**TF7L2 rs7903146–macronutrient interaction in obese individuals’ responses to a 10-wk randomized hypoenergetic diet**¹–³

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**ABSTRACT**

**Background:** Transcription factor 7-like 2 (TF7L2) rs7903146 associates with type 2 diabetes and may operate via impaired glucose-like peptide 1 secretion, which is stimulated more by fat than by carbohydrate ingestion.

**Objective:** The objective was to examine the interaction between TCF7L2 rs7903146 and dietary fat and carbohydrate [high-fat, low-carbohydrate: 40–45% of energy as fat (HF); compared with low-fat, high-carbohydrate: 20–25% of energy as fat (LF)] in obese individuals’ responses to a 10-wk hypoenergetic diet (−600 kcal/d).

**Design:** European, obese participants (n = 771) were randomly assigned to receive an HF or an LF diet. Body weight, fat mass (FM), fat-free mass (FFM), waist circumference (WC), resting energy expenditure (REE), fasting fat oxidation in percentage of REE (FatOx), homeostasis model assessed insulin release (HOMA-β), and HOMA–insulin resistance (HOMA-IR) were determined at baseline and after the intervention; 739 individuals were genotyped for rs7903146.

**Results:** Average weight loss was 6.9 kg with the LF and 6.6 kg with the HF (difference between diets, NS) diet. Among individuals who were homozygous for the T-risk allele, those in the HF diet group experienced smaller weight losses (Δweight: 2.6 kg; P = 0.009; n = 622), smaller ΔFFM (1.6 kg; P = 0.027; n = 609), smaller ΔWC (3.3 cm; P = 0.010; n = 608), and a smaller ΔHOMA-IR (1.3 units; P = 0.004; n = 615) than did the LF diet group. For C allele carriers, there were no differences between the HF and LF diet groups. For the HF diet group, each additional T allele was associated with a reduced loss of FM (0.67 kg; P = 0.019; n = 609). TCF7L2 rs7903146 was not associated with ΔREE, ΔFatOx, ΔHOMA-β, or dropout.

**Conclusion:** Our results suggest that obese individuals who are homozygous for the TCF7L2 rs7903146 T-risk allele are more sensitive to LF than to HF weight-loss diets.

To support their findings, Grau et al. conducted a study on 771 obese participants, randomly assigned to either a high-fat (HF) or low-fat (LF) diet for 10 weeks. The participants were genotyped for TCF7L2 rs7903146 to assess dietary preferences and genetic interaction with macronutrient consumption.

**INTRODUCTION**

It is of potential importance to identify individuals with a genetic pattern that influences the response to weight reduction therapy. The NUGENOB study, which investigated gene–diet interactions in human obesity: implications for dietary guidelines, aimed to find such patterns. The study was supported by the European Community (contract no. QLK-CT-2000-00618).

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changes in resting energy expenditure (ΔREE), insulin release (ΔHOMA-β), and insulin sensitivity (ΔHOMA-IR).

Transcription factor 7-like 2 (TCF7L2) was identified as a type 2 diabetes (T2D) locus by the deCODE genetics group (4), and this finding was subsequently confirmed in Europeans in 5 genome-wide association studies and also in other ethnic groups (5). Of the 3 markers originally identified, rs7903146 is the SNP most strongly associated to T2D (6, 7) and is considered, if not the causal variant itself, to be the closest known correlate (7, 8). Weight gain does not seem to be part of the causal pathway from rs7903146 to development to T2D (9), but obesity seems to modify the association between rs7903146 and T2D (10). In a study by Cauchi et al (10), the allelic odds ratio (OR) of T2D was 1.89 (95% CI: 1.67, 2.14) for nonobese and 1.30 (95% CI: 1.14, 1.48) for obese individuals. The main effects of TCF7L2 rs7903146 on baseline values and changes in BMI, insulin release, and insulin resistance (11) and on postabsorptive REE and 3-h postprandial REE (12) was previously analyzed in the NUGENOB cohort; no associations were found.

TCF7L2 is essential for transcription of the proglucagon gene and thus for glucagon-like peptide 1 (GLP-1) synthesis (13). In one study, no difference was found in either basal GLP-1 concentrations or in GLP-1 concentrations during an oral-glucose-tolerance test (OGTT) between carriers and noncarriers of the rs7903146 T-risk allele (14). Another study found non-significantly lower GLP-1 concentrations during a mixed meal in T-risk allele carriers than in noncarriers (15). Both studies found a reduced insulinotropic effect of GLP-1 in T allele carriers. GLP-1 may contribute to body weight regulation in various ways—through appetite (16, 17), adipose tissue metabolism (18, 19), and insulin signaling (20). Because its release is stimulated differentially by fat and carbohydrate, GLP-1 concentrations are higher after ingestion of fat than after ingestion of carbohydrate in healthy (21) and insulin-resistant (22) individuals, it is possible that functional variants in TCF7L2 may alter the responsiveness to weight-loss diets differing in fat and carbohydrate composition. We decided to examine whether TCF7L2 rs7903146 is related to diet-induced weight loss and associated phenotypes including possible interactions with the macronutrient content of the weight-loss diet in the NUGENOB cohort.

SUBJECTS AND METHODS

Participants and study design

The NUGENOB study was a randomized, parallel, 2-arm, open-label, 10-wk dietary intervention (trial registration: ISRCTN25867281) of 2 hypoenergetic diets with either a low (LF) or high (HF) fat content. NUGENOB was a multicenter study including 8 clinical centers in 7 European countries [Sweden, Denmark, United Kingdom, Netherlands, Czech Republic, France (2 clinical centers), and Spain] and has been described previously (www.nugenob.org) (1, 2).

Participants were recruited from May 2001 until September 2002 through the media, from waiting lists, ongoing population studies, by self-referral, and referral from a general physician or other clinical units and local obesity organizations. Inclusion criteria were as follows: body mass index (BMI; in kg/m²) ≥ 30 and age 20–50 y. Exclusion criteria were as follows: weight change >3 kg within 3 mo before the study start; hypertension, diabetes or hyperlipidemia treated by drugs; untreated thyroid disease; surgically or drug-treated obesity; pregnancy; participation in other trials; and alcohol or drug abuse.

The target macronutrient composition of the 2 diets was as follows: LF diet (20–25% of total energy from fat, 15% from protein, and 60–65% from carbohydrate) and HF diet (40–45% of total energy from fat, 15% from protein, and 40–45% from carbohydrate). Both diets were designed to provide 600 kcal/d (2510 kJ/d) less than the individually estimated daily energy requirement based on an initial resting metabolic rate multiplied by 1.3. Subjects were given oral and written instructions relating to these targets based on either a template (see details at www.nugenob.org) or exchange system (23). Instructions were also given to minimize differences between the 2 diets in other components such as sources and type of fat, amount and type of fiber, type of carbohydrate, fruit and vegetables, and meal frequency and participants were requested to abstain from alcohol consumption. Dietary instructions were reinforced weekly.

In total, 771 obese white Europeans (579 women) were included and randomly assigned to receive 1 of the 2 intervention diets by stratified block randomization. The randomization list was computer generated at the coordinating center, and the block size was unknown to the clinical centers. Informed written consent was obtained before study participation, and the study was approved by the ethics committee at each of the participating centers.

Phenotypes

Before randomization to the weight-loss intervention and after completion of the intervention, participants underwent a clinical investigation protocol starting at 0800 after a 12-h overnight fast. The first clinical investigation was preceded by a 3-d dietary run-in period, during which participants had to keep to their habitual diet and avoid excessive physical activity and alcohol consumption. The second clinical investigation was conducted in the 10th week after the start of the dietary weight-loss intervention program.

Anthropometric measures and body composition were assessed after the subjects voided their bladder. Body weight was measured on calibrated scales. Waist circumference (WC) was measured while the participants were wearing only nonrestrictive underwear. Body height was measured with a calibrated stadiometer. The mean of 3 measurements was recorded for each variable. Fat mass (FM) and fat-free mass (FFM) were assessed by multifrequency bioimpedance (QuadScan 4000; Bodystat, Isle of Man, British Isles).

REE and respiratory quotient (RQ) were measured by indirect calorimetry with open-circuit ventilated hood systems routinely used at each center for 30 min. The experimental room was kept thermoneutral at 25 °C. All equipment and procedures were standardized for the different centers, and a standardized validation program was used to facilitate pooling of the results from the different centers. Before the start of the study, validation was assessed by using 10 alcohol-burning tests per center. Within-subject variation was assessed by running repeated measurements on the same day from 10 lean and/or obese fasting subjects per center. The mean (±SD) variation in RQ was 0.668 ± 0.006. Likewise the within-subject CV was 2.73 ± 1.10 and 2.89 ±
1.19% for RQ and REE, respectively. REE was calculated according to the equation of Weir (24). Fat oxidation (FatOx) was calculated according to the equations of Frayn (25). In these calculations, nitrogen excretion was assumed to be similar to daily nitrogen intake. At the second clinical investigation day, indirect calorimetry measurements on some of or all participants completing the intervention were carried out in 6 of 8 centers (Sweden, United Kingdom, Czech Republic, both centers in France, and Spain). After participants rested supine for 15 min, venous blood samples were drawn to determine fasting plasma glucose and fasting plasma insulin. Plasma glucose concentrations (ABX Diagnostics, Montpellier, France) were measured on a COBAS MIRA automated spectrophotometric analyzer (Roche Diagnostica, Basel, Switzerland). Plasma insulin concentrations were measured with a double-antibody radioimmunoassay (Insulin RIA 100; Kabi-Pharmacia, Uppsala, Sweden).

Homeostasis model assessment was used to estimate insulin release (HOMA-β) and insulin resistance (HOMA-IR) (26–28).

Genotyping

Samples of buffy coat were sent on dry ice to the Steno Diabetes Center in Copenhagen, where DNA was extracted. Extracted DNA samples were diluted in Tris/EDTA buffer to a stock DNA solution of 100 ng/μL and a working DNA solution of 10 ng/μL. Stock solutions were stored at −80 °C, and working solutions were stored at 4°C. DNA samples were stored and handled in locations free of contaminating polymerase chain reaction products.

Helgason et al (7) identified a variant of TCF7L2, HapA, represented by haplotypes with rs10885406 A and rs7903146 C, which was associated with BMI. In European individuals, however, there is no difference in the associations of the rs7903146 C allele and of the HapA haplotype with obesity (11). In the present study, only rs7903146 was genotyped.

High-throughput genotyping of the rs7903146 variant was performed by using the TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA). The polymerase chain reaction primers and TaqMan probes were designed by Primer Express and optimized according to the manufacturer’s protocol. The genotype success rate was >98%. No duplicate samples were made in the NUGENOB cohort to determine the error rate, but the error rate was 0% when the same laboratory genotyped rs7903146 in a previous study of 384 control and 384 T2D individuals (11). The rs7903146 allele frequencies were in Hardy-Weinberg equilibrium (P = 0.91) (see Supplementary Table S1 under “Supplemental data” in the online issue).

Statistical methods

 Examination of Hardy-Weinberg equilibrium was carried out while taking into account center differences by summing up Pearson chi-square statistics for each center and making comparisons with a chi-square distribution with 8 df.

First, general genetic models with no assumption of a specific effect in individuals heterozygous and homozygous for the T allele compared with noncarriers (null hypothesis) were analyzed. The results from these analyses were then used to decide whether to proceed with analyzing models assuming a particular effect of the T-risk allele compared with the noncarrier: dominant effect (noncarrier = 0, heterozygous and homozygous = 1), codominant effect (assessed as an additive effect: noncarrier = 0, heterozygous = 1, homozygous = 2), or recessive effect (noncarrier = 0, heterozygous = 0, homozygous = 1). Models examining the main effects of rs7903146 were made followed by models examining the interaction between genotype and diet.

Changes (Δ) in body weight (kg), ΔFFM (kg), ΔFFM (kg), ΔWC (cm), ΔHOMA-β, ΔHOMA-IR, ΔREE (kcal/24 h) and ΔFatOx (%) were calculated by subtracting measurements recorded immediately before randomization from the measurement recorded at the completion of the intervention. Main effects (with 95% CIs) of rs7903146 genotype (CC, CT, or TT) and interactions between genotype and assigned hypoenergetic diet (HF or LF) in relation to Δweight, ΔFFM, ΔWC, ΔHOMA-β, ΔHOMA-IR, ΔREE, and ΔFatOx were estimated by separate linear regression models for each outcome variable. Drop-out was similarly analyzed in logistic regression models. In models for changes in phenotypes, we controlled for respective baseline values (linear; separate effect of weight, WC, FM, and FFM for men and women; FM, HOMA-β, and HOMA-IR log transformed), age (linear in models of ΔFFM, ΔHOMA-β, ΔHOMA-IR, ΔREE, and ΔFatOx; linear and squared in models of Δweight, ΔFFM, and ΔWC), sex, and center (Gaussian random effect). When models for ΔFFM and ΔFFM were also adjusted for height and baseline weight, the same results were obtained (data not shown). Models for ΔREE and ΔFatOx were also adjusted for baseline FFM and ΔFFM. ΔWC was additionally analyzed as the change in BMI-adjusted WC residuals (the difference between residuals from regression models with BMI at baseline as independent variable and WC at baseline as dependent variable and residuals from regression models with BMI after intervention as an independent variable and WC after intervention as a dependent variable) [Δ(WC - BMI)]. Models for ΔREE, ΔFatOx, and ΔHOMA-IR were also performed while additionally adjusting for baseline FFM and ΔFFM or for baseline FFM, baseline FM, ΔFFM, and ΔFFM, but this did not change the results from the analyses. Odds ratios (ORs) for drop-out according to genotype and randomized diet combined were analyzed with adjustment for baseline BMI (linear), age (linear), sex, and center.

Assessments of main effects was conducted by including the genotypes as covariates and as a separate covariate the diet group to which the participants had been randomly assigned. Gene-diet interactions were tested in analyses that also included a product term for genotype × diet. The product term is then the genotype-specific difference in mean Δweight, ΔFFM, ΔWC, ΔHOMA-β, ΔHOMA-IR, ΔREE, and ΔFatOx, respectively—adjusting as described above—between the LF and the HF and then comparing the differences in mean Δweight, ΔFFM, ΔWC, ΔHOMA-β, ΔHOMA-IR, ΔREE, and ΔFatOx, respectively, for CT and/or TT with CC or CC and CT depending on the assumed genetic model. Estimates from models including a categorical genotype-diet variable are used to present combined effects of genotype and diet when interactions are found.

The statistical software program STATA version 9.0 (Stata, College Station, TX) was used for all statistical analyses. The detectable genotype-diet interaction effect sizes for Δweight, ΔFFM, ΔWC, and ΔHOMA-IR were estimated by using QUANTO version 1.2 (29), and they were 2.82 kg for Δweight, 1.11 kg for ΔFFM, 1.99 kg for ΔFFM, 3.72 cm for ΔWC, and
1.31 for ΔHOMA-IR (see also Supplementary Table S2 under “Supplemental data” in the online issue).

RESULTS

*TCF7L2* rs7903146 was genotyped successfully in 739 out of 771 obese subjects. Overall, 117 of the successfully genotyped subjects failed to complete the 10-wk weight-loss intervention, but dropout was not associated with genotype (P = 0.49; n = 739).

Unadjusted mean values for anthropometric, body-composition, and metabolic variables at baseline and the mean change from baseline to after intervention are presented in Table 1 according to genotype and diet. Pearson correlations partialled for age, sex, and study center for all dependent variables are presented in Supplementary Table S3 under “Supplemental data” in the online issue.

The results of the regression analyses with no assumption about genetic model are presented for all analyzed outcome variables in Supplementary Table S4 under “Supplemental data” in the online issue, which suggests that the associations between the SNP and weight loss, ΔFFM, ΔWC, and ΔHOMA-IR are better reflected by a recessive model than by the alternative models (codominant or dominant models) and ΔFM by a codominant model. The combined effects of genotype and diet on Δweight, ΔFFM, ΔFM, ΔWC, and Δ-IR are presented in Figure 1. Among subjects who completed the intervention and for whom *TCF7L2* rs7903146 was genotyped, we found significant interactions between genotype (TT compared with CC and CT) and diet in relation to Δweight (P for interaction: 0.023; n = 622), ΔFM (per T-allele effect; P for interaction: 0.048; n = 609), ΔFFM (P for interaction: 0.032; n = 609), ΔWC (P for interaction: 0.023; n = 608), and Δ-IR (P for interaction: 0.0025; n = 615).

Weight loss

Mean Δweight was −6.81 kg. For subjects with the *CC/CT* genotype, there was no difference in weight loss between the HF and LF diet (P = 0.35). In individuals with the *TT* genotype, weight loss was 2.57 kg smaller (P = 0.0088) with the HF than with the LF diet. With the HF diet, weight loss was 2.08 kg smaller (P = 0.010) for the *TT* genotype than for the *CC/CT* genotype.

Loss of fat-free mass

Mean ΔFFM was −1.48 kg. For subjects with the *CC/CT* genotype, there was no difference in ΔFFM between the HF and LF diet groups (P = 0.94). For the *TT* genotype, loss of FFM was 1.55 kg smaller (P = 0.027) with the HF than with the LF diet. With the HF diet, loss of FFM was 1.31 kg smaller (P = 0.022) with the *TT* genotype than with the *CC/CT* genotype.

Loss of fat mass

Mean ΔFM was −5.35 kg. For subjects randomly assigned to the HF diet, loss of FM decreased by 0.67 kg for each additional *T* allele (P = 0.019). With the LF diet, the *T* allele was not associated with ΔFM (P = 0.73). For subjects with the *CT* and *TT* genotypes, loss of FM was 0.63 and 1.39 kg smaller, respectively (P = 0.031 and P = 0.021), with the HF than with the LF diet in the codominant model.

Decrease in waist circumference

Mean ΔWC was −6.33 cm. For subjects with the *CC/CT* genotype, there was no difference in ΔWC between the HF and LF diets (P = 0.43). For the *TT* genotype, the decrease in WC was 3.33 cm smaller (P = 0.010) with the HF than with the LF diet. With the HF diet, the decrease in WC was 2.40 cm smaller (P = 0.024) in subjects with the *TT* genotype than with the *CC/CT* genotype. We found no effect of *TCF7L2* rs7903146 or diet on ΔWC (BMI).

Decrease in insulin resistance and release

Mean ΔHOMA-IR was −0.31 units. For subjects with the *CC/CT* genotype, there was no difference in ΔHOMA-IR between the HF and LF diet groups (P = 0.42). For the *TT* genotype, the decrease in HOMA-IR was 1.33 units smaller (P = 0.004) with the HF than with the LF diet. With the HF diet, the decrease in HOMA-IR was 1.26 units smaller (P = 0.001) for the *TT* genotype than for the *CC/CT* genotype. We found no effect of *TCF7L2* rs7903146 on ΔHOMA-β. Mean ΔHOMA-β among successfully genotyped participants completing the intervention was −7.83 units.

Decrease in REE and fat oxidation

We found no effect of *TCF7L2* rs7903146 on ΔREE or ΔFatOx. Among successfully genotyped participants completing the intervention, ΔREE decreased on average by 114.8 kcal/24 h, whereas FatOx increased by 2.3% points from 46.7% REE to 49.0% REE.

DISCUSSION

In the present weight-loss intervention study, we found statistically significant interactions between *TCF7L2* rs7903146 genotype and the macronutrient content of the hypoenergetic diet (HF and LF) in relation to changes in 5 (Δweight, ΔFFM, ΔFM, ΔWC, and ΔHOMA-IR) of 8 investigated obesity-related phenotypes.

Some, but not all, previous studies found that the *T*-risk allele at *TCF7L2* rs7903146 associated with estimates of decreased insulin release (30–33) and surrogate measures of decreased insulin sensitivity (30). In the present study, rs7903146 was associated with ΔHOMA-IR and with changes in anthropometric and body-composition variables, but not with ΔHOMA-β. It is possible that this inconsistency with the literature was due to the fact that the present study addressed diet-induced weight loss in obese patients, showing effects of the gene variant that differ from those inducing the elevated risk of T2D as mentioned in the Introduction (10).

The effects were fairly consistent for the 5 phenotypes, although the effects on ΔFM fit better with a codominant genetic model, whereas effects on Δweight, ΔFFM, ΔWC, and ΔHOMA-IR were found in recessive models. In most studies of *TCF7L2* rs7903146 and T2D, the genetic effect appeared to be codominant (11), although other studies suggest a recessive (31) or dominant (33) effect. The biological


<table>
<thead>
<tr>
<th>Genotype at rs7903146</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>P for main effect of gene-diet interaction²</th>
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<tr>
<td></td>
<td>LF (n = 168)</td>
<td>HF (n = 149)</td>
<td>LF (n = 125)</td>
<td>HF (n = 134)</td>
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<td>1886 ± 314</td>
<td>1878 ± 315</td>
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<td></td>
<td>Change</td>
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<td>-117 ± 182</td>
<td>-101 ± 190</td>
</tr>
<tr>
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<td></td>
<td>Change</td>
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<td>12 ± 410</td>
<td>-32 ± 458</td>
</tr>
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<td>0.5 ± 23.1</td>
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</tbody>
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¹ All values are means ± SDs. Baseline values are for those who completed the intervention. Change was defined as the value at week 10 minus the baseline value. HF, high-fat, low-carbohydrate hypoenergetic diet (−600 kcal/d); LF, low-fat, high-carbohydrate hypoenergetic diet (−600 kcal/d); HOMA-IR, homeostasis model assessed insulin resistance; HOMA-β, homeostasis model assessed insulin release; FatOx, fat oxidation as a percentage of resting energy expenditure; REE, resting energy expenditure.

² P values from linear regression analyses carried out for the variables relevant for the objective of the study: change (Δ) in weight, Δfat-free mass (FFM), Δfat mass (FM), Δwaist circumference (WC), ΔHOMA-β, ΔHOMA-IR, ΔREE, and ΔFatOx. Adjusted for baseline weight (linear; separate effect for men and women), FFM (linear; separate effect for men and women), FM (log transformed; linear; separate effect for men and women), WC (linear; separate effect for men and women), HOMA-β (logarithm transformed; linear), HOMA-IR (logarithm transformed; linear), REE (linear), and FatOx (linear), respectively, along with age (linear in models of ΔFFM, ΔHOMA-β, ΔHOMA-IR, REE, and ΔFatOx; linear and squared in models of Δweight, ΔFM, and ΔWC), sex, and center (Gaussian random effect).

³ Genetic model: recessive.

⁴ Genetic model: codominant.

⁵ No assumption of genetic model.

⁶ Models for ΔHOMA-IR were also performed with additional adjustment for baseline FFM and ΔFFM or for baseline FFM, baseline FM, ΔFFM and ΔFM, but this did not change the results from the analyses.

⁷ After the intervention, REE and FatOx were measured in ~66% of the subjects who completed the intervention. (CC, LF: n = 110; CC, HF: n = 98; CT, LF: n = 69; CT, HF: n = 85; TT, LF: n = 19; TT, HF: n = 13).
processes leading to T2D may, however, be different from those investigated here. The figures for Δweight and ΔWC are almost identical; 1-kg differences in Δweight mirrored as 1-cm difference in ΔWC. The TCF7L2 variant and diet had no effect on Δ(WC|BMI).

Considering that ≈75% of the mean weight loss in the whole group was loss of FM and ≈25% was loss of FFM, the difference in ΔFFM explained a relatively large part (≈50%) of the difference in Δweight (~2.6 kg) between the LF and HF diets for the TT genotype.

FIGURE 1. Combined effects of TCF7L2 rs7903146 genotype (CC, CT, and TT) and dietary fat and carbohydrate (high-fat, low-carbohydrate (high fat) compared with low-fat, high-carbohydrate (low fat) hypoenergetic diets] on changes in weight (Δweight), fat-free mass (ΔFFM), fat mass (ΔFM), waist circumference (ΔWC), and homeostasis model assessed insulin resistance (ΔHOMA-IR) from linear regression models. The genetic models were recessive (CC/CT compared with TT) for Δweight, ΔFFM, ΔWC, and ΔHOMA-IR and codominant (additive effect of the T allele) for ΔFM. For Δweight, ΔFFM, ΔWC, and ΔHOMA-IR, 95% CIs are for comparison with the CC/CT genotype with the low-fat diet. For ΔFM, 95% CIs are for comparison with the genotype with one less T-risk allele within each diet group. Values in the comparison group are the mean values of that genotype-diet group. Differences in Δweight, ΔFFM, ΔFM, ΔWC, and ΔHOMA-IR between one genotype-diet group and the reference group are from linear regressions adjusted for baseline weight (linear; separate effect for men and women), baseline FFM (linear; separate effect for men and women), baseline FM (logarithm transformed; linear; separate effect for men and women), baseline WC (linear; separate effect for men and women), and baseline HOMA-IR (logarithm transformed; linear), respectively, along with age (linear and squared for Δweight, ΔFM, ΔWC; linear for ΔFFM and ΔHOMA-IR), sex, and center (Gaussian random effect). The analysis of ΔHOMA-IR was also performed while additionally adjusting for baseline FFM and ΔFFM or for baseline FFM, baseline FM, ΔFFM and ΔFM; the interaction remained. %E, percentage of energy.
The effects of $TCF7L2$ rs7903146 on weight loss in the present study were larger than any effect found of 43 SNPs in 27 genes (including $FTO$) previously analyzed in relation to weight loss in NUGENOB (2, 3), where the largest significant difference in weight loss between HF and LF diets was 1.7 kg for $KCNJ11$ rs5219 (2). No previous associations between $TCF7L2$ variants and weight loss or related phenotypes were found, but our finding of greater weight loss and increased insulin sensitivity depending on genotype and diet may be important for T2D prevention. Because we previously found effects of a variant in $FTO$ activity depending on genotype and diet may be important for T2D prevention. Because we previously found effects of a variant in $FTO$ (13), and the insulinotropic action of GLP-1 is reduced in $TCF7L2$ rs7903146 T-risk allele carriers (14, 15). Differential GLP-1 release stimulated by fat and carbohydrate together with $TCF7L2$ rs7903146 altering GLP-1 action may explain the observed interaction between the rs7903146 genotype and macronutrient composition. Part of the effects we observed may be related to the lack of GLP-1-mediated effects on satiety and/or adipose tissue metabolism affecting fatty acid handling. Meal ingestion is the primary physiologic stimulus for GLP-1 secretion (37), and, because the response is greater after ingestion of fat than of carbohydrate (21, 22), it is plausible that the adverse effect of risk variants of $TCF7L2$ are more pronounced with an HF than with an LF diet. Recent data indicate that increased endogenous GLP-1 release reduces fatty acid flux from adipose tissue during fasting (18) and increases postprandial lipolysis and FatOx, ie, affects adipose tissue and skeletal muscle metabolism (19). It can be speculated that subjects with the $TCF7L2$ variant may take longer to adapt the postprandial FatOx to the relatively high-fat diet, irrespective of the absence of effects on fasting FatOx, which leads to a less-negative fat energy balance and less weight loss only with the high-fat diet. In addition, GLP-1 may also directly affect lipid absorption at the gut level and may directly affect insulin signaling at the adipose tissue level (20).

It is possible that the participants changed their behaviors during the trial (eg, physical activity and smoking). Because these other behaviors cannot be properly controlled for, we cannot exclude the possibility that such changes might explain part of the effects of the intervention. If change in behavior was different between the 2 diet groups, it could explain the observed interactions. On the other hand, we do find it unlikely that the tendency to make such changes independent of the dietary intervention was skewed between the 2 randomized groups because of the fairly large sample size.

Analyzing the allocated diet (HF or LF) rather than the actual diet may have obscured the associations examined in the present study. Although average weight loss was as expected, which indicated that the participants, on average, complied with the 600 kcal/d energy deficit, the interindividual variation in weight loss suggested that not all subjects complied fully with the energy restriction—some individuals had a higher energy intake and others a lower energy intake than targeted (1). Similarly, concerning compliance with fat and carbohydrate intakes with the HF and LF diets, the differences in changes in blood lipids between diets were as expected, but there were considerable interindividual differences in reported fat and carbohydrate composition within both diets (2). However, the use of reported intakes has its own limitations, eg, potential misreporting, which may challenge the advantage of the randomized design.

In the present study, multiple tests were conducted. One SNP was analyzed in relation to 9 outcomes (dropout from intervention, weight loss, loss of FM, loss of FF, and decreases in WC, insulin secretion, insulin release, REE, and FatOx). Interactions as well as main effects were investigated. In total, 18 associations between the SNP and the outcome variables were analyzed. Because the multiple statistical testing done for the $TCF7L2$ variant is not analogous to repeated testing of the same null hypothesis multiple times and because the choice of analyzing these variants is clearly hypothesis-driven, it can be argued that adjustment for multiple testing is not required. However, if such a correction were made by the very conservative Bonferroni method, only $P$ values <0.0028 would be statistically significant. As an alternative to the Bonferroni correction, we have considered the expected proportions of type I errors, which is a 0.9 false-positive association when analyzing 18 associations.

Apart from $TCF7L2$ rs7903146 the effects of 48 SNPs in 30 genes have been studied in relation to changes in anthropometric and/or metabolic variables during the NUGENOB weight loss intervention (2, 3) (unpublished data for 5 SNPs in 3 genes, 2008 and 2009) and more SNPs will follow. If the analyses of all these SNPs are considered, repeated testing of the same null hypothesis multiple times all nominally significant associations would be expected to be nonsignificant.

Taking into account that the statistical testing has an element of post hoc analysis by choosing the recessive model on the basis of the observed data, our results must be interpreted with caution and be considered only as leads to further studies of the interactions between the function of the $TCF7L2$ gene and diet composition.

Although the CIs of the present study are fairly narrow, it should be noted that, because of the size and thus the power of the study, it is possible that we have missed some clinically relevant effects of $TCF7L2$ rs7903146.

Previous analyses comparing the 2 diets in the NUGENOB study, but not including genetic factors, led to the conclusion that the LF and HF hypoenergetic diet produced similar mean weight loss and that both diets produced favorable changes in fasting insulin and glucose (1). The effect of $TCF7L2$ rs7903146 on weight loss, which was also reflected in loss of FF, loss of FM, and a decrease in WC and the effect we found on HOMA-IR suggest that an LF hypoenergetic diet may be preferable to an HF hypoenergetic diet for healthy obese carriers of the $TCF7L2$ rs7903146 T-risk allele who wish to lose weight and may reduce...
their risk of developing T2D. However, because there have been no previous reports of TCF7L2 rs7903146-macronutrient interactions in relation to weight status or weight loss, these findings need to be replicated in a different population.

The authors’ responsibilities were as follows—TIA, OP, and AA: responsible for initiating the NUGENOB study; TIAS: responsible for obtaining funding; TIAS, PF, OP, AA, WHMS, and DL: responsible for the conception and design of the study; TIAS, PF, OP, AA, JAM, WHMS, J-MO, SR, IAM, DL, EK, and EB: involved in the conduct of the NUGENOB study; PF and SC: responsible for TCF7L2 rs7903146 genotyping; CH: responsible for data management and data harmonization; KG, CH, and TIAS: responsible for the data analysis; KG and TIAS: responsible for drafting the manuscript; and KG, TIAS, PF, SC, OP, AA, JAM, WHMS, J-MO, PA, SR, IAM, DL, EK, and EB: contributed to the data interpretation and revised the manuscript for important intellectual content. No conflicts of interest were declared.

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