Modulation by Dietary Fat and Carbohydrate of IRS1 Association With Type 2 Diabetes Traits in Two Populations of Different Ancestries

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OBJECTION — Insulin receptor substrate 1 (IRS1) is central to insulin signaling pathways. This study aimed to examine the association of IRS1 variants with insulin resistance (IR) and related phenotypes, as well as potential modification by diet.

RESEARCH DESIGN AND METHODS — Two IRS1 variants (rs7578326 and rs2943641) identified by genome-wide association studies as related to type 2 diabetes were tested for their associations with IR and related traits and interaction with diet in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study (n = 820) and the Boston Puerto Rican Health Study (BPRHS) (n = 844).

RESULTS — Meta-analysis indicated that rs7578326 G-allele carriers and rs2943641 T-allele carriers had a lower risk of IR, type 2 diabetes, and metabolic syndrome (MetS). Significant interactions on IR and MetS were found for these two variants and their haplotypes with diet. In GOLDN, rs7578326 G-allele carriers and rs2943641 T-allele carriers and their haplotype G-T carriers had a significantly lower risk of IR and MetS than noncarriers only when the dietary saturated fatty acid-to-carbohydrate ratio was low (≤0.24). In both GOLDN (P = 0.0008) and BPRHS (P = 0.011), rs7578326 G-allele carriers had a lower risk of MetS than noncarriers only when dietary monounsaturated fatty acids were lower than the median intake of each population.

CONCLUSIONS — IRS1 variants are associated with IR and related traits and are modulated by diet in two populations of different ancestries. These findings suggest that IRS1 variants have important functions in various metabolic disorders and that dietary factors could modify these associations.
the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study and the Boston Puerto Rican Health Study (BPRHS).

**RESEARCH DESIGN AND METHODS**

**Study populations**

The GOLDN participants were predominately of European ancestry and recruited from two genetically homogeneous centers (Minneapolis, MN, and Salt Lake City, UT). In this study, only 820 participants (406 men and 414 women) of European ancestry were included in our analyses. The primary aim of GOLDN was to examine the influence of genetic and dietary factors on the response of individuals to lenofibrate. Baseline data obtained from subjects before they entered the intervention were selected for this analysis. The study details and related methodology of GOLDN have been described (19). Dietary intake was collected using a diet history questionnaire, which was developed by the National Cancer Institute and was validated in two studies (20,21). Calculation of dietary glycemic load (GL) and glycemic index (GI) was according to the method described previously (22). The protocol was approved by the institutional review boards at the University of Alabama, the University of Minnesota, the University of Utah, and Tufts University.

The BPRHS is a longitudinal cohort study of stress, nutrition, health, and aging, for which study participants were self-identified as Puerto Rican and living in the Boston and metropolitan area (23). The ancestry composition of the BPRHS is 57.2% European, 27.4% African, and 15.4% Native American (24). For this study, we included 844 participants (239 men and 605 women) with complete genotype and dietary data. Dietary intake was assessed by a validated food frequency questionnaire (FFQ) that was designed for and validated in this population (25). Dietary GL and GI were calculated per the method previously used in this population (26). The study protocol was approved by the institutional review boards at Tufts University and Northeastern University.

**Biochemistry and anthropometric measurements**

Blood samples were drawn after an overnight fast. In GOLDN, fasting insulin was obtained using a radioimmunoassay by a commercial kit (Linco Research), and fasting glucose was measured using a hexokinase-mediated reaction on the Hitachi commercial kit (Roche Diagnostics). Measurements of blood lipids, including triglycerides and HDL cholesterol (HDL-C), have been described (27). In the BPRHS, fasting insulin was measured using an Immulite 1000 Insulin Kit (LKIN1) on the Immulite 1000 (Seimens Medical Solution Diagnostics), and the Olympus Au400e with Olympus glucose reagents (Olympus America Inc.) were used to measure fasting glucose. Fasting triglycerides and HDL-C were measured with Olympus HDL-C reagents (OSR6195) and Olympus triglyceride reagents (OSR6033).

For both GOLDN and the BPRHS, homeostasis model assessment of insulin resistance (HOMA-IR, calculated as fasting glucose × fasting insulin/22.5) was used to represent insulin resistance. Type 2 diabetes was defined as fasting glucose ≥126 mg/dL or use of diabetes medication. Normal fasting glucose was defined as individuals without diabetes and with fasting glucose <100 mg/dL, and impaired fasting glucose (IFG) was defined as individuals without diabetes but with 100≤ fasting glucose <126 mg/dL. As the prevalence of type 2 diabetes in GOLDN was low, thereby limiting the power to detect the main association and gene-diet interaction, IFG/T2D was defined as the combined IFG and type 2 diabetes in GOLDN; IFG/T2D was also treated as an outcome in the BPRHS to be comparable with GOLDN. MetS was defined as having at least three of the following five criteria: waist circumference ≥102 cm for men or ≥88 cm for women, elevated triglycerides ≥150 mg/dL or drug treatment for elevated triglycerides, low HDL-C (<40 mg/dL for men or <50 mg/dL for women) or drug treatment for reduced HDL-C, high blood pressure (systolic ≥130 mmHg or diastolic ≥85 mmHg) or antihypertensive medication, and elevated fasting glucose ≥100 mg/dL or drug treatment for elevated glucose (3).

**DNA isolation, genotyping, and haplotype analysis**

DNA was obtained from blood samples with Gentra Puregene Blood Kits (Gentra Systems) in GOLDN and with QIAamp DNA Blood Mini Kits (Qiagen) in the BPRHS. For GOLDN, Affymetrix Genome-Wide Human SNP Array 6.0 was used for genome-wide genotyping; for the BPRHS, Illumina HumanOmni1-Quad GWAS arrays were used to conduct the genome-wide genotyping. Genotypes of two IRS1 single nucleotide polymorphisms (SNPs) (rs7578326 and rs2943641) were selected for these analyses in both populations. Haplotype frequencies were estimated by the expectation-maximization algorithm, using the haplo.stats package in R (version 2.15.0).

Population admixture of the BPRHS population was calculated by selecting 100 SNPs as ancestry-informative markers (24). We adjusted for population admixture for all analyses in the BPRHS.

**Statistical analyses**

All continuous dependent variables were Box-Cox transformed to obtain normal distribution before statistical analysis (28). $\chi^2$ tests were conducted to examine the Hardy-Weinberg equilibrium for IRS1 variants. Dietary factors, including carbohydrate, monounsaturated fatty acid (MUFA), saturated fatty acid (SFA), total fat, and SFA-to-carbohydrate ratio were all expressed as percentages of total energy intake and dichotomized based on the median intake of each population for the interaction analysis. In GOLDN, the GENMOD procedure in SAS was used to adjust for family relationships, and a generalized estimating equation approach with exchangeable correlation structure was used in GENMOD. A multivariate interaction model was used to assess the interactions of IRS1 variants with dietary factors, while adjusting for potential confounders, including age, sex, waist circumference, study center, smoking status, alcohol drinking, type 2 diabetes, physical activity, and family relationships. In the BPRHS, multivariate logistic regression models were used to assess the association of IRS1 variants with binary outcomes, and the interaction of these variants with diet. For continuous outcomes, multivariate linear regression models were used, with control for age, sex, waist circumference, smoking status, alcohol drinking, type 2 diabetes, physical activity, and population structure. All statistical analyses were conducted using SAS 9.2 (SAS Institute Inc., Cary, NC).

Meta-analysis was conducted with the Meta-Analysis Helper (METAL) (http://www.sph.umich.edu/csg/abecasis/metal/) under fixed-effects models. For binary outcomes, we used meta-analysis to combine the effect size estimates ($\beta$ coefficients) from GOLDN and...
weinberg equilibrium expectation in either
population (P > 0.05). This pair of IR1
variants was in strong linkage disequi-
librium (LD) in both GOLDN (r² = 0.714)
and the BPRHS (r² = 0.458).

Meta-analysis of IR1 variants with
HOMA-IR, fasting insulin, type 2
diabetes, IFG/T2D, and MetS
For SNP rs7578326, G-allele carriers had
significantly lower HOMA-IR (z =
-3.102, P = 0.002) and fasting insulin
(z = -3.648, P = 0.0003) than A-allele
homozygotes (Supplementary Table 1).
For SNP rs2943641, T-allele carriers
had a significantly lower HOMA-IR (z =
-3.08, P = 0.002) and fasting insulin (z =
-2.932, P = 0.003) than C-allele
homozygotes. No significant heterogeneity
was observed (P heterogeneity >0.1).
The pooled odds ratios (ORs) of type
2 diabetes (pooled OR 1.83 [95% CI
[1.06–2.05], P = 0.024; rs2943641: P = 0.008) (Fig. 1), SFA-to-carbohydrate
ratio (rs7578326: P = 0.019; rs2943641:
P = 0.01), total fat (rs7578326: P = 0.038;
rs2943641: P = 0.01), carbohydrate
(rs7578326: P = 0.009; rs2943641: P =
0.002), and SFA-to-carbohydrate ratio
(rs7578326: P = 0.003; rs2943641: P =
0.003) for HOMA-IR (Table 3). SNP
rs7578326 G-allele carriers and/rs2943641 T-allele carriers, compared
with noncarriers, had significantly lower
HOMA-IR when consuming low MUFA,
low total fat, or low SFA-to-carbohydrate
ratio. SNP rs7578326 also interacted with
dietary carbohydrate (P = 0.027) and
SFA-to-carbohydrate ratio (P = 0.017)
for fasting insulin, whereas rs2943641
interacted with dietary MUFA (P = 0.033),
carbohydrate (P = 0.004), and SFA-to-
carbohydrate ratio (P = 0.014) for fasting
insulin (data not shown). To further
explore the potential influence of
the dietary GL and GI for HOMA-IR and fasting
insulin, but with no significant results (data
not shown).
In the BPRHS, rs7578326 tended to
interact with dietary MUFA for HOMA-IR
(P = 0.07) (Fig. 1), and rs7578326 G-
allele carriers showed lower HOMA-IR
compared with A-allele homozygotes
only when MUFA intake was low
(≤11.0% energy, P = 0.011), but not
when it was high (>11.0% energy). In
addition, this SNP significantly interacted
with dietary GL on HOMA-IR (P = 0.038)
and fasting insulin (P = 0.014). HOMA-IR
for G-allele carriers of rs7578326 was
significantly lower than noncarriers when
dietary GL was low (≤141.2, P = 0.007),

### RESULTS

#### Characteristics of the study populations and IR1 variants
In both GOLDN and BPRHS populations,
men had a significantly higher physical
activity score (P = 0.007) and dietary
MUFA intake, % 13.6
dietary carbohydrate intake were lower in
women, whereas HDL-C and
HOMA-IR 3.87
systolic blood pressure, mmHg 119 ± 18

#### Table 1—Characteristics of participants in the GOLDN and BPRHS populations

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>GOLDN (n = 406)</th>
<th>BPRHS (n = 605)</th>
</tr>
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<tbody>
<tr>
<td>Age, years</td>
<td>48.8 ± 15.9</td>
<td>57.6 ± 7.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.6 ± 4.7</td>
<td>29.9 ± 5.1</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>101 ± 14</td>
<td>102 ± 14</td>
</tr>
<tr>
<td>Type 2 diabetes, n (%)</td>
<td>33 (8.1)</td>
<td>106 (44.4)</td>
</tr>
<tr>
<td>Impaired fasting glucose, n (%)</td>
<td>210 (51.7)</td>
<td>89 (26.0)</td>
</tr>
<tr>
<td>MetS, n (%)</td>
<td>177 (43.6)</td>
<td>141 (59.0)</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>33 (8.1)</td>
<td>70 (29.91)</td>
</tr>
<tr>
<td>Current drinker, n (%)</td>
<td>199 (49.0)</td>
<td>121 (50.6)</td>
</tr>
<tr>
<td>Physical activity score</td>
<td>34.9 ± 7.3</td>
<td>32.8 ± 6.1</td>
</tr>
<tr>
<td>Energy intake, kcal/day</td>
<td>2,505 ± 1,501</td>
<td>2,900 ± 1,518</td>
</tr>
<tr>
<td>Total fat intake, %</td>
<td>35.9 ± 6.8</td>
<td>32.4 ± 6.9</td>
</tr>
<tr>
<td>SFA intake, %</td>
<td>12.1 ± 2.7</td>
<td>9.8 ± 2.7</td>
</tr>
<tr>
<td>MUF A intake, %</td>
<td>13.6 ± 2.8</td>
<td>11.3 ± 2.2</td>
</tr>
<tr>
<td>Carbohydrate, %</td>
<td>47.5 ± 8.6</td>
<td>49.1 ± 8.2</td>
</tr>
<tr>
<td>SFA-to-carbohydrate ratio</td>
<td>0.27 ± 0.01</td>
<td>0.21 ± 0.09</td>
</tr>
<tr>
<td>Dietary GL</td>
<td>145.8 ± 86</td>
<td>186 ± 65</td>
</tr>
<tr>
<td>Dietary GI</td>
<td>49.6 ± 3.8</td>
<td>57.4 ± 3.7</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>106 ± 22</td>
<td>125 ± 52</td>
</tr>
<tr>
<td>Fasting insulin, mU/L</td>
<td>14.6 ± 8.4</td>
<td>17.9 ± 16.8</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.87 ± 2.6</td>
<td>5.95 ± 7.69</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>41.0 ± 9.6</td>
<td>40.3 ± 11.9</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>153 ± 112</td>
<td>180 ± 166</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>71.0 ± 9.1</td>
<td>83.1 ± 11.6</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>119 ± 14</td>
<td>139 ± 19</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, or n (%). 2 P < 0.01 different from men within the population (Student t test).
but not for high GL (＞141.2). No significant interactions with GI or other dietary factors were observed for rs7578326. For rs2943641, no significant gene-diet interaction was observed (data not shown).

### Interaction of IRS1 variants with diet for type 2 diabetes, IFG/T2D, and MetS

In the GOLDN population, no significant interaction between IRS1 variants and dietary factors for the risk of type 2 diabetes or IFG/T2D was observed, whereas both rs7578326 and rs2943641 significantly interacted with dietary total fat, carbohydrate, and SFA-to-carbohydrate ratio to modulate risk of MetS (Table 3). Only when dietary SFA-to-carbohydrate ratio was low (≤0.24) did subjects with rs7578326 G allele have a lower risk of MetS compared with AA carriers (OR 0.52 [95% CI 0.34–0.80]), and only when the ratio was low (≤0.24) did rs2943641 T-allele carriers, compared with the CC carriers, have a lower risk of MetS (0.63 [0.41–0.99]). There was no significant interaction for dietary GL or GI for these outcomes in GOLDN.

For the BPRHS population, no significant interaction was observed for IRS1 variants and dietary factors for type 2 diabetes, IFG/T2D, or MetS.

### Haplotype analyses for IRS1 variants

**Main genetic associations.** For rs7578326 and rs2943641, four haplotypes were observed in both GOLDN and the BPRHS, with the frequencies ranging from 0.016 to 0.629 in GOLDN, and from 0.056 to 0.581 in the BPRHS (Supplementary Table 2). Meta-analysis indicated that haplotype G-T carriers had lower HOMA-IR (z = 2.817, P = 0.003) and fasting insulin (z = 3.072, P = 0.002) than noncarriers (Supplementary Table 3). Haplotype A-C carriers had a higher risk of type 2 diabetes (pooled OR 1.62 [95% CI 1.10–2.38], P = 0.014), IFG/T2D (1.46 [1.06–2.01], P = 0.02), and MetS (1.46 [1.09–1.96], P = 0.012), compared with noncarriers (Supplementary Table 2). No significant heterogeneity was observed for the meta-analysis (P heterogeneity > 0.1).

**Haplotype-diet interaction.** For GOLDN, haplotype G-T significantly interacted with SFA (P = 0.031), carbohydrate (P = 0.007), and the SFA-to-carbohydrate ratio (P = 0.005) on HOMA-IR (Supplementary Table 4). Haplotype G-T also significantly interacted with dietary total fat (P = 0.032), carbohydrate (P = 0.008), and the SFA-to-carbohydrate ratio (P = 0.002), influencing the risk of MetS. Haplotype A-C interacted with total fat intake (P = 0.048) and GL (P = 0.006) for risk for MetS (P = 0.048). Subjects not carrying haplotype A-C had a lower risk of MetS compared with A-C carriers when dietary GL was low (≤111.5, OR 0.38 [95% CI 0.18–0.63]), but not with high GL (>111.5, 1.36 [0.10–2.84]) (Supplementary Table 5). No interaction for type 2 diabetes or IFG/T2D was observed.

For the BPRHS, haplotype A-C marginally interacted with dietary GL for HOMA-IR (P = 0.065) and fasting insulin (P = 0.065) (data not shown). Subjects not carrying haplotype A-C had lower HOMA-IR than carriers only when dietary GL was low (≤141.2, P = 0.007), but not for high dietary GL. Haplotype A-C also interacted with dietary GI for MetS risk (P = 0.034) (Supplementary Table 4). Subjects not carrying haplotype A-C had a lower risk of MetS than A-C carriers only with low dietary GI (≤57.1, OR 0.50 [95% CI 0.28–0.87]). In addition, haplotype G-T had a marginally significant interaction with dietary carbohydrate (P = 0.051) for type 2 diabetes risk. Haplotype G-T carriers had a lower risk of type 2 diabetes compared with noncarriers when consuming high carbohydrate (>51.5, 0.65 [0.43–1.00]), but not when consuming low carbohydrate (≤51.5, 1.22 [0.80–1.87]). No significant interaction between IRS1 haplotypes and other dietary factors for MetS.
either outcome was observed in this population.

CONCLUSIONS—In the current study, we found that genetic variants at IRS1 were associated with insulin resistance, fasting insulin, type 2 diabetes, IFG/T2D, and MetS. Haplotype analyses further confirmed these associations. Our findings are consistent with previous GWAS (5,7) in European populations. Rung et al. (7) reported that the C allele of rs2943641 was associated with insulin resistance, hyperinsulinemia, and a higher risk of diabetes in French, Danish, and Finnish populations. For rs7578326, the A allele was associated with a higher risk of diabetes in populations of European ancestry (5). The results from previous GWAS were successfully replicated not only in GOLDN (a white population of European descent), but also in the BPRHS, whose genetic background is quite different from the European populations (24). In addition, to our knowledge, this was the first study to reveal that these two IRS1 variants were also associated with the risk of MetS in two independent populations.

Impaired regulation of insulin signaling is considered to be a major contributor to insulin resistance and type 2 diabetes, and phosphorylation of IRS plays a key role in the insulin signaling pathways (9). IRS1 and IRS2 are major IRS proteins associated with glucose homeostasis, and IRS1 is the major protein initiating the stimulation of glucose transport in both muscle and adipose tissues (10). In addition to the well-established role of the IRS1 protein in insulin signaling, previous evidence supports a link between the IRS1 genotype and dysregulation of glucose metabolism. For example, the diabetogenic C allele of rs2943641 was associated with decreased IRS1 protein expression in Danish twins (7). The same study reported that, after in vivo insulin infusion, the rs2943641 C allele was associated with reduced IRS1-associated PI3K-activity and with reduced insulin sensitivity (7). Therefore, the associations between rs2943641 and insulin resistance and type 2 diabetes observed in the current study may be attributed to the dysregulation of IRS1 protein expression and impaired insulin signaling. Similarly, we observed that the C allele of rs2943641 was associated with a higher risk of MetS, which is plausible because insulin resistance is a component of MetS, and type 2 diabetes is also closely related to MetS (16). These findings are consistent with a previous study (29). A missense mutation at IRS1, rs1801278 (G972R), was associated with MetS (29), and this SNP was also associated with insulin resistance and type 2 diabetes (30,31). However, these two SNPs, rs2943641 and rs1801278, are 567 kbp apart and not in LD (7). The mechanisms for their associations with insulin resistance, type 2 diabetes, and MetS may be quite different and need further clarification. Another study (32) indicated that the T allele of the IRS1 variant rs2943650 (in complete LD with rs2943641) was associated with a decreased risk for several MetS components, including body fat, insulin resistance, and dyslipidemia. Therefore, our results confirmed the prior findings and suggested a decreased risk of MetS associated with the rs2943641 T allele. The second SNP (rs7578326) tested in the current study is in strong LD with rs2943641 in both GOLDN and the BPRHS. Therefore, rs7578326 may regulate insulin signaling through rs2943641, or both SNPs combined may represent new causal genetic variant at IRS1 affecting insulin resistance and related phenotypes.

In addition to our analyses of genetic associations, we also explored interactions between dietary intake and IRS1 SNPs. SNPs rs7578326 G allele and rs2943641 T allele showed more beneficial effects on HOMA-IR, fasting insulin, and MetS than the CC genotype only when the SFA-to-carbohydrate ratio was low. These results were further confirmed by the haplotype analyses. Our findings, consistent with another observational study (14), suggested a protective effect of a diet high in carbohydrate or low in fat on diabetes for men with the rs2943641 T allele. In contrast, rs2943641 showed a different interaction pattern with dietary carbohydrate and fat for HOMA-IR and fasting insulin in an intervention study (13). Specifically, the CC genotype carriers had a greater improvement of insulin and HOMA-IR than the other genotypes when consuming high-carbohydrate and low-fat diets. The inconsistencies between the current study and the previous intervention study may be attributed to the different ranges of dietary intake and the study designs. For example, average carbohydrate intake in the high-carbohydrate, low-fat dietary group was 65% energy in the previous intervention study (13), whereas the median carbohydrate intake was only 49.1% energy in GOLDN and 51.5% energy in the BPRHS. Of concern, high-quality carbohydrate-rich foods with a low GI were used in that study (13), whereas combined sources of carbohydrate intake were evaluated in our study, and we, for the first time, reported significant interactions between dietary GI and GI and IRS1 variants for insulin resistance and related phenotypes. Therefore, carbohydrate quantity and quality may be the most relevant sources of inconsistencies between the current study and the previous one. However, the precise mechanism for the observed inconsistencies still needs further investigation. In addition, the previous intervention study (13) did not explicitly evaluate macronutrients separately because fat and carbohydrate were both altered simultaneously. Our study clearly shows that the dietary SFA-to-carbohydrate ratio and
carbohydrate quantity and quality were the important dietary factors contributing to the interactions with IRS1 SNPs. In addition, dietary MUFA was found to interact with IRS1 variants for insulin resistance in both populations. Our results provide consistent evidence that the T allele of rs2943641 and the G allele of rs7578326 were associated with lower levels of insulin resistance or its related phenotypes under certain dietary conditions, including a low SFA-to-carbohydrate ratio, low MUFA intake, or low GL or GI.

The potential mechanisms for these interactions may be related to lipid-induced insulin resistance (33). A high-fat diet was associated with a reduction in tyrosine phosphorylation and an increase in serine phosphorylation of IRS1, thus leading to the suppression of downstream PI3K activity and decreased insulin sensitivity (33). When dietary fat intake was low, reduced levels of IRS1 protein associated with carrying the risk allele C of rs2943641 could still suppress the downstream PI3K activity (7), thereby increasing insulin resistance. In contrast, the T allele tended to be protective and associated with the enhancement of PI3K activity. These hypotheses may provide a plausible explanation for our results. Insulin resistance for subjects carrying the rs2943641T allele and with low fat (MUFA, total fat, or SFA) intake was lower compared with subjects carrying the CC genotype or compared with subjects carrying either genotype with high fat intake. In addition, the interaction of IRS1 variants with GL or GI was also plausible, as high-GI foods could induce higher blood glucose and be associated with insulin resistance and diabetes (34). It might be that the protective effect of the IRS1 nonrisk allele was enhanced only when dietary GL or GI was low. However, the precise mechanism for the interaction remains to be clarified. Another concern is whether the interaction of IRS1 with dietary fat on MetS was confirmed by the correlation between triglycerides, HDL-C, and dietary fat. However, our analyses ensured that the significant interaction was independent of the main effect of dietary fat, and no significant interaction for triglycerides, HDL-C, or other MetS components was found.

The current study has several limitations. First, the GOLDN and BPRHS populations have quite different ancestries and lifestyles. For example, the dietary intakes differ significantly, and this could explain the different forms of gene-diet interactions observed. However, we found consistent trends across the two populations in terms of the main genetic associations and gene-diet interactions, and these relationships are all biologically plausible. Second, moderate sample sizes for GOLDN and BPRHS limited the statistical power. Nevertheless, to our knowledge, this is the first study revealing the interactions between IRS1 variants (rs7578326 and rs2943641) and dietary factors to modulate insulin resistance, risk of type 2 diabetes, and MetS in observational studies. More replications in other populations are clearly warranted.

In conclusion, IRS1 variants rs7578326 G allele and rs2943641 T allele were associated with a lower risk of insulin resistance, type 2 diabetes, and MetS in two independent populations of different ancestries. Notably, these associations appeared to be modulated by dietary factors, especially the dietary SFA-to-carbohydrate ratio, MUFA, and carbohydrate quantity and quality. If replicated, these results may eventually

### Table 3—Interaction between IRS1 variants and diet on HOMA-IR and risk of MetS in the GOLDN population

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total energy, %</th>
<th>HOMA-IR</th>
<th>P\text{trend}</th>
<th>P\text{interaction}</th>
<th>MetS OR (95% CI)</th>
<th>P\text{trend}</th>
<th>P\text{interaction}</th>
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<tbody>
<tr>
<td>rs7578326</td>
<td></td>
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<td></td>
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<tr>
<td>MUFA</td>
<td>≤13.2</td>
<td>3.86 ± 0.21 (193)</td>
<td>3.22 ± 0.13 (216)</td>
<td>0.0008</td>
<td>0.024</td>
<td>0.65 (95% CI 0.42–1.00)</td>
<td>0.052</td>
</tr>
<tr>
<td></td>
<td>&gt;13.2</td>
<td>3.78 ± 0.19 (181)</td>
<td>3.66 ± 0.17 (228)</td>
<td>0.906</td>
<td></td>
<td>1.17 (95% CI 0.72–1.91)</td>
<td>0.519</td>
</tr>
<tr>
<td>SFA</td>
<td>≤11.8</td>
<td>3.94 ± 0.20 (194)</td>
<td>3.23 ± 0.14 (215)</td>
<td>0.0009</td>
<td>0.019</td>
<td>0.68 (95% CI 0.44–1.04)</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>&gt;11.8</td>
<td>3.69 ± 0.21 (180)</td>
<td>3.65 ± 0.16 (229)</td>
<td>0.933</td>
<td></td>
<td>1.08 (95% CI 0.69–1.67)</td>
<td>0.740</td>
</tr>
<tr>
<td>Total fat</td>
<td>≤35.7</td>
<td>3.79 ± 0.18 (196)</td>
<td>3.15 ± 0.12 (213)</td>
<td>0.002</td>
<td>0.038</td>
<td>0.60 (95% CI 0.39–0.92)</td>
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</tr>
<tr>
<td></td>
<td>&gt;35.7</td>
<td>3.85 ± 0.23 (178)</td>
<td>3.71 ± 0.17 (231)</td>
<td>0.908</td>
<td></td>
<td>1.19 (95% CI 0.74–1.93)</td>
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</tr>
<tr>
<td>Carbohydrate</td>
<td>≤49.1</td>
<td>3.82 ± 0.24 (171)</td>
<td>3.67 ± 0.17 (238)</td>
<td>0.761</td>
<td>0.009</td>
<td>1.22 (95% CI 0.74–2.03)</td>
<td>0.434</td>
</tr>
<tr>
<td></td>
<td>&gt;49.1</td>
<td>3.82 ± 0.17 (203)</td>
<td>3.18 ± 0.12 (206)</td>
<td>0.0005</td>
<td></td>
<td>0.60 (95% CI 0.40–0.89)</td>
<td>0.012</td>
</tr>
<tr>
<td>SFA-to-carbohydrate ratio</td>
<td>≤0.24</td>
<td>3.88 ± 0.18 (203)</td>
<td>3.10 ± 0.12 (206)</td>
<td>0.0001</td>
<td>0.003</td>
<td>0.52 (95% CI 0.34–0.80)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>&gt;0.24</td>
<td>3.75 ± 0.23 (171)</td>
<td>3.75 ± 0.17 (238)</td>
<td>0.499</td>
<td></td>
<td>1.37 (95% CI 0.82–2.28)</td>
<td>0.229</td>
</tr>
<tr>
<td>rs2943641</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUFA</td>
<td>≤13.2</td>
<td>3.88 ± 0.23 (175)</td>
<td>3.25 ± 0.12 (234)</td>
<td>0.0005</td>
<td>0.008</td>
<td>0.73 (95% CI 0.46–1.16)</td>
<td>0.183</td>
</tr>
<tr>
<td></td>
<td>&gt;13.2</td>
<td>3.80 ± 0.21 (163)</td>
<td>3.66 ± 0.16 (246)</td>
<td>0.719</td>
<td></td>
<td>1.22 (95% CI 0.76–1.94)</td>
<td>0.400</td>
</tr>
<tr>
<td>SFA</td>
<td>≤11.8</td>
<td>3.99 ± 0.21 (178)</td>
<td>3.24 ± 0.13 (231)</td>
<td>0.0005</td>
<td>0.010</td>
<td>0.72 (95% CI 0.46–1.12)</td>
<td>0.147</td>
</tr>
<tr>
<td></td>
<td>&gt;11.8</td>
<td>3.67 ± 0.23 (160)</td>
<td>3.67 ± 0.15 (249)</td>
<td>0.906</td>
<td></td>
<td>1.19 (95% CI 0.77–1.84)</td>
<td>0.436</td>
</tr>
<tr>
<td>Total fat</td>
<td>≤35.7</td>
<td>3.85 ± 0.19 (177)</td>
<td>3.17 ± 0.11 (232)</td>
<td>0.0006</td>
<td>0.010</td>
<td>0.68 (95% CI 0.43–1.06)</td>
<td>0.085</td>
</tr>
<tr>
<td></td>
<td>&gt;35.7</td>
<td>3.83 ± 0.25 (161)</td>
<td>3.74 ± 0.16 (248)</td>
<td>0.690</td>
<td></td>
<td>1.24 (95% CI 0.79–1.95)</td>
<td>0.346</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>≤49.1</td>
<td>3.85 ± 0.26 (150)</td>
<td>3.68 ± 0.16 (259)</td>
<td>0.355</td>
<td>0.002</td>
<td>1.31 (95% CI 0.81–2.12)</td>
<td>0.271</td>
</tr>
<tr>
<td></td>
<td>&gt;49.1</td>
<td>3.85 ± 0.18 (188)</td>
<td>3.20 ± 0.12 (221)</td>
<td>0.0002</td>
<td></td>
<td>0.67 (95% CI 0.43–1.02)</td>
<td>0.063</td>
</tr>
<tr>
<td>SFA-to-carbohydrate ratio</td>
<td>≤0.24</td>
<td>3.89 ± 0.16 (189)</td>
<td>3.14 ± 0.11 (220)</td>
<td>0.0001</td>
<td>0.003</td>
<td>0.63 (95% CI 0.41–0.99)</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>&gt;0.24</td>
<td>3.78 ± 0.26 (149)</td>
<td>3.73 ± 0.16 (260)</td>
<td>0.463</td>
<td></td>
<td>1.35 (95% CI 0.83–2.22)</td>
<td>0.229</td>
</tr>
</tbody>
</table>

1Values are means ± SEM or OR and 95% CI. 2P values were adjusted for age, sex, waist circumference, study center, smoking status, alcohol drinking, type 2 diabetes, physical activity, and family relationships. 3P values were adjusted for age, sex, study center, smoking status, alcohol drinking, physical activity, and family relationships.
provide useful information for the prevention of insulin resistance, type 2 diabetes, and MetS and could help develop effective dietary recommendations in different populations.

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No potential conflicts of interest relevant to this article were reported.

J.-S.Z. analyzed data, wrote the manuscript, and was primarily responsible for the final content. D.K.A., K.L.T., and J.M.O. designed and conducted the research. L.D.P., C.E.S., and D.L. wrote the manuscript. I.B.B. designed and was primarily responsible for the opportunity provider and employer.

IRS1 variants and diet interaction on diabetes traits


