular basis of such gene-nutrient interactions and their impact on cellular fatty acid metabolism and insulin resistance.

PUFA, especially the n-3 PUFA, are associated with insulin sensitivity (55, 56) and lower TAG concentrations (57). We did not observe any interaction between any dietary PUFA components and genotype on TAG. However, we demonstrated that the detrimental effect conferred by ACSL1 rs9997745 GG homozygosity on insulin resistance was abolished against a high PUFA background. Some animal studies have shown that increased hepatic ACSL1 content does not result in insulin resistance and that mice lacking hepatic ACSL1 are not protected against insulin resistance when liver TAG is elevated (58, 59). Thus, the gene-nutrient interactions observed in the current study may be more relevant to adipose rather than liver ACSL1. Recent functional analyses revealed that ACSL1 knockout adipocytes display alterations consistent with insulin resistant glucose uptake (60). Compared with control adipocytes, the ACSL1-silenced adipocytes displayed increased phosphorylation of protein kinase C θ and c-Jun NH2-terminal kinase, reduced insulin signaling, and decreased insulin-stimulated glucose uptake (60). Retinoic acid receptor agonists act in a similar fashion as PPAR-γ agonists (61), and recent work has highlighted the potential of retinoic acid receptor ligands to modulate ACSL1 expression, leading to enhanced insulin sensitivity (62). Thus, it is reasonable to hypothesize that the apparent “normalization” of insulin resistance by the GG homozygotes observed in the context of a high dietary PUFA background may be, in part, achieved through such a mechanism, which may be more active in adipose tissue rather than in the liver. There is no functional data on ACSL1 rs9997745; thus, we can only speculate about mechanisms underlying our findings. The intrinsic location of this SNP has the potential to affect mRNA stability or modulate ACSL1 gene transcriptional activity. It is also possible that this SNP may be a surrogate marker for other functional ACSL1 SNPs in the region.

In conclusion, this study provides new data on ACSL1 genotype and MetS risk. Further investigation of these novel associations and gene-nutrient interactions may help to improve therapeutic efficacy of dietary recommendations with a personalized nutrition approach, wherein the genetic profile may determine choice of dietary therapy to aid responsiveness to dietary fatty acid interventions.

V. Pirisi, B. Gleize, and AM. Lorec are acknowledged for handling of plasma biochemical analyses.

REFERENCES

1. Moller, D. E., and K. D. Kaufman. 2005. Metabolic syndrome: a clinical and molecular perspective. Annu. Rev. Med. 56: 45–62.
2. Roche, H. M., C. Phillips, and M. J. Gibney. 2005. The metabolic syndrome: the crossroads of diet and genetics. Proc. Nutr. Soc. 64: 371–377.
3. Unger, R. H. 2002. Lipotoxic diseases. Annu. Rev. Med. 53: 319–336.
4. Ghanim, H., A. Aljada, D. Hofmeyer, T. Syed, P. Mohanty, and P. Dandona. 2004. Circulating mononuclear cells in the obese are in a proinflammatory state. Circulation. 110: 1564–1571.
5. Guilermer, A., J. V. Virbasius, V. Pur, and M. P. Czeck. 2008. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Natl. Rev. Mol. Cell. Biol. 9: 367–377.
6. Sahiel, A. R. 2000. Series introduction: the molecular and physiological basis of insulin resistance: emerging implications for metabolic and cardiovascular diseases. J. Clin. Invest. 106: 163–164.
7. Schinner, S. A. Schebaumm, S. R. Bornstein, and A. Barthel. 2005. Molecular mechanisms of insulin resistance. Diabet. Met. 22: 674–682.
8. Hall, A. M., A. J. Smith, and D. A. Bernlohr. 2003. Characterization of the Acyl-CoA synthetase activity of purified murine fatty acid transport protein 1. J. Biol. Chem. 278: 43908–43913.
9. Mcgarry, J. D. 2002. Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. Diabetes. 51: 7–18.
10. Shimabukuro, M., Y. T. Zhou, M. Levi, and R. H. Unger. 1998. Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. Proc. Natl. Acad. Sci. USA 95: 2498–2502.
11. Coleman, R. A., T. M. Lewin, and D. M. Muoio. 2000. Physiological and nutritional regulation of enzymes of triacylglycerol synthesis. Annu. Rev. Nutr. 20: 77–103.
12. Mashek, D. G., L. O. Li, and R. A. Coleman. 2006. Rat long-chain acyl-CoA synthetase mRNA, protein, and activity vary in tissue distribution and in response to diet. J. Lipid Res. 47: 2004–2010.
13. Martin, G., K. Schoonjans, A. M. Lefebvre, B. Staels, and J. Auwerx. 1997. Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARalpha and PPARgamma activators. J. Biol. Chem. 272: 28210–28217.
14. Schoonjans, K., B. Staels, and J. Auwerx. 1996. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. J. Lipid Res. 37: 907–925.
15. Kabagambe, E. K., M. Y. Tsai, P. N. Hopkins, J. M. Ordovas, J. M. Peacock, I. B. Borecki, and D. K. Arnett. 2008. Erythrocyte fatty acid composition and the metabolic syndrome. Genes Nutr. 3: 173–176.
16. Phillips, C., J. Lopez-Miranda, F. Perez-Jimenez, R. McManus, and H. M. Roche. 2006. Genetic and nutrient determinants of the metabolic syndrome. Curr. Opin. Cardiol. 21: 185–193.
17. Szabo de Edelenyi, F., L. Goumidi, S. Bertrais, C. Phillips, R. Macmanus, H. Roche, R. Planells, and D. Lairon. 2008. Prediction of the metabolic syndrome status based on dietary and genetic parameters, using Random Forest. Genes Nutr. 3: 173–176.
18. Yesby, B. 2003. Dietary fat, fatty acid composition in plasma and the metabolic syndrome. Curr. Opin. Lipidol. 14: 15–19.
with polyunsaturated fatty acids to modulate risk of metabolic syndrome. Am. J. Clin. Nutr. 90: 1665–1673.
38. Le Moullac N. D. M., P. Preziosi, P. Monteiro, P. Valeix, M-F. Rolland-Cachera, G. Potier De Courey, J. F. Christides, F. Cherouvrier, P. Galan, S. Hercberg. 1996. Validation du manuel-photos utilisé pour l’enquête alimentaire de l’étude SU.VI.MAX. Cahiers de Nutrition et de Diététique. 31: 158–164.
39. Feinberg, M., J. C. Favier, and J. Ireland-Ripert. 1991. Répertoire général des aliments. Fondation Française pour la Nutrition; Centre Informatique sur la Qualité des Aliments. Institut National de la Recherche Agronomique: Technique et Documentation - Lavoisier, France.
40. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). 2001. JAMA 285: 2486–2497.
41. Shaw, D. I., A. C. Tieneyer, S. McCarthy, J. Uprichard, S. Vermunt, H. L. Gulseth, C. A. Drevon, E. B. Blaak, W. H. Sarris, B. Karlstrom, et al. 2008. LIPGENE food-exchange model for alteration of dietary fat quantity and quality in free-living participants from eight European countries. Br. J. Nutr. XXX: 1–10.
42. Matthews, D. R., J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Turner, and R. C. Hattersley. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 28: 112–119.
43. Perseghin, G., A. Caumo, M. Caloni, G. Testolin, and L. Luzi. 2001. Incorporation of the fasting plasma FFA concentration into QUICKI improves its association with insulin sensitivity in non-obese individuals. J. Clin. Endocrinol. Metab. 86: 4770–4771.
44. Phillips, C. M., L. O. Li, J. M. Ellis, H. A. Paich, S. Wang, N. Gong, G. N. Feinberg, M., D. J. Fesker, R. T. Chan, J. B. German, and M. A. Roberts. 2002. Dietary saturated fat modulates the association between STAT3 polymorphisms and abdominal obesity in men. J. Nutr. 132: 2011–2017.
45. de Bakker, P. I., R. Velyvis, J. P’e’er, S. B. Gabriel, M. J. Daly, and D. Altshuler. 2005. Efficiency and power in genetic association studies. Nat. Genet. 37: 1217–1223.
46. Liang, K. Y., and S. L. Zeger. 1993. Regression analysis for correlated data. Annu. Rev. Public Health. 14: 43–68.
47. Tregouet, D. A., and V. Garelle. 2007. A new JAVA interface implementation of THESIAS: testing haplotype effects in association studies. Bioinformatics. 23: 1038–1039.
48. Sarawathi, V., and A. H. Hasty. 2009. Inhibition of long-chain acyl coenzyme A synthetases during fatty acid loading induces lipotoxicity in macrophages. Arterioscler. Thromb. Vasc. Biol. 29: 1937–1943.
49. International HapMap Consortium. 2003. The International HapMap Project: Nature. 426: 780–796.
50. Picard, F., and J. Amieux. 2002. PPARGamma and glucose homeostasis. Annu. Rev. Nutr. 22: 167–197.
51. Staels, B., J. Dalongeville, J. Auwerx, K. Schoonjans, E. Leitersdorf, and J. C. Fruchart. 1998. Mechanism of action of fibrates on lipid and lipoprotein metabolism. Circulation. 98: 2088–2093.
52. Staels, B., and J. C. Fruchart. 2005. Therapeutic roles of peroxisome proliferator-activated receptor agonists. Diabetes. 54: 2460–2470.
53. Clemens, M., N. Frost, M. Schuppan, S. Caron, A. Foryst-Ludwig, C. Bohm, M. Hartge, R. Gust, B. Staels, T. Unger, et al. 2008. Liver-specific peroxisome proliferator-activated receptor alpha target gene regulation by the angiotensin type 1 receptor blocker telmisartan. Diabetes. 57: 1405–1413.
54. Forman, B. M., J. Chisholm, and R. M. Evans. 1997. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. Proc. Natl. Acad. Sci. USA. 94: 4312–4317.
55. Feskens, E. J., J. G. Loeb er, and D. Kromhout. 1994. Diet and physical activity as determinants of hyperinsulinemia: the Zutphen Elderly Study. Am. J. Epidemiol. 140: 750–768.
56. Feskens, E. J., C. H. Bowles, and D. Kromhout. 1991. Inverse association between fish intake and risk of glucose intolerance in normoglycemic elderly men and women. Diabetes. 14: 935–941.
57. Harris, W. S. 1997. n-3 fatty acids and serum lipoproteins: human studies. Am. J. Clin. Nutr. 65: 1645S–1658S.
58. Li, L., J. M. Ellis, H. A. Paich, S. Wang, N. Gong, G. N. Altschuller, R. T. Chan, J. B. German, S. M. Watkins, D. M. Muiao, et al. 2009. Liver-specific loss of long-chain acyl-CoA
synthetase-1 decreases triacylglycerol synthesis and beta-oxidation, and alters phospholipid fatty acid composition. *J. Biol. Chem.* **284**: 27816–27826.

59. Neschen, S., K. Morino, L. E. Hammond, D. Zhang, Z. X. Liu, A. J. Romanelli, G. W. Cline, R. L. Pongratz, X. M. Zhang, C. S. Choi, et al. 2005. Prevention of hepatic steatosis and hepatic insulin resistance in mitochondrial acyl-CoA:glycerol-sn-3-phosphate acyltransferase 1 knockout mice. *Cell Metab.* **2**: 55–65.

60. Lobo, S., B. M. Wiczer, and D. A. Bernlohr. 2009. Functional analysis of long-chain acyl-CoA synthetase 1 in 3T3-L1 adipocytes. *J. Biol. Chem.* **284**: 18347–18356.

61. Mukherjee, R., P. J. Davies, D. L. Crombie, E. D. Bischoff, R. M. Cesario, L. Jow, L. G. Hamann, M. F. Boehm, C. E. Mondon, A. M. Nadzan, et al. 1997. Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists. *Nature* **386**: 407–410.

62. Martin, G., H. Poirier, N. Hennuyer, D. Crombie, J. C. Fruchart, R. A. Heyman, P. Besnard, and J. Auwerx. 2000. Induction of the fatty acid transport protein 1 and acyl-CoA synthase genes by dimer-selective rexinoids suggests that the peroxisome proliferator-activated receptor-retinoid X receptor heterodimer is their molecular target. *J. Biol. Chem.* **275**: 12612–12618.