Sex-specific interactions between the IRS1 polymorphism and intakes of carbohydrates and fat on incident type 2 diabetes

Ulrika Ericson, Gull Rukh, Ivana Stojkovic, Emily Sonestedt, Bo Gullberg, Elisabet Wirfält, Peter Wallström, and Marju Orho-Melander

ABSTRACT

Background: The minor T allele of rs2943641 near the gene encoding for insulin receptor substrate 1 (IRS1) has been associated with decreased risk of type 2 diabetes (T2D) and adiposity in genome-wide association studies. Dietary intake can influence the regulation of IRS1, and studies have indicated sex-specific associations between IRS1 and adiposity.

Objective: The objective was to examine the interaction between IRS1 rs2943641 and macronutrient intakes on incident T2D and percentage body fat in the Malmö Diet and Cancer cohort.

Design: The study included 15,227 women and 9614 men aged 45–74 y without prevalent diabetes. Dietary data were collected with a modified diet history method. During 12 y of follow-up, 1567 incident T2D cases were identified.

Results: The T allele was associated with lower incidence of T2D (P-trend = 0.003) and, in men, with higher percentage body fat (P-trend = 0.00002). We observed 3-way interactions between sex, rs2943641, and carbohydrate intake (P = 0.01) as well as between sex, rs2943641, and fat intake (P = 0.01) on incident T2D. Among women, the T allele was associated with decreased risk only in the lower tertiles of carbohydrate intake (P-interaction = 0.01, P-trend = 0.01). In contrast, among men, the T allele was associated with decreased risk in the lowest tertile of fat intake (P-interaction = 0.02). No interaction was observed between macronutrient intakes and rs2943641 on percentage body fat.

Conclusions: Our results indicate that IRS1 rs2943641 interacts with carbohydrate and fat intakes on incident T2D in a sex-specific fashion. A protective association between the rs2943641 T allele and T2D was restricted to women with low carbohydrate intake and to men with low fat intake.

INTRODUCTION

Insulin receptor substrate 1 (IRS1) plays a key role in insulin signaling. When insulin binds to the insulin receptor, it acts by phosphorylating IRS1 and a cascade of phosphorylation reactions is initiated, finally leading to metabolic effects. Genetic variation near the gene encoding IRS1 (IRS1) has been associated with type 2 diabetes, insulin resistance, hyperinsulinemia, and adiposity in genome-wide association studies (1, 2). The minor T allele of IRS1 rs2943641 was first found to be associated with increased insulin sensitivity and decreased incidence of type 2 diabetes (1). More recently, another variant near IRS1 (rs2943650), in complete linkage disequilibrium with rs2943641 ($r^2 = 1.00$), was associated with higher body fat content. The association with body fat percentage indicated a significant sex difference, with a more pronounced effect in men (2).

Dietary intake and weight changes may influence the expression of insulin receptors and thereby affect IRS1 phosphorylation and insulin signaling (3). Diets with low energy content have shown positive effects on insulin sensitivity, and the relative intakes of macronutrients may affect regulation of IRS1 and insulin sensitivity (4–8). However, the importance of dietary macronutrient composition on risk of type 2 diabetes is unclear (9), and taking genetic variation in genes that are associated with type 2 diabetes into account could help to clarify this issue.

In line with this, a recent 2-y randomized trial [Preventing Overweight Using Novel Strategies (POUNDS LOST)] indicated that the IRS1 rs2943641 polymorphism could modify insulin sensitivity response to weight-loss diets among overweight individuals (5). A greater improvement of insulin sensitivity in response to a high-carbohydrate/low-fat weight-loss diet was seen in individuals homozygous for the major C allele than in carriers of the minor T allele, whereas a low-carbohydrate/high-fat diet tended to have a more positive effect on insulin sensitivity in T allele carriers.

The primary aim of this study was to examine the interaction between the IRS1 rs2943641 polymorphism and dietary macronutrient composition on the incidence of type 2 diabetes and prevalent body fat percentage at baseline in the Malmö Diet and Cancer (MDC) cohort. Because genetic variation in the IRS1 locus has indicated sex differences in associations with anthropometric and metabolic traits (2) and because our recent results

1 From the Department of Clinical Sciences in Malmö, Diabetes and Cardiovascular Disease, Genetic Epidemiology (UE, GR, IS, ES, and MO-M) and the Department of Clinical Sciences in Malmö, Nutrition Epidemiology (BG, EW, and PW), Lund University, Malmö, Sweden.

2 Supported by the Swedish Research Council, the Region Skåne, the Skåne University Hospital, the Novo Nordic Foundation, the Albert Pahlsson Research Foundation.

3 Address correspondence to U Ericson, Clinical Research Centre, Building 60:13, SUS in Malmö, Jan Waldenströms gata 35, SE-205 02 Malmö, Sweden. E-mail: ulrika.ericson@med.lu.se.

4 Abbreviations used: EPIC, European Prospective Investigation into Cancer and Nutrition; GI, glycemic index; IRS1, insulin receptor substrate 1; MDC, Malmö Diet and Cancer; POUNDS LOST, Preventing Overweight Using Novel Strategies.

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from the MDC cohort propose that sex may modify associations between carbohydrate intake and risk of type 2 diabetes (10), we also explored whether putative interactions between IRS1 and macronutrient intakes show sex specificity.

SUBJECTS AND METHODS

Study population and data collection

The MDC study is a population-based, prospective cohort study in Malmö, a city in the south of Sweden. Baseline examinations were conducted between 1991 and 1996. All women who were born during 1923–1950 and all men born during 1923–1945 who lived in the city of Malmö were invited to participate. Details of the cohort and the recruitment procedures are described elsewhere (11, 12). The only exclusion criteria were mental incapacity and inadequate Swedish language skills. The participants filled out questionnaires that covered socioeconomic, lifestyle, and dietary factors; recorded their meals; and underwent a diet history interview. Blood pressure and anthropometric measurements were conducted by nurses. Weight was measured by using a balance-scale while subjects were wearing light clothing and no shoes. Standing height was measured with a fixed stadiometer calibrated in centimeters. Waist circumference was measured midway between the lowest rib margin and the iliac crest. Body composition was estimated with a bioelectrical impedance analyzer (single-frequency analyzer, BIA 103; RJL Systems). Body fat percentage was calculated by using an algorithm provided by the manufacturer. During the screening period, 28,098 participants (40% of the eligible persons) completed all baseline examinations. From this population we excluded 958 individuals with prevalent type 2 diabetes. After exclusion of patients with prevalent type 2 diabetes we were left with 27,140 individuals, of whom 24,841 had available DNA and were genotyped successfully for IRS1 rs2943641; these patients constituted our study population. Prevalent diabetes was determined on the basis of self-reported diabetes diagnosis, self-reported diabetes medication, or information from medical data registries indicating a date of diabetes diagnosis preceding the baseline examination date. A random 50% subsample of those who participated between 1991 and 1994 were invited to be involved in additional baseline examinations. All additional measurements were made at baseline with a median time lag of 7 mo after the first visit. In total, 6103 individuals participated in the additional examinations. Measurements included fasting whole-blood glucose and fasting insulin. HOMA-IR was used as a measure of insulin resistance and was calculated with the following formula: fasting insulin × fasting glucose/22.5 (13). The ethical committee at Lund University approved the study (no. LU 51-90), and the participants provided written informed consent.

Because, of the 28,098 participants in the MDC cohort, 1758 incident diabetes cases and 1578 controls are included in the European Prospective Investigation into Cancer and Nutrition (EPIC) InterAct Consortium for the study of genetic factors and gene-lifestyle interactions with regard to incident diabetes, we describe the main differences between our study design and data as compared with MDC data included in EPIC InterAct. MDC is a cohort study, whereas EPIC InterAct has a case-control study design. The dietary data used within EPIC InterAct are made uniform, and many details found in the MDC dietary data that are used in the current study and described below are lacking in these harmonized data. That is, differences in study design and size, extensive information on confounding variables, the possibility to exclude individuals with reported dietary change, and uniform dietary data of high relative validity ensure the uniqueness of the present study in comparison with the pooled analyses that may be performed within the EPIC InterAct.

Dietary data

The MDC study used an interview-based, modified diet history method that combined the following: 1) a 7-d menu book for recording intake of meals that varied from day to day (usually lunch and dinner meals), cold beverages, and nutrient supplements; 2) a 168-item questionnaire for assessment of consumption frequencies and portion sizes of regularly eaten foods that were not covered by the menu book; and 3) a 45-min interview, which completed the dietary assessment.

Mean daily intake of foods was calculated on the basis of frequency and portion-size estimates from the questionnaire and menu book. Food intake was converted to energy and nutrient intakes by using the MDC nutrient database in which the majority of the nutrient information comes from a food database (PC-KOST2-93; www.slv.se/) from the National Food Administration in Uppsala, Sweden. The MDC method is described in detail elsewhere (14, 15).

The coding routines of dietary data were slightly altered in September 1994 to shorten the interview time. The change did not have any major influence on the ranking of individuals (15). The relative validity of the MDC method was evaluated in the Malmö Food Study in 1984–1985 (16). Pearson correlation coefficients between the reference method and the MDC method in men and women, respectively, and adjusted for total energy, were as follows: for protein, 0.53 and 0.54; for fat, 0.69 and 0.64; for carbohydrates, 0.70 and 0.66, and for fiber, 0.69 and 0.74 (16).

We used the following variables for nutrient intakes in this study: total energy (kcal; including energy from fat, carbohydrates, protein, alcohol, and fiber), nonalcohol energy (kcal), carbohydrates (% of energy), fat (% of energy), protein (% of energy), and fiber (g/1000 kcal). Nutrient densities were calculated by dividing total nutrient intakes by nonalcohol energy intake. Tertiles were used as exposure categories of macronutrients.

Genotyping

IRS1 rs2943641 was genotyped by using the TaqMan PCR (polymerase chain reaction) method (Applied Biosystems) according to the manufacturer’s instructions. The ABI Prism Sequence Detection System ABI 7900HT (Applied Biosystems) was used for post-PCR allelic discrimination by measuring allele-specific fluorescence. Genotypes were successfully determined for 97% of the individuals, and the minor allele frequency was 37%. The genotype concordance rate within 8306 duplicate samples was 99.0%. The genotype distribution did not deviate from Hardy-Weinberg equilibrium (P = 0.74).

Diabetes case ascertainment

Among the individuals with available DNA who were successfully genotyped for IRS1 rs2943641 and were without prevalent
diabetes at baseline \((n = 24,841)\), we identified 1567 incident cases of type 2 diabetes during 292,821 person-years of follow-up via at least 1 of 3 registries. The mean follow-up time was 12 y. The subjects contributed person-time from date of enrollment until date of diabetes diagnosis, death, migration from Sweden, or end of follow-up (December 2006), whichever occurred first. We used information on the date of diagnosis from the registries, which was prioritized in the following order: 1) the regional Diabetes 2000 Registry of Scania (33%) (17), 2) the Malmö Hemoglobin A1c Registry (49%) (18), and 3) the Swedish National Diabetes Registry (18%) (19). The Diabetes 2000 Registry and the national registry required a physician’s diagnosis according to established diagnostic criteria (fasting plasma glucose concentration \(\geq 7.0 \text{ mmol/L}\) or fasting whole-blood concentration \(\geq 6.1 \text{ mmol/L}\), measured on 2 different occasions). Individuals with \(\geq 2\) glycated hemoglobin values \(>6.0\%\) according to the Swedish Mono-S standardization system (which corresponds to 6.9\% according to the US National Glycohemoglobin Standardization Program and to 52 \text{ mmol/mol} with the International Federation of Clinical Chemistry and Laboratory Medicine units) (20, 21) were categorized as diabetes cases in the Malmö Hemoglobin A1c Registry.

Other variables

Information on age was obtained from the Swedish national identification number. BMI (kg/m²) was calculated from direct measurement of weight and height. Leisure-time physical activity was assessed by asking the participants to estimate the number of minutes per week they spent on 17 different activities. The duration was multiplied with an activity-specific intensity coefficient, and an overall leisure-time physical activity score was created. The score was divided into sex-specific tertiles and categorized as low, medium, and high. Smoking status of the participants was defined as smokers (including irregular smokers), ex-smokers, and never-smokers. Total consumption of alcohol was defined by a 4-category variable. Participants who reported zero consumption in the menu book, and who indicated no consumption of any type of alcohol during the previous year, were categorized as zero-reporters. The other category ranges for alcohol intake were as follows: \(<15\) g/d for women and \(<20\) g/d for men (low), 15–30 g/d for women and 20–40 g/d for men (medium), >30 g/d for women and >40 g/d for men (high). Participants were divided into 4 categories according to their highest level of education (\(\leq 8\) y, 9–10 y, 11–13 y, or university degree). Season was defined as season of dietary data collection: winter (December–February), spring (March–May), summer (June–August), and fall (September–November). Diet method version was defined as data collection before or after the change in coding routines in September 1994. Dietary change in the past (yes or no) was based on the question, “Have you substantially changed your eating habits because of illness or some other reasons?” Participants who report dietary changes may have unstable food habits. Their reported dietary habits may reflect a short period of their lives and may therefore have less influence on the development of chronic disease.

Statistical methods

The SPSS statistical computer package (version 20.0; IBM Corporation) was used for all statistical analyses. We examined baseline characteristics across \(\text{IRSI}\) genotypes for continuous variables by using the general linear model with adjustments for age and sex when applicable. Plasma insulin and HOMA-IR were log transformed (e-log) before analysis. Chi-square test was used for categorical variables. We performed the analyses for men and women separately. We also performed tests for interactions between \(\text{IRSI}\) genotype and sex.

Body fat percentage according to tertiles of energy-adjusted dietary intakes and \(\text{IRSI}\) was examined by using the general linear model. The basic model included adjustments for age (continuous) and sex, if applicable. The full multivariate model also included adjustments for total energy intake (continuous) and the following categorical variables: diet method version, season, leisure-time physical activity, smoking, alcohol intake, and education. We performed tests for interactions between \(\text{IRSI}\) genotype and dietary intakes with regard to body fat percentage [genotype \(\times\) diet tertile (treated as continuous variables)].

We used a Cox proportional hazards model to calculate HRs of diabetes incidence according to non-sex-specific tertiles of energy-adjusted dietary intakes and \(\text{IRSI}\) genotype. Years of follow-up was used as the underlying time variable. The basic model included adjustments for age (continuous) and sex, if applicable. The full multivariate model also included adjustments for BMI (continuous) and total energy intake (continuous) and the following categorical variables: diet method version, season, leisure-time physical activity, smoking, alcohol intake, and education. These covariates were identified from the literature and indicated potential confounding in the MDC cohort due to their associations with diabetes incidence and dietary intakes. Analyses of macronutrient intakes were additionally adjusted for protein or fiber intakes when applicable. We performed tests for interactions between \(\text{IRSI}\) genotype and dietary intakes with regard to diabetes incidence [genotype \(\times\) diet tertile (treated as continuous variables)]. In addition, 3-way interactions were examined by introducing multiplicative factors of sex, genotype, and dietary intakes (sex \(\times\) genotype, sex \(\times\) dietary intake, genotype \(\times\) dietary intake, and sex \(\times\) genotype \(\times\) dietary intake). We also performed all analyses for men and women separately. In sensitivity analyses we also examined sex-specific tertiles of dietary intakes. In addition, we excluded individuals with reported dietary changes in the past.

Pearson correlation coefficients between energy-adjusted intakes of nutrients were computed. All statistical tests were 2-sided, and significance was assumed at \(P < 0.05\).

RESULTS

The \(\text{IRSI}\) rs2943641 T allele was associated with decreased incidence of type 2 diabetes (HR for TT compared with CC: 0.82; 95\% CI: 0.70, 0.96; \(P\)-trend across genotypes = 0.003), and the trend was significant in women \((P\)-trend = 0.006) but not in men \((P\)-trend = 0.17) (Table 1).

At baseline, the carriers of the T allele had lower HOMA-IR and plasma insulin concentrations (Table 2). The trend of decreased HOMA-IR across genotypes was strengthened after adjustments for body fat percentage \((P\)-trend = 0.04 and 0.01 in women and men, respectively). In women, the T allele tended to associate with lower lean body mass, but no interaction with sex was observed. We found significant interactions between rs2943641 and sex on age \((P = 0.03)\), fat mass \((P = 0.01)\), and
body fat percentage ($P = 0.01$). In men, the T allele was associated with greater age, fat mass, and body fat percentage. The IRS1 genotype was not associated with macronutrient intakes or other lifestyle factors (see Supplementary Table 1 under “Supplemental data” in the online issue).

We observed a significant 3-way interaction between sex, rs2943641, and carbohydrate intake on incidence of type 2 diabetes ($P = 0.01$) as well as between sex, rs2943641, and fat intake ($P = 0.01$) (Table 3). In women, the minor T allele was associated with lower incidence of type 2 diabetes among those in the low ($P$-trend across genotypes = 0.01) and mid ($P$-trend = 0.01) tertiles of carbohydrate intakes but not among those in the highest tertile ($P$-trend = 0.76) ($P$-interaction for carbohydrate intake $\times$ rs2943641 = 0.01). In addition, low carbohydrate intake was associated with decreased risk among women homozygous for the T allele ($P$-trend = 0.04), whereas no significant associations were seen among women with CC or CT genotypes. Although the interaction between fat intake and IRS1 genotype in women did not reach significance ($P = 0.14$), we observed a risk pattern for fat intake that was consistent with that of carbohydrate intake (Pearson correlation coefficient between intakes of carbohydrates and fat = −0.91). The minor T allele was associated only with lower incidence of type 2 diabetes among women in the highest tertile of fat intake ($P$-trend = 0.03). In addition, high fat intake tended to be associated with decreased risk among women homozygous for the T allele ($P$-trend = 0.06) but not among women with CC or CT genotypes.

Also, in men, we observed an interaction with diet but in the opposite direction compared with that in women. The T allele was associated only with lower incidence of type 2 diabetes among men in the highest tertile of carbohydrate intake ($P$-trend across genotypes = 0.04; $P$-interaction for carbohydrate intake $\times$ rs2943641 = 0.10) and among those in the lowest tertile of fat intake ($P$-trend = 0.01; $P$-interaction for fat intake $\times$ rs2943641 = 0.02). In addition, low fat intake tended to be associated with decreased incidence of type 2 diabetes in men who were homozygous for the T allele ($P$-trend = 0.06).

Among women, the lowest risk estimate was seen for TT carriers with diets low in carbohydrates (HR: 0.51; 95% CI: 0.32, 0.81), whereas the lowest risk estimate among men was seen for TT carriers with diets low in fat (HR: 0.62; 95% CI: 0.37, 1.02).

No interaction was seen between IRS1 genotype and protein intake, and the observations did not differ between women and men. Furthermore, we did not observe any significant interactions between IRS1 genotype and fiber intake on type 2 diabetes.

The results did not differ significantly from the presented results when sex-specific tertiles of dietary intakes were examined, and the 3-way interactions between sex, IRS1 genotype, and intakes of carbohydrates and fat remained significant. Our observations were similar in analyses with or without inclusion of BMI (see Supplementary Table 2 under “Supplemental data” in the online issue) or body fat percentage in the statistical models, and adjustments for intakes of protein or fiber did not change the results. Finally, sensitivity analyses that excluded individuals who reported dietary changes in the past did not differ significantly from the presented results.

We did not detect any significant interactions between dietary intakes and IRS1 genotype with regard to body fat percentage (Table 4). Among men, the rs2943641 T allele was associated with higher body fat percentage independently of dietary intakes, whereas no significant associations were seen in any strata of dietary intakes among women. No significant 3-way interaction was seen between rs2943641, macronutrient intake, and sex on body fat percentage.

Pearson correlation coefficients were as follows—between energy-adjusted intakes of carbohydrates and intakes of fat, protein, and fiber: $−0.91$, $−0.20$, and 0.52, respectively ($P < 0.001$); between intakes of fat and intakes of protein and fiber: $−0.21$ and $−0.58$, respectively ($P < 0.001$); and between intakes of protein and fiber: 0.14 ($P < 0.001$). No major differences were seen between sexes (see Supplementary Table 3 under “Supplemental data” in the online issue).

**DISCUSSION**

In this study we replicated earlier observed associations from genome-wide association studies between IRS1 rs2943641 and type 2 diabetes, as well as male-specific association with body fat percentage. In addition, we detected significant 3-way interactions between sex, IRS1 rs2943641, and intakes of carbohydrates and fat on incidence of type 2 diabetes. More precisely, the rs2943641 T allele was associated with lower incidence of type 2 diabetes among women with diets low in carbohydrates or high in fat but not among women with diets high in carbohydrates or low in fat. In contrast, the T allele was significantly associated only with decreased risk in men with diets high in carbohydrates or low in fat. Furthermore, low carbohydrate intake was associated with decreased risk of type 2 diabetes in women with the TT genotype, whereas low fat intake tended to be associated with decreased risk in men with the TT genotype.

We are not aware of any previous population-based observational study examining the interaction between macronutrient intake and genetic variation of IRS1 on incident type 2 diabetes. Our results showing a lower risk of type 2 diabetes at low carbohydrate intakes among women with the rs2943641 TT genotype are in line with observations in the POUNDS LOST trial, which suggests that a low–carbohydrate diet has a more positive effect on insulin sensitivity among T allele carriers than among CC carriers (5), whereas a high–carbohydrate diet has a more positive effect among CC carriers. However, comparison between

### TABLE 1

HRs (95% CIs) of type 2 diabetes according to IRS1 rs2943641 genotypes

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>No. of cases</td>
<td>686</td>
<td>691</td>
<td>190</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>0.87 (0.78, 0.97)</td>
<td>0.82 (0.70, 0.96)</td>
</tr>
</tbody>
</table>

HRs were calculated by using a Cox proportional hazards model and adjusted for age and sex when applicable.
Baseline characteristics across rs2943641 genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Age (y)</th>
<th>BMI (kg/m²)</th>
<th>Fat mass (kg)</th>
<th>Body fat (%)</th>
<th>Fasting blood glucose (mmol/L)</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>24,841</td>
<td>58.0</td>
<td>25.7</td>
<td>10.9</td>
<td>7.0</td>
<td>49.89</td>
<td>1.65</td>
</tr>
<tr>
<td>CT</td>
<td>5,802</td>
<td>58.2</td>
<td>25.7</td>
<td>10.9</td>
<td>7.0</td>
<td>49.89</td>
<td>1.65</td>
</tr>
<tr>
<td>TT</td>
<td>2,939</td>
<td>58.7</td>
<td>26.2</td>
<td>12.3</td>
<td>7.9</td>
<td>49.89</td>
<td>1.65</td>
</tr>
</tbody>
</table>

Values derived by using a general linear model adjusted for age and sex when applicable.

<table>
<thead>
<tr>
<th>Trait</th>
<th>n</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.00002</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Fat mass</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Body fat</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.007</td>
<td></td>
</tr>
</tbody>
</table>

Significant interactions between sex and genotype on age (P = 0.03), fat mass (P = 0.01), and body fat percentage (P = 0.001).

It is possible that the different observations among women and men in our study occurred because of biological differences, such as concentrations of sex hormones, which may influence the importance of genetic predisposition as well as optimal carbohydrate and fat intakes with regard to macronutrient metabolism and prevention of diabetes development. Endogenous sex hormones may play a role in the development of type 2 diabetes, but their importance seems to differ in women and men (23). Several studies have also shown that genetic variations have different effects on physiologic traits and development of diseases in women and men (24). In addition to previous sex-dependent observations between IRS1 and anthropometric and metabolic traits (2) and the sex-dependent interactions with diet found in our study, one study also reported an interaction between genetic variation near IRS1 (rs1522813) and physical activity on type 2 diabetes among women but not among men (25). However, despite biological differences and sex-dependent associations with IRS1 in some studies, our results might also be explained, at least partially, by discrepancies in food choices or dietary reporting. Regardless of the small differences in the distribution of macronutrient intakes between women and men in this study, dissimilarities in food patterns have previously been seen in the MDC cohort (26). Moreover, other studies have indicated that food choices and accuracy of diet reporting vary with sex (27, 28). However, in a subpopulation of the MDC cohort, dairy products, meat, margarines, pastry, and bread were the major contributors to total fat intake in both sexes, although women appeared to consume more dairy products but less meat than did men (29). Regarding carbohydrate intake, women from the MDC cohort reported higher intakes of fruit and vegetables (30) and protein intakes did not change our findings. Even if it has been suggested that the fat quality may be of minor importance despite biological differences and sex-dependent associations, our study suggested that the different observations among women and men in our study occurred because of biological differences, such as concentrations of sex hormones, which may influence the importance of genetic predisposition as well as optimal carbohydrate and fat intakes with regard to macronutrient metabolism and prevention of diabetes development. Endogenous sex hormones may play a role in the development of type 2 diabetes, but their importance seems to differ in women and men (23). Several studies have also shown that genetic variations have different effects on physiologic traits and development of diseases in women and men (24). In addition to previous sex-dependent observations between IRS1 and anthropometric and metabolic traits (2) and the sex-dependent interactions with diet found in our study, one study also reported an interaction between genetic variation near IRS1 (rs1522813) and physical activity on type 2 diabetes among women but not among men (25). However, despite biological differences and sex-dependent associations with IRS1 in some studies, our results might also be explained, at least partially, by discrepancies in food choices or dietary reporting. Regardless of the small differences in the distribution of macronutrient intakes between women and men in this study, dissimilarities in food patterns have previously been seen in the MDC cohort (26). Moreover, other studies have indicated that food choices and accuracy of diet reporting vary with sex (27, 28). However, in a subpopulation of the MDC cohort, dairy products, meat, margarines, pastry, and bread were the major contributors to total fat intake in both sexes, although women appeared to consume more dairy products but less meat than did men (29). Regarding carbohydrate intake, women from the MDC cohort reported higher intakes of fruit and vegetables (30) but lower intakes of refined cereal products than did men (10).

The opposite associations with diabetes for carbohydrates and fat intakes were not unexpected, because the intakes of these macronutrients show a very strong inverse correlation. Correlations with protein intakes were less pronounced, and adjustments for protein intake did not change our findings. Even if it has been suggested that the fat quality may be of minor importance with regard to effects related to IRS1 (31), intakes of specific fatty acids and food sources of fat may also be of importance, and these aspects need to be addressed in future studies. It has been indicated that replacing saturated fat with monounsaturated fat may have positive effects on insulin sensitivity comparable to replacement with carbohydrates (32), and polyunsaturated fat seems to be beneficial (33). However, other studies have indicated that diets low in carbohydrates could improve insulin sensitivity (34), but it may be difficult to separate carbohydrate...
TABLE 3
HRs (95% CIs) of type 2 diabetes according to macronutrient intakes and IRS1 rs2943641 genotype1

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Women</th>
<th>Men</th>
<th>P-trend2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>686</td>
<td>691</td>
<td>190</td>
<td>347</td>
</tr>
<tr>
<td>Carbohydrate (mean)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First tertile (40.2% of energy)</td>
<td>1.00 (Ref)</td>
<td>0.96 (0.72, 1.03)</td>
<td>0.78 (0.59, 1.02)</td>
<td>0.04</td>
</tr>
<tr>
<td>Second tertile (46.7% of energy)</td>
<td>1.01 (1.00, 1.13)</td>
<td>0.91 (0.60, 1.04)</td>
<td>0.79 (0.60, 1.04)</td>
<td>0.05</td>
</tr>
<tr>
<td>Third tertile (53.3% of energy)</td>
<td>0.80 (0.70, 0.90)</td>
<td>0.79 (0.60, 1.03)</td>
<td>0.79 (0.60, 1.04)</td>
<td>0.26</td>
</tr>
<tr>
<td>Fiber (mean)</td>
<td>0.74 (0.56, 0.97)</td>
<td>0.47</td>
<td>0.96</td>
<td>0.75</td>
</tr>
<tr>
<td>First tertile (6.6 g/100 kcal)</td>
<td>0.95 (0.75, 1.21)</td>
<td>0.87 (0.65, 1.16)</td>
<td>0.95 (0.73, 1.24)</td>
<td>0.27</td>
</tr>
<tr>
<td>Second tertile (9.0 g/100 kcal)</td>
<td>1.02 (0.87, 1.19)</td>
<td>0.89 (0.65, 1.16)</td>
<td>0.95 (0.73, 1.24)</td>
<td>0.21</td>
</tr>
<tr>
<td>Third tertile (12.3 g/100 kcal)</td>
<td>1.01 (0.87, 1.18)</td>
<td>0.90 (0.66, 1.22)</td>
<td>0.95 (0.73, 1.24)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

1 HRs were calculated by using a Cox proportional hazards model.
2 Adjusted for age and sex when applicable.
3 Joint effect model adjusted for age, sex, diet method version, season, BMI, education, alcohol intake, smoking, total energy intake, and leisure-time physical activity.
4 Adjusted for age, sex, diet method version, season, BMI, education, alcohol intake, smoking, total energy intake, and leisure-time physical activity.
TABLE 4
Mean body fat percentage (95% CI) according to macronutrient intake and IRS1 rs2943641 genotype

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th></th>
<th>Women</th>
<th></th>
<th></th>
<th>Men</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>P-trend</td>
<td>n</td>
<td>n</td>
<td>P-trend</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td>CC</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First tertile</td>
<td>26.6 (26.1, 27.1)</td>
<td>26.7 (26.2, 27.2)</td>
<td>27.1 (26.6, 27.6)</td>
<td>0.02</td>
<td>31.1 (30.5, 31.7)</td>
<td>31.1 (30.5, 31.7)</td>
<td>31.4 (30.7, 32.1)</td>
</tr>
<tr>
<td>Second tertile</td>
<td>26.5 (26.0, 27.0)</td>
<td>26.1 (26.1, 27.0)</td>
<td>26.9 (26.4, 27.5)</td>
<td>0.03</td>
<td>31.3 (30.6, 31.9)</td>
<td>31.3 (30.6, 31.9)</td>
<td>31.5 (30.7, 32.1)</td>
</tr>
<tr>
<td>Third tertile</td>
<td>26.2 (25.7, 26.7)</td>
<td>26.3 (25.8, 26.8)</td>
<td>26.5 (25.9, 27.0)</td>
<td>0.17</td>
<td>30.9 (30.3, 31.5)</td>
<td>30.9 (30.3, 31.5)</td>
<td>31.0 (30.3, 31.7)</td>
</tr>
<tr>
<td>(P-interaction)²</td>
<td>(0.70)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fat (% of energy) |     |            |                |            |            |               |            |            |         |
| First tertile | 26.5 (26.0, 27.0) | 26.6 (26.1, 27.0) | 26.7 (26.2, 27.3) | 0.28 | 31.2 (30.6, 31.8) | 31.2 (30.6, 31.8) | 31.3 (30.6, 32.0) | 0.86 | 21.4 (20.5, 22.2) | 21.6 (20.8, 22.4) | 21.9 (21.0, 22.9) | 0.05 |
| Second tertile | 26.4 (25.9, 26.9) | 26.6 (26.1, 27.1) | 26.9 (26.4, 27.5) | 0.001 | 31.1 (30.5, 31.7) | 31.3 (30.6, 31.9) | 31.5 (30.8, 32.2) | 0.08 | 21.5 (20.7, 22.3) | 21.7 (20.9, 22.5) | 22.2 (21.4, 23.1) | 0.002 |
| Third tertile | 26.5 (26.0, 27.0) | 26.4 (25.9, 26.9) | 26.9 (26.3, 27.4) | 0.14 | 30.9 (30.3, 31.6) | 30.7 (30.1, 31.4) | 31.0 (30.4, 31.7) | 0.94 | 19.9 (21.1, 22.8) | 21.9 (21.1, 22.8) | 22.6 (21.7, 23.5) | 0.01 |
| (P-interaction)² | (0.94) |            |                |            |            |               |            |            |         |

Protein (% of energy) |     |            |                |            |            |               |            |            |         |
| First tertile | 25.9 (25.4, 26.4) | 26.0 (25.6, 26.5) | 26.2 (25.7, 26.8) | 0.05 | 30.4 (29.7, 31.0) | 30.6 (29.9, 31.2) | 30.7 (30.0, 31.3) | 0.29 | 21.4 (20.5, 22.2) | 21.5 (20.6, 22.3) | 21.8 (20.9, 22.7) | 0.05 |
| Second tertile | 26.4 (25.9, 26.9) | 26.3 (25.8, 26.8) | 26.7 (26.1, 27.3) | 0.33 | 31.1 (30.5, 31.7) | 30.8 (30.2, 31.4) | 31.2 (30.5, 31.9) | 0.76 | 21.5 (20.7, 22.3) | 21.6 (20.8, 22.4) | 22.1 (21.1, 23.0) | 0.05 |
| Third tertile | 27.0 (26.5, 27.4) | 27.1 (26.6, 27.6) | 27.5 (27.0, 28.1) | 0.004 | 31.6 (31.0, 32.2) | 31.7 (31.1, 32.3) | 31.8 (31.2, 32.5) | 0.26 | 22.1 (21.2, 22.9) | 22.3 (21.5, 23.1) | 23.3 (22.4, 24.2) | <0.001 |
| (P-interaction)² | (0.44) |            |                |            |            |               |            |            |         |

Fiber (g/1000 kcal) |     |            |                |            |            |               |            |            |         |
| First tertile | 26.6 (26.1, 27.1) | 26.6 (26.1, 27.1) | 27.0 (26.4, 27.5) | 0.02 | 31.0 (30.4, 31.6) | 31.0 (30.4, 31.6) | 31.2 (30.5, 32.0) | 0.36 | 21.9 (21.1, 22.8) | 21.9 (21.1, 22.7) | 22.5 (21.7, 23.4) | 0.02 |
| Second tertile | 26.5 (26.0, 27.0) | 26.7 (26.2, 27.2) | 27.0 (26.4, 27.5) | 0.006 | 31.3 (30.7, 31.9) | 31.3 (30.7, 31.9) | 31.5 (30.8, 32.2) | 0.62 | 21.4 (20.6, 22.2) | 21.9 (21.0, 22.7) | 22.3 (21.4, 23.2) | <0.001 |
| Third tertile | 26.2 (25.7, 26.7) | 26.2 (25.8, 26.7) | 26.5 (25.9, 27.0) | 0.45 | 30.9 (30.3, 31.5) | 30.9 (30.3, 31.5) | 31.1 (30.4, 31.8) | 0.67 | 21.3 (20.4, 22.1) | 21.4 (20.5, 22.2) | 21.7 (20.8, 22.7) | 0.26 |
| (P-interaction)² | (0.64) |            |                |            |            |               |            |            |         |

¹ Calculations were made by using the general linear model and adjusted for age and sex when applicable.
² Adjusted for age, sex, diet method version, season, education, alcohol intake, smoking, total energy intake, and leisure-time physical activity.
quantity from carbohydrate quality. We did not detect any interaction between rs2943641 and fiber intake, and adjustments for fiber intake did not change our findings. However, studies that focus on food sources of carbohydrates would be valuable, as would experimental studies on GI exposure. It is difficult to estimate individual GI exposure in epidemiologic studies (35). The dietary assessment methods are commonly not designed for estimating GI, and GI values for many foods are not available.

With regard to the associated effects of IRS1 genotype on body fat percentage, we did not observe any interaction with macronutrient intakes, but we provide further evidence that strengthens the initial findings of a male-specific association between the type 2 diabetes–protective T allele and increased body fat (2). Concerning the lack of interaction on body fat percentage, it is important to remember that we were restricted to the much less robust cross-sectional analysis of body fat percentage.

Strengths of our study include the high-quality dietary data compared with those of other epidemiologic studies (16, 36) and the extensive information on potential confounding factors. Furthermore, the prospective design with regard to type 2 diabetes minimizes selection bias and reverse causation. As discussed above, a weakness is the lack of anthropometric measurements after the baseline examinations. It can also be argued that the reported dietary habits may only reflect short periods of the study participants’ lives and may thereby have limited influence on the development of chronic disease. On the other hand, studies on long-term reproducibility indicate that single measurements of dietary intakes can be used as proxies of long-term exposures (37, 38). In addition, information on dietary changes in the past is an advantage of our study, and we observed similar results after the exclusion of individuals who tended to have unstable food habits. It also needs to be underlined that we did not study extreme diets. The mean fat intakes in the lowest and highest tertiles were 31% and 44% of energy, respectively, and the mean carbohydrate intakes in the lowest and highest tertiles were 40% and 53% of energy, respectively. Mean intakes in the sex-specific tertiles differed at most by 1% of energy. Interestingly, our results indicate sex-dependent interactions between IRS1 rs2943641 and carbohydrate and fat intakes. A protective association between the T allele and type 2 diabetes was restricted to women with diets low in carbohydrates or high in fat but to men with diets high in carbohydrates or low in fat. Biological differences seem to determine the importance of the associated effect by the variant in the IRS1 locus on body fat percentage and may also lie behind the distinct interactions with macronutrient intakes on future risk of type 2 diabetes. However, other sex-specific dietary characteristics might also have contributed to the observations, and our findings need to be replicated in future studies.

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The authors’ responsibilities were as follows—UE and MO-M: designed the research; UE: performed the statistical analysis, wrote the manuscript, and had primary responsibility for final content; GR, IS, ES, BG, EW, PW, and MO-M: contributed to the interpretation of results and to the revision of the manuscript; and BG: provided statistical advice. All authors read and approved the final version. MO-M is a senior scientist at the Swedish Research Council. None of the funders had any role in the study design, data collection and analysis, interpretation of data, decision to publish, or preparation of the manuscript. None of the authors had any conflicts of interest.

REFERENCES


