RESEARCH ARTICLE

# Insulin receptor substrate-2 gene variants in subjects with metabolic syndrome: Association with plasma monounsaturated and *n*-3 polyunsaturated fatty acid levels and insulin resistance

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**Scope**: Several insulin receptor substrate-2 (*IRS-2*) polymorphisms have been studied in relation to insulin resistance and type 2 diabetes. To examine whether the genetic variability at the *IRS-2* gene locus was associated with the degree of insulin resistance and plasma fatty acid levels in metabolic syndrome (MetS) subjects.

**Methods and results:** Insulin sensitivity, insulin secretion, glucose effectiveness, plasma fatty acid composition and three *IRS-2* tag-single nucleotide polymorphisms (SNPs) were determined in 452 MetS subjects. Among subjects with the lowest level of monounsaturated (MUFA) (below the median), the rs2289046 A/A genotype was associated with lower glucose effectiveness ($p<0.038$), higher fasting insulin concentrations ($p<0.028$) and higher HOMA IR ($p<0.038$) as compared to subjects carrying the minor G-allele (A/G and G/G). In contrast, among subjects with the highest level of MUFA (above the median), the A/A genotype was associated with lower fasting insulin concentrations and HOMA-IR, whereas individuals carrying the G allele and with the highest level of ω-3 polyunsaturated fatty acids (above the median) showed lower fasting insulin ($p<0.01$) and HOMA-IR ($p<0.02$) as compared with A/A subjects.

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**Abbreviations: CVD,** cardiovascular disease; **DI,** disposition index; **HOMA-B,** homeostasis model assessment of β-cell; **HOMA-IR,** homeostasis model assessment of insulin resistance; **IRS-2,** insulin receptor substrate-2; **IVGTT,** insulin-modified intravenous glucose tolerance test; **MetS,** metabolic syndrome; **MUFA,** monounsaturated fatty acids; **Sg,** glucose effectiveness; **Si,** insulin sensitivity index; **SNPs,** single-nucleotide polymorphisms

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**Conclusion:** The rs2289046 polymorphism at the *IRS2* gene locus may influence insulin sensitivity by interacting with certain plasma fatty acids in MetS subjects.

**Keywords:**
Fatty acids / Metabolic syndrome / Nutrigenomics / *n*-3 PUFA / Polymorphism

## 1 Introduction

The metabolic syndrome (MetS) is a constellation of metabolic risk factors reflecting overnutrition and sedentary lifestyle. Its prevalence is increasing to epidemic proportions not only in the Western world but also in developing countries. Most studies show that this syndrome is associated with increased risk of cardiovascular disease (CVD) and of type 2 diabetes [1, 2]. The pathogenesis of the MetS is complex and not completely understood but the interaction of dietary and lifestyle, obesity, and genetic factors are known to contribute [3]. The number of studies investigating gene–nutrient interactions related to this syndrome continues to grow, and holds a potential for reducing disease risk at the level of the individual genotype [4–7]. Evidence suggests that some people are genetically predisposed to insulin resistance [8], a possible underlying mechanism for these metabolic disturbances. Thus, it would be expected that general dietary recommendations may not be beneficial for all individuals with MetS.

The insulin receptor substrate (IRS) is a major insulin-signalling molecule [9]. Recently, we demonstrated that the G972R polymorphism at the *IRS-1* gene determines insulin sensitivity as a function of the type of diet [10]. Thus, in healthy G972R subjects, the consumption of a high-carbohydrate, low-fat diet induced greater peripheral sensitivity than did high-fat diets. Together with *IRS-1*, the *IRS-2* gene has also been considered to be a candidate for metabolic diseases such as type 2 diabetes and obesity [11]. However, to date, there are no studies exploring the interaction of polymorphisms in the *IRS-2* gene with fatty acid composition as a determinant of insulin resistance. In view of the physiological role of IRS-2 in glucose homeostasis and the link between insulin resistance and dietary fat, we hypothesized that genetic variation of *IRS-2* might be associated with variability in insulin response to different fatty acids among MetS patients.

## 2 Subjects and methods

The design of the present study is described in recent articles from the LIPGENE cohort [5–7, 12].

### 2.1 Subjects

About 452 participants (200 men, 252 women), aged 35–70 years and BMI 20–40 $kg/m^2$, were recruited for the LIPGENE dietary intervention study from eight European countries (Ireland, UK, Norway, France, The Netherlands, Spain, Poland and Sweden). Informed consent was obtained from all participants in accordance with the Helsinki Declaration of 1975, as revised in 1983. The study was registered with The US National Library of Medicine Clinical Trials registry (NCT00429195). Subject eligibility was determined using a modified version of the NCEP criteria for the MetS [13], where subjects were required to fulfil at least three of the following five criteria: waist circumference $>102$ cm (men) or $>88$ cm (women); fasting plasma concentration of glucose 5.5–7.0 mmol/L; TAG $\geq 1.5$ mmol/L; HDL cholesterol $<1.0$ mmol/L (men) or $<1.3$ mmol/L (women) and blood pressure $\geq 130/85$ mm Hg or pharmacological treatment of previously diagnosed hypertension. The design of and baseline data from the LIPGENE dietary intervention cohort have been published elsewhere [14, 15]. Briefly, subjects completed 3-d weighed food diaries and FFQ to assess habitual dietary intake. Based on these, nutritionists advised subjects individually on recommended food choices for their allocated diet. Fortnightly, 24 h recalls were taken and the frequency of study food consumption was monitored. Anthropometric measurements were recorded according to a standardized protocol for the LIPGENE study and blood pressure was measured according to the guidelines of European Society of Hypertension [16].

### 2.2 Biochemical measurements

Plasma, serum, and buffy coat were prepared from 12 h fasting blood samples in each subject. Serum insulin was measured by solid-phase, two-site fluoroimmunometric assay on a 1235 automatic immunoassay system (AutoDELFIA kits, Wallac Oy, Turku, Finland). Plasma glucose concentrations were measured using the IL TestTM Glucose Hexokinase Clinical Chemistry kit (Instrumentation Laboratories, Warrington, UK). Homeostasis model assessment of insulin resistance (HOMA-IR) was derived from fasting glucose and insulin levels [(fasting plasma glucose $\times$ fasting serum insulin)/22.5] [17]. As HOMA-IR takes into account both insulin and glucose levels, it may be a more complete index than fasting serum insulin. Homeostasis model assessment of β-cell function (HOMA-B) was calculated as [(20 $\times$ fasting serum insulin)/(fasting plasma glucose $-$ 3.5)]. An insulin-modified intravenous glucose tolerance test (IVGTT)

 

was performed [18]. Insulin sensitivity (sensitivity index, SI) and glucose effectiveness were determined using the MINMOD Millenium Program (version 6.02, Richard N Bergman) [19]. The acute insulin response to glucose (AIRg = first-phase insulin response) was defined as the incremental area under the curve from time 0–8 min. Disposition index (DI) was calculated as the product of AIRg and insulin sensitivity.

Cholesterol and triglycerides were quantified using the IL TestTM Cholesterol kit and IL TestTM Triglycerides kit (Instrumentation Laboratories).

Plasma fatty acid composition was determined as a biomarker of habitual dietary fat intake and reflects the combination of dietary fat consumption and endogenous de novo fatty acid biosynthesis and metabolism. Fatty acids were extracted from plasma and transmethylated with boron trifluoride in methanol. Fatty acid methyl esters were analyzed by gas chromatography on a Shimadzu GC-14A (Shimadzu, Kyoto, Japan) fitted with a Shimadzu C-r6A integrator and a 25M BP 21 polar aluminium silica column. Detector and injector temperatures were 260 and 250°C respectively. Fatty acids were identified by the comparison of the relative retention times of plasma fatty acid methyl esters with fatty acid methyl ester standards. Fatty acid mass was measured as a relative percentage of the total quantified fatty acids [20]. Total plasma *n*-3 PUFA was calculated from the sum of C18:3 (*n*-3), C18:4(*n*-3), C20:4 (*n*-3), C20:5 (*n*-3), C22:5 (*n*-3) and C22:6 (*n*-3). Long chain (LC) n-3 PUFA was calculated from C20:5 (*n*-3) and C22:6(*n*-3); and *n*-6 PUFAs from the sum of 18:2, 18:3*n*-6, 20:3, 20:4*n*-6, and 22:4.

### 2.3 SNP selection and genotyping

*IRS-2* genotype data from HapMap v1.1 (www.hapmap.org) was uploaded into HITAGENE, a web-based combined database and genetic analysis software suite. Haplotype frequencies were estimated by implementation of the expectation maximization algorithm. Using a 5% cut-off for individual haplotype frequency and >70% for the sum of all haplotype frequencies, haplotype tagged (HT) SNPs were identified using SNP tagger (www.broad.mit.edu/mpg/tagger/server.html). Three SNPs (rs2289046, rs1865434 and rs4771646) were genotyped by Illumina (San Diego, CA, USA). DNA was extracted from buffy coat samples using the AutoPure LS automated system (Gentra Systems, Minneapolis, MN, USA), and low yielding samples (<10 ng) were subjected to whole genome amplification using the REPLI-g kit (Qiagen, West Sussex, UK). Adherence to Hardy–Weinberg equilibrium (HWE) at each SNP locus was determined using the $\chi^2$ test with 1 degree of freedom. All SNPs were used in the analyses, although results are presented only for the rs2289046 showing the most interesting and significant findings.

### 2.4 Statistical analysis

Biochemical variables were assessed for normality of distribution, and skewed variables were normalised by $\log_{10}$ or square root transformation as appropriate. Statistical analysis was carried out using SPSS version 18.0 for Windows (SPSS, Chicago, IL, USA). Data were presented as mean ± SEM for continuous variables and as frequencies or percentages for categorical variables. Differences in mean values were assessed by analysis of variance and unpaired *t*-tests. Furthermore, comparisons of frequencies between qualitative variables were carried out using the $\chi^2$ test. Gene–nutrient interactions were tested by using an SNP–fatty acid interaction term in a univariate general linear model. The effect of each SNP interacting with groups of total plasma fatty acids (saturated, monounsaturated and polyunsaturated fatty acids, divided into total *n*-3 and *n*-6) on each biochemical variable was investigated using the median of all different groups of plasma fatty acids to dichotomize the sample, and using the resulting groups (above or below the median) as a fixed factor in combination with the SNP genotypes in an univariate ANOVA analysis. Bonferroni's test was used where post-hoc analyses were required. A linear regression model was used to create predicted values. All the analyses were adjusted for potential confounders factors (age, sex, BMI, and LIPGENE centre of origin) and $p<0.05$ was considered significant.

## 3 Results

### 3.1 Characteristics of study participants

Baseline demographic and biochemical characteristics according to the rs2289046 SNP at the *IRS-2* gene locus are presented in Table 1. Genotype distributions did not deviate from Hardy–Weinberg expectations. The genotype frequencies were 216 A/A, 187 A/G and 49 G/G. Given the low genotype frequencies of individuals homozygous for the minor alleles, and because the analysis did not suggest a recessive mode of action, we analyzed the rs2289046 SNP using two genotype categories. Thus, for the *IRS-2* rs2289046, carriers of the minor G-allele displayed lower BMI than subjects homozygous for the major allele A (Table 1). No other significant baseline differences were observed in relation to age, fasting lipids, glucose, and insulin concentration by genotype.

### 3.2 Insulin sensitivity

IVGTT and HOMA indices were used to estimate insulin sensitivity (insulin sensitivity index (Si) and HOMA-IR). In addition to the insulin sensitivity parameters we also calculated the glucose effectiveness (Sg), which is the ability of glucose per se to enhance its rate of disappearance and to inhibit hepatic glucose production.

 

**Table 1.** Baseline characteristics of participants according to the rs2289046 SNP

| | rs2289046 | | |
|---|---|---|---|
| | A/A | A/G+G/G | *p*-Value |
| *n* (men/women) | 216 (92/124) | 236 (108/128) | |
| Age( years) | 54.56 (0.6) | 54.37 (0.5) | 0.817 |
| BMI (kg/m$^2$) | 33.13 (0.2) | 31.85 (0.2) | **0.001** |
| Total cholesterol (mmol/L) | 5.32 (0.06) | 5.36 (0.06) | 0.610 |
| LDL-C (mmol/L) | 3.26 (0.07) | 3.27 (0.06) | 0.888 |
| HDL-C (mmol/L) | 1.08 (0.01) | 1.12 (0.01) | 0.155 |
| TG (mmol/L) | 1.81 (0.06) | 1.77 (0.05) | 0.587 |
| Apo B (g/L) | 1.02 (0.01) | 1.01 (0.01) | 0.794 |
| Apo A-1 (g/)L | 1.40 (0.01) | 1.39 (0.01) | 0.764 |
| Fasting glucose (mmol/L) | 5.96 (0.06) | 5.94 (0.05) | 0.791 |
| Insulin (mU/L) | 10.57 (0.3) | 9.69 (0.3) | 0.102 |

BMI, body mass index; TG, triglycerides; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; Apo, apolipoprotein. Bold font indicates: statistically significant.

Gene–nutrient interactions between the rs2289046 *IRS-2* and plasma level of plasma MUFA and n-3 PUFA were observed. In the whole cohort, among subjects with the lowest level of MUFA (below the median), the A/A genotype was associated with higher fasting insulin concentrations ($p<0.028$), higher HOMA-IR ($p<0.038$) and lower glucose effectiveness ($p<0.038$), as compared with subjects carrying the minor G-allele (A/G and G/G) (Fig. 1). In contrast, A/A subjects with the highest level of MUFA (above the median) exhibited lower fasting insulin concentrations and HOMA-IR (Fig. 1). Moreover, individuals carrying the G allele and with the highest level of *n*-3 PUFA (above the median) showed lower fasting insulin ($p<0.018$) and HOMA-IR ($p<0.021$), as compared with A/A subjects (Fig. 2).

Si did not differ between participants with different genotypes. There were no significant interactions between other groups of plasma fatty acids and the rs2289046 *IRS-2* on glucose metabolism.

A linear regression model including the original covariates was applied to create predicted values of HOMA-IR according to genotype at the rs2289046 SNP (Fig. 3). The genotype groups exhibited striking differences in the predicted changes in HOMA-IR in relation to plasma MUFA and *n*-3 PUFA concentrations. Thus, from baseline data, the model predicts that in A/A individuals, an increase in plasma MUFA would elicit a considerable decrease in HOMA-IR (Fig. 3A). This decrease would not be seen in subjects carrying the minor G-allele. However, in this group of patients carrying the G allele, an increase in plasma concentration of *n*-3 PUFA would elicit a reduction in HOMA-IR, as compared with A/A subjects (Fig. 3B).

Although we explored the genetic component independently of the nutrient effect, no differences were observed.

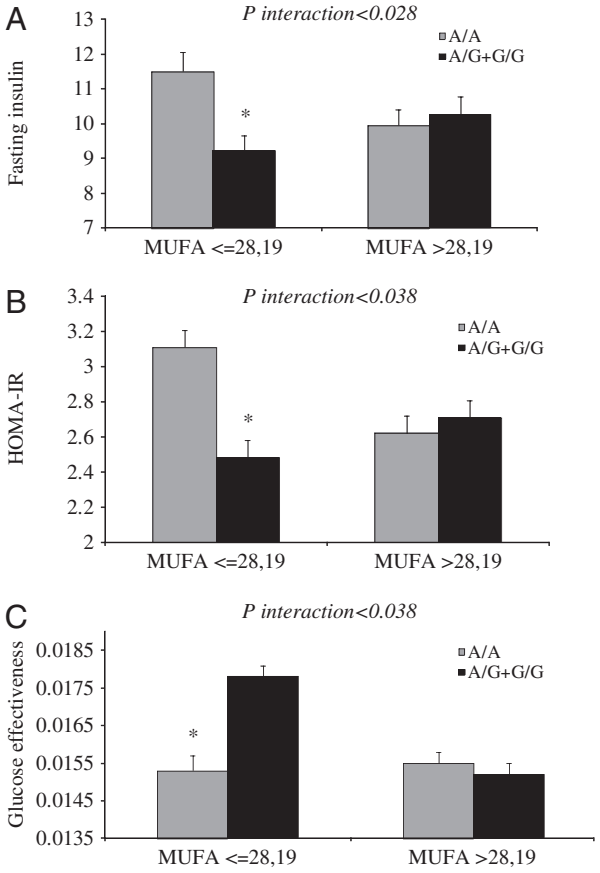


**Figure 1.** Interaction between the rs2289046 SNP at the *IRS-2* gene locus and plasma levels of monounsaturated fatty acids (MUFA), above or below the median within the same genotype group of fasting insulin (A), homeostasis model assessment of insulin resistance (HOMA-IR) (B), and glucose effectiveness) (C). Values represent mean ± SEM. *p*-Values were adjusted for age, sex, BMI and LIPGENE centre of origin. $^*p<0.05$ A/A versus A/G +G/G.

### 3.3 Insulin secretion

We examined the effect of the rs2289046 SNP on insulin secretion according to plasma levels of different fatty acids. For that purpose we measured acute insulin response to glucose (AIRg), disposition index (DI) and HOMA-B. In contrast to the insulin sensitivity findings, we did not observe any gene–nutrient interactions for these parameters. However, subjects carrying the minor G-allele had lower HOMA-B compared with A/A subjects, 75.81 ± 6.3 and 95.52 ± 4.2 ($p<0.011$), respectively.

## 4 Discussion

The shift towards personalized nutrition may be a fruitful approach for the epidemic of cardiometabolic diseases. Our study supports the notion that rs2289046 SNP interacted

 

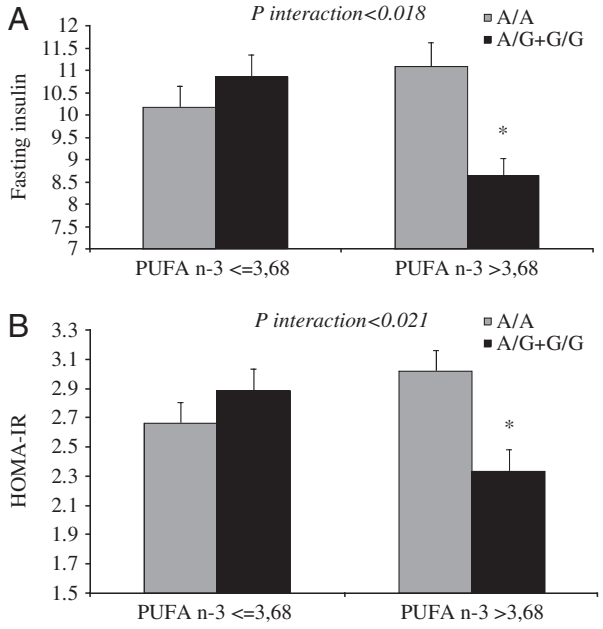


**Figure 2.** Interaction between the rs2289046 SNP at the *IRS-2* gene locus and plasma levels of ω-3 polyunsaturated fatty acids (*n*-3 PUFA), above or below the median within the same genotype group of fasting insulin (A), and homeostasis model assessment of insulin resistance (HOMA-IR) (B). Values represent mean ± SEM. *p*-Values were adjusted for age, sex, BMI and LIPGENE centre of origin. $^{*}p<0.05$ A/A versus A/G+G/G.

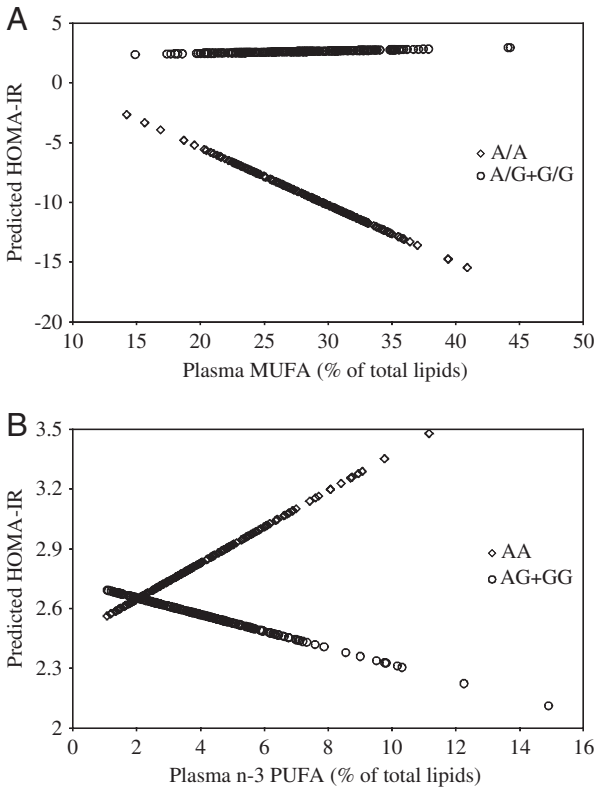


**Figure 3.** Predicted values for homeostasis model assessment of insulin resistance (HOMA-IR) for the rs2289046 SNP at the *IRS-2* gene locus. (A) A difference was observed between the genotype groups, with the A/A genotype group (triangles) appearing to be ''high responders'' to plasma levels of monounsaturated fatty acids (MUFA) and the minor-G allele group (circles) appearing to be ''low responders''. (B) A difference was observed between the genotype groups, with the minor G-allele group (circles) appearing to be ''high responders'' to plasma level of ω-3 polyunsaturated fatty acids (*n*-3 PUFA) and the A/A genotype group (triangles) appearing to be ''low responders''.

with total plasma MUFA and *n*-3 PUFA at certain levels, which were significantly associated with insulin resistance. We observed that among subjects with the lowest level of MUFA, the A/A genotype was associated with lower glucose effectiveness, higher fasting insulin concentrations and higher HOMA-IR as compared with subjects carrying the minor G-allele. In contrast, among subjects with the highest level of MUFA, the A/A genotype was associated with lower fasting insulin concentrations and HOMA-IR. However, individuals carrying the G allele and with the highest level of *n*-3 PUFA showed lower fasting insulin and HOMA-IR as compared with A/A subjects.

Genetic background may interact with habitual dietary fat composition, affecting predisposition to the MetS and individual responsiveness to changes in dietary fat intake. Recently we have demonstrated an association between several variants of candidate genes and insulin resistance in response to dietary fatty acids in healthy [21, 22] and MetS subjects [7, 23]. The *IRS-2* gene encodes the insulin receptor substrate 2, a cytoplasmic signaling molecule that mediates the effects of insulin, insulin-like growth factor 1 and other cytokines by acting as a molecular adaptor. In the last decade evidence indicates that polymorphisms in the *IRS-2* gene may contribute to developing type 2 diabetes and other metabolic traits, such as obesity, polycystic ovary syndrome, and has also been related with breast cancer [24], but with contradictory results [25]. Feigelson et al. conducted a case–control study among post-menopausal women and observed among the cases that the minor-G allele for *IRS2* rs2289046 was associated with less weight gain in adulthood as compared with the A allele [24]. In the same line we observed that carriers of the minor G-allele displayed lower BMI than subjects homozygous for the major allele A. These evidences together with data from a meta-analysis showing the linkage of BMI and the 13q region near *IRS2* suggest that *IRS2* regulates body weight control [26]. However, new studies are needed to confirm these findings. On the other hand, we observed a gene–fatty acid interaction between the same rs2289046 SNP and the proportion of MUFA and *n*-3 PUFA in plasma, suggesting possible sensitivity of this SNP to dietary factors. Furthermore, our results showed that MetS subjects may benefit in a different way according to their genetic background. Thus, among subjects with the highest level of MUFA, the A/A genotype was associated with lower fasting insulin concentrations and HOMA-IR,

 

whereas among subjects with the highest level of *n*-3 PUFA, the G allele was associated with lower fasting insulin and HOMA-IR compared with A/A subjects. Our data suggest a beneficial effect of increasing the amount of MUFA in the diet of A/A subjects and of *n*-3 PUFA in those carrying the minor G-allele. The mechanisms underlying the observed associations for this polymorphism need to be elucidated. IRS-2 is involved in preserving insulin action in multiple cell types, whereas reduction in IRS expression and/or function may cause development of insulin resistance, obesity, β-cell failure and diabetes [9]. Although we did not perform functional studies, one explanation of our findings may be that this gene–fatty acids interaction is related to increased expression, or alternatively increased activity of the *IRS-2* gene. Owing to a lack of information on the effect of this polymorphism on the molecular function of *IRS-2*, explanations for the observed interactions remain speculative. However, IRS-2 was also shown to play an important role in insulin secretion. IRS-2 knockout mice had an impaired glucose-stimulated insulin secretion [27]. Furthermore, IRS-2 signaling was shown to be important for development of β-cells and regulation of β-cell mass. Thus, we measured acute insulin response to glucose, disposition index and HOMA-B. In contrast to the insulin sensitivity findings, we did not observe any gene–nutrient interactions for these parameters. Consequently, our findings suggest that this gene–nutrient interaction modifies only insulin resistance and not insulin secretion. This is interesting because insulin resistance is believed to be the main factor that links metabolic abnormalities in MetS subjects. Moreover, many authors have suggested that insulin resistance is the primary abnormality and that β-cell dysfunction is a late event that arises from the prolonged, increased secretory demand placed on the β-cell by insulin resistance [11].

The LIPGENE cohort is a carefully characterized population, and the multicentre origin of the patients allows extrapolation of the results to the European population. However, the major limitation of our present study is that it is cross-sectional, without the possibility to prove causality. Nevertheless, identified gene–nutrient interactions may lay the ground for future studies.

In conclusion, our results support the notion that fatty acids are key metabolic regulators, which may interact with genes or transcription factors and influence glucose metabolism. Thus, MUFA and *n*-3-PUFA may play a role in triggering insulin resistance by interacting with a genetic variant at *IRS-2* gene locus in subjects with the MetS. Although we cannot perform interventions to change genetic constitution, dietary interventions may reduce insulin resistance, and individuals with the risk allele might attenuate harmful effects by dietary changes.

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## 5 References

[1] Grundy, S. M., Metabolic syndrome: connecting and reconciling cardiovascular and diabetes worlds. *J. Am. Coll. Cardiol.* 2006, *47*, 1093–1100.

[2] Cornier, M. A., Dabelea, D., Hernandez, T. L., Lindstrom, R. C. et al., The metabolic syndrome. *Endocr. Rev.* 2008, *29*, 777–822.

[3] Roche, H. M., Phillips, C., Gibney, M. J., The metabolic syndrome: the crossroads of diet and genetics. *Proc. Nutr. Soc.* 2005, *64*, 371–377.

[4] Delgado-Lista, J., Perez-Martinez, P., Garcia-Rios, A., Phillips, C. M. et al., Pleiotropic effects of TCF7L2 gene variants and its modulation in the metabolic syndrome: from the LIPGENE study. *Atherosclerosis* 2011, *214*, 110–116.

[5] Ferguson, J. F., Phillips, C. M., McMonagle, J., Perez-Martinez, P. et al., NOS3 gene polymorphisms are associated with risk markers of cardiovascular disease, and interact with omega-3 polyunsaturated fatty acids. *Atherosclerosis* 2010, *211*, 539–544.

[6] Ferguson, J. F., Phillips, C. M., Tierney, A. C., Perez-Martinez, P. et al., Gene-nutrient interactions in the metabolic syndrome: single nucleotide polymorphisms in ADIPOQ and ADIPOR1 interact with plasma saturated fatty acids to modulate insulin resistance. *Am. J. Clin. Nutr.* 2010, *91*, 794–801.

[7] Perez-Martinez, P., Delgado-Lista, J., Garcia-Rios, A., Ferguson, J. F. et al., Calpain-10 interacts with plasma saturated fatty acid concentrations to influence insulin resistance in individuals with the metabolic syndrome. *Am. J. Clin. Nutr.* 2011, *93*, 1136–1141.

[8] Lopez-Miranda, J., Perez-Martinez, P., Marin, C., Fuentes, F. et al., Dietary fat, genes and insulin sensitivity. *J. Mol. Med.* 2007, *85*, 213–226.

 

[9] Brady, M. J., IRS2 takes center stage in the development of type 2 diabetes. *J. Clin. Invest.* 2004, *114*, 886–888.

[10] Marin, C., Perez-Martinez, P., Delgado-Lista, J., Gomez, P. et al., The insulin sensitivity response is determined by the interaction between the G972R polymorphism of the insulin receptor substrate 1 gene and dietary fat. *Mol. Nutr. Food Res.* 2011, *55*, 328–335.

[11] Kahn, S. E., The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia* 2003, *46*, 3–19.

[12] Perez-Martinez, P., Moreno-Conde, M., Cruz-Teno, C., Ruano, J. et al., Dietary fat differentially influences regulatory endothelial function during the postprandial state in patients with metabolic syndrome: from the LIPGENE study. *Atherosclerosis* 2010, *209*, 533–538.

[13] 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). *J. Am. Med. Assoc.* 2001, *285*, 2486–2497.

[14] Shaw, D. I., Tierney, A. C., McCarthy, S., Upritchard, J. et al., LIPGENE food-exchange model for alteration of dietary fat quantity and quality in free-living participants from eight European countries. *Br. J. Nutr.* 2009, *101*, 750–759.

[15] Tierney, A. C., McMonagle, J., Shaw, D. I., Gulseth, H. L. et al., Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome-LIPGENE: a European randomized dietary intervention study. *Int. J. Obes. (London)* 2011, *35*, 800–809.

[16] Mancia, G., De Backer, G., Dominiczak, A., Cifkova, R. et al., 2007 ESH-ESC Practice Guidelines for the Management of Arterial Hypertension: ESH-ESC Task Force on the Management of Arterial Hypertension. *J. Hypertens.* 2007, *25*, 1751–1762.

[17] Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A. et al., Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985, *28*, 412–419.

[18] Gulseth, H. L., Gjelstad, I. M., Tierney, A. C., Lovegrove, J. A. et al., Serum vitamin D concentration does not predict insulin action or secretion in European subjects with the metabolic syndrome. *Diabetes Care* 2010, *33*, 923–925.

[19] Boston, R. C., Stefanovski, D., Moate, P. J., Sumner, A. E. et al., MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. *Diabetes Technol. Ther.* 2003, *5*, 1003–1015.

[20] Tully, A. M., Roche, H. M., Doyle, R., Fallon, C. et al., Low serum cholesteryl ester-docosahexaenoic acid levels in Alzheimer's disease: a case-control study. *Br. J. Nutr.* 2003, *89*, 483–489.

[21] Perez-Martinez, P., Lopez-Miranda, J., Cruz-Teno, C., Delgado-Lista, J., Adiponectin gene variants are associated with insulin sensitivity in response to dietary fat consumption in Caucasian men. *J. Nutr.* 2008, *138*, 1609–1614.

[22] Gomez, P., Perez-Jimenez, F., Marin, C., Moreno, J. A., The -514 C/T polymorphism in the hepatic lipase gene promoter is associated with insulin sensitivity in a healthy young population. *J. Mol. Endocrinol.* 2005, *34*, 331–338.

[23] Perez-Martinez, P., Delgado-Lista, J., Garcia-Rios, A., Mc Monagle, J., Glucokinase regulatory protein genetic variant interacts with omega-3 PUFA to influence insulin resistance and inflammation in metabolic syndrome. *PLoS One* 2011, *6*, e20555.

[24] Feigelson, H. S., Teras, L. R., Diver, W. R., Tang, W. et al., Genetic variation in candidate obesity genes ADRB2, ADRB3, GHRL, HSD11B1, IRS1, IRS2, and SHC1 and risk for breast cancer in the Cancer Prevention Study II. *Breast Cancer Res.* 2008, *10*, R57.

[25] Bernal, D., Almind, K., Yenush, L., Ayoub, M. et al., Insulin receptor substrate-2 amino acid polymorphisms are not associated with random type 2 diabetes among Caucasians. *Diabetes* 1998, *47*, 976–979.

[26] Saunders, C. L., Chiodini, B. D., Sham, P., Lewis, C. M. et al., Meta-analysis of genome-wide linkage studies in BMI and obesity. *Obesity (Silver Spring)* 2007, *15*, 2263–2275.

[27] Withers, D. J., Gutierrez, J. S., Towery, H., Burks, D. J. et al., Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 1998, *391*, 900–904.

 