**TFAP2B-Dietary Protein and Glycemic Index Interactions and Weight Maintenance after Weight Loss in the DiOGenes Trial**

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**Key Words**
Transcription factor AP-2 beta · Obesity · Body weight changes · Diet · Dietary proteins · Dietary carbohydrates · Glycemic index

**Abstract**

**Background:** TFAP2B rs987237 is associated with obesity and has shown interaction with the dietary fat-to-carbohydrate ratio, which has an effect on weight loss. We investigated interactions between rs987237 and protein-to-carbohydrate ratio or glycemic index (GI) in relation to weight maintenance after weight loss. **Methods:** This study included 742 obese individuals from 8 European countries who participated in the Diet, Obesity, and Genes (DiOGenes) trial, lost \textgreater8% of their initial body weight during an 8-week low-calorie diet and were randomized to one of 5 ad libitum diets with a fixed energy percentage from fat: either low-protein/low-GI, low-protein/high-GI, high-protein/low-GI, or high-protein/high-GI diets, or a control diet for a 6-month weight maintenance period. Using linear regression analyses and additive genetic models, we investigated main and dietary interaction effects of TFAP2B rs987237 in relation to weight maintenance. **Results:** In total, 468 completers of the trial were genotyped for rs987237. High-protein diets were beneficial for weight maintenance in the AA genotype group (67% of participants), but in the AG and GG groups no differences were observed for low- or high-protein diets. On the high-protein diet, carriers of the obesity risk allele (G allele) regained 1.84 kg (95% CI: 0.02; 3.67, \(p = 0.047\)) more body weight per risk allele than individuals on a low-protein diet. There was no interaction effect between rs987237 and GI on weight maintenance. **Conclusion:** TFAP2B rs987237 and dietary protein/carbohydrate interacted to modify weight maintenance. Considering the carbohydrate proportion of the diet, the interaction was different from the previously reported rs987237-fat-to-carbohydrate ratio interaction for weight loss. Thus, TFAP2B-macronutrient interactions might diverge depending on the nutritional state.

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Introduction

In genome-wide association studies, rs987237 located in the transcription factor AP-2 beta gene (TFAP2B) on chromosome 6 has been associated with body mass index (BMI), obesity defined by BMI, and waist circumference [1–3]. In the randomized Nutrient-Gene Interactions in Human Obesity (NUGENOB) trial of obese individuals, we recently reported a small main effect ($p = 0.04$) of rs987237 on weight loss but a stronger interaction effect ($p = 0.00007$) between the SNP and an energy-restricted diet either low or high in fat-to-carbohydrate ratio in relation to weight loss [4]. Individuals with the obesity risk allele (AG and GG genotypes) reduced their weight more on the high-fat/low-carbohydrate diet than individuals without the obesity risk allele (AA genotype), who reduced their weight more on the low-fat/high-carbohydrate diet. Replication of this finding in the 8-week weight loss phase of the Diet, Obesity and Genes (DiOGenes) study showed a similar but nonsignificant pattern for the interaction between rs987237 and dietary fat, despite the different study design using a low-calorie diet (LCD) for all participants [4].

TFAP2B encodes a transcription factor that is mainly expressed in adipose tissue, where its regulation of adipocyte function and adipokine expression is believed to be the functional link to obesity [1, 5]. The mechanisms by which TFAP2B potentially interact with dietary macronutrients are unknown, but observations from the NUGENOB trial indicate an interplay between TFAP2B function and the metabolism of dietary fat or carbohydrates. This interaction might be specific to weight loss under energy restriction or may apply to other dynamic or stable weight conditions. The weight loss phase in the DiOGenes study was followed by a weight maintenance phase in a two-by-two factorial design, in which participants received 1 of 5 ad libitum diets with low or high protein content and low or high glycemic index (GI) or a control diet [6]. The diets were standardized to a moderate fat content but with different protein-to-carbohydrate ratios in the low-protein and high-protein groups. The similar participant characteristics in the NUGENOB and DiOGenes studies as well as the differences in macronutrient proportion and study design encourage further examination of TFAP2B-macronutrient interactions in the DiOGenes weight maintenance setting.

The aim of this study was to investigate rs987237 and other SNPs in the TFAP2B loci and their interaction with dietary protein and GI in relation to weight maintenance and changes in body composition in the DiOGenes trial. We hypothesized that any interaction between rs987237 and low-/high-protein diets, i.e. low/high protein-to-carbohydrate ratio, would show the same pattern as the rs987237-fat-to-carbohydrate ratio interaction in the NUGENOB trial, considering the low/high carbohydrate proportions of the randomized diets in the DiOGenes and NUGENOB studies, respectively.

Materials and Methods

Dietary Intervention

The DiOGenes trial is a multicenter European study with the objective to investigate the effects of protein and GI on weight maintenance after weight loss in obese individuals. The study has been previously described in detail elsewhere [7]. Briefly, families with one or two parents aged 18–65 years with a BMI of 27–45 and one or more healthy child aged 5–17 years were recruited to the study at 1 of 8 European study centers. The present study includes the parents from these families. Exclusion criteria were characteristics that could potentially influence the results, such as a change in weight of more than 3 kg within 2 months prior to the study, pregnancy, and medications or certain diseases. In total, 934 participants passed the criteria and started an LCD regimen for 8 weeks. The LCD contained products from Modifast® (Nutrition et Santé) and an additional maximum of 400 g vegetables, which altogether provided approximately 880 kcal/day with an energy percent from fat of 20%, 54% from carbohydrates, and 26% from protein. The goal was to lose at least 8% of body weight. A total of 774 participants achieved a weight loss of ≥8% of their initial body weight and were randomized to 1 of 3 ad libitum dietary regimens, all containing 25–30 energy percent from fat. The 5 diets consisted of low-protein/low-GI, low-protein/high-GI, high-protein/low-GI, and high-protein/high-GI groups as well as a control group according to the national dietary guidelines of the respective country. The difference in protein intake between the low- and high-protein groups was targeted at 12% of total energy from protein, and the difference between the low- and high-GI diets was targeted at 15 GI units. The families received dietary counseling including recipes, cooking instruction, and behavioral advice every other week during the first 6 weeks and monthly thereafter up to 6 months in 6 study centers and 12 months in 2 study centers. The 2 centers with a longer follow-up applied free-of-charge shopping in laboratory supermarkets similar to commercial grocery stores but run by the study center. The present analysis includes follow-up for 6 months. The main results for the DiOGenes trial showing beneficial effects of a diet high in protein and/or with a low GI on weight maintenance have been reported previously [6].

Anthropometric Measurements

Weight was measured at baseline and at every dietary counseling session (up to 9 times during the follow-up period), and waist and body composition were measured before and after the 8-week weight loss phase and the 6-month weight maintenance phase. Waist circumference was measured between the bottom of the ribs and the top of the hip bone, and body composition was measured by dual energy X-ray absorptiometry (Lunar Radiation, Madison, Wisc., USA) at 2 centers, by bioelectrical impedance analysis (Quad Scan 4000; Bodystat, Douglas, Isle of Man, UK) at 5 centers.
and by dual energy X-ray absorptiometry and bioelectrical impedance analysis at 1 center. Measurements of body composition were unsuccessful or missing in 96 participants in the present study.

**Gene Loci Selection and Genotyping**

DNA was extracted from EDTA blood buffy coats stored at −80°C by KBioscience. Genotyping was performed at the Centre National de Génotypage (Évry, France) using the Illumina 660k quad chip, which covered TFAP2B rs987237 (chromosome 6, position 50911009). Autosomal SNPs were included if they had a call rate >98% (16,706 SNPs excluded), were in Hardy-Weinberg equilibrium (p < 10^{-7}, 47 SNPs excluded), and had a minor allele frequency (MAF) >98% (16,706 SNPs excluded), leaving a total of 500,745 autosomal SNPs passing quality control criteria. In addition to rs987237 that was of primary interest for this study, we included genotyped SNPs within ±40 kb from rs987237 with a MAF >5%. After the exclusion of 2 SNPs (rs9381908 and rs2636900) in high linkage disequilibrium (LD) (r^2 > 0.90) with another selected SNP (rs2817402), 6 SNPs available from the 660k quad chip were included: rs2143081, rs2206272, rs2635727, rs4715209, rs6930924, and rs9367415. Reasons for missing genotyping in individuals were little or low quality of DNA or exclusions after quality control (duplicates, sex discrepancy, non-European, etc.). LDs between SNPs included in the study, their individual MAF, and Hardy-Weinberg equilibrium calculated by Fisher’s exact test are reported in online supplementary table 1 (www.karger.com/doi/10.1159/000353591).

**Statistical Analysis**

Differences in baseline BMI and TFAP2B rs987237 genotype distribution between completers and noncompleters of the weight loss phase and the weight maintenance phase were computed by two-sample t test for BMI and by Pearson χ^2 statistics for genotype distribution.

We investigated the main SNP effects and SNP-diet interactions in relation to changes in weight, waist circumference, fat mass, and fat-free mass. The 5 randomized diets were recoded into 3 indicator variables (dichotomous, 0/1), accounting for indications of the high-protein, high-GI, and control diets, respectively. Thus, SNP-diet interactions were assessed for low versus high dietary protein or low versus high GI. Hence, no protein-GI interactions were analyzed. The SNP-diet interaction variables were formed as standard product based on the indicator variables and the SNP variable. We applied a standard linear regression model based on the assumption of an additive genetic effect (SNP coded as 0/1/2) and adjusted for sex, family structure (in categories), intervention center (in categories), baseline age, baseline BMI, weight change during the LCD phase, length of the ad libitum dietary intervention phase (linear and quadratic), and measures of the outcome variable at the beginning of the intervention. For analyses of weight change, we additionally applied a longitudinal model, using up to 9 measurements of weight for an individual during the weight maintenance period at weeks 0, 2, 4, 6, 10, 14, 18, 22, and 26 (approximation on the individual level). The fitting procedure was quasi-least squares, which is based on the generalized estimating equations approach, as implemented in Stata through the xtols command [8]. Within individual weight change-related covariance, matrices were fitted based on the Markovian structure, and robust standard errors were estimated. These models were similar to the models using two measures of weight, except that the fitted effects of included variables – when applicable in the sense of not already being the case by definition – were modeled as (linearly) dependent on time since start of the ad libitum dietary intervention to facilitate sensible interpretations.

We visualized the results graphically (fig. 1) through adjusted means over genotype and protein groups based on the underlying interaction model. In order to display only the protein-related effects, we fixed the prediction model to pseudo-low GI individuals.
not being part of the control group, whereas all other variables were set either to their mean values (continuous variables) or to be proportionally averaged over groups (categorical variables). The interaction effect is then the observed change of the estimated protein/carbohydrate effect for each added G allele to the genotype with AA as the reference. Two-sided p values < 0.05 were considered significant. Statistical analyses were performed in Stata 12.1 (StataCorp LP, College Station, Tex., USA; www.stata.com).

Results

In total, 548 participants completed the weight maintenance phase. Characteristics of successfully genotyped completers of the weight loss phase (653 of 774 completers, 84%) and genotyped completers of the weight maintenance phase (468 of 548 completers, 85%) are shown in table 1. There was no significant difference for baseline BMI or TFAP2B rs987237 genotype distribution between completers and noncompleters of the weight loss phase (p > 0.05, data not shown). Weight loss was on average 11.0 kg (standard deviation, SD = 3.6) during the weight loss phase, and weight change after the weight loss phase till the end of the weight maintenance phase ranged from 0.5 kg (SD = 6.6) weight loss in the high-protein/low-GI group to 2.3 kg (SD = 4.8) weight gain in the low-protein/high-GI group. Dietary intake at baseline, before the weight loss phase, and during the weight maintenance phase in participants randomized to low- or high-protein diets are shown in table 2.

TFAP2B rs987237 interacted with protein intake in relation to weight maintenance (p = 0.047). The adjusted mean weight change among AA allele carriers (n = 251) was 1.5 kg (95% CI: 0.7; 2.2) if they had been assigned to a low-protein diet, but –0.9 kg (95% CI: –1.7; 0.0) if they had been assigned to a high-protein diet (fig. 1). This beneficial effect of a high-protein intake on weight was not shown among carriers of the G allele; however, the GG genotype group was very small (n = 12). Based on linear regression analysis, participants in the randomized high-protein groups gained 1.84 kg (95% CI: 0.02; 3.67) more weight per G allele, i.e. per obesity risk-allele, compared to participants in the low-protein groups (table 3). In longitudinal analyses of the weight maintenance period, the rs987237-protein interaction was somewhat weaker (p = 0.07) than in analyses using weight only at the start and the end of the weight maintenance period. No main effects of rs987237 or interactions with diet were significant in rela-
This study examined the interaction effect of TFAP2B rs987237 and an ad libitum diet either low or high in protein or low or high in GI in relation to weight regain and changes in body composition over 6 months after an initial 8-week weight loss period. TFAP2B rs987237 and dietary protein interacted as participants without the previously known TFAP2B risk allele for obesity (AA genotype) [1–3] maintained their weight loss on the high-protein diet but not on the low-protein diet, while this effect was not observed among participants with the obesity risk allele (AG and GG genotypes). However, the gene-diet effect was small, with p values ranging from 0.047 to 0.070 depending on the statistical model used. Additionally, the small sample sizes of AG (n = 110, 29%) and GG (n = 12, 3%) genotypes raise uncertainty about the effects in these groups.

This study used a hypothesis-based approach initiated after results had been obtained from the NUGENOB study, which is a 10-week randomized trial of 2 energy-restricted diets either low or high in fat-to-carbohydrate ratio. In that study, participants with the rs987237 AA genotype lost more weight on the low-fat/high-carbohydrate diet, while the AG and GG groups lost more weight on the high-fat/low-carbohydrate diet (p for interaction = 0.00007) [4]. Considering the direction of interaction between rs987237 and the carbohydrate proportion, the results of the DiOGenes study differ from those of the NUGENOB trial. In the DiOGenes trial, participants with the AA genotype maintained their weight loss better on the high-protein/low-carbohydrate diet than on the low-protein/high-carbohydrate diet. Whilst acknowledging the much weaker gene-diet interaction in the present study, we did not expect to find an interaction that was in the opposite direction of our previous findings from the NUGENOB study. Our observations indicate that the interaction between rs987237 and diet is different under energy restriction and ad libitum energy intake conditions. Nonetheless, combining the results from the DiOGenes and NUGENOB studies suggests that rs987237 may not interact solely with the carbohydrate proportion of a diet. As we did not find any significant effects of interactions between rs987237 and GI, the carbohydrate quality does not seem to be of substantial importance for the interaction with rs987237; however, the interaction between dietary carbohydrates, fat, protein, and rs987237 might interplay in a more complex way. However, little is known about the functional effects of TFAP2B, except that it has been associated with type 2 diabetes [9] and is mainly expressed in adipose tissue, and that the overexpression of TFAP2B results in triglyceride accumulation and insulin resistance [10].

Since the discovery of the fat mass and obesity-associated gene (FTO) in 2007, it has been the locus with the strongest association with obesity-related traits [2, 11, 12]. Compared to TFAP2B rs987237 that is associated with a 0.1 higher BMI per risk allele, FTO (rs1558902) is associated with a 0.4 higher BMI per risk allele [2]. Not surprisingly, FTO is the gene that has been most frequently investigated for gene-diet interaction in obesity, but only a few studies of adults have reported on the interaction with

### Table 2. Mean (SD) dietary intake before the 8-week LCD weight loss phase and during weeks 4 and 26 of the 6-month weight maintenance phase for randomized low- and high-protein diets

<table>
<thead>
<tr>
<th></th>
<th>Randomized low-protein diets (n = 161)</th>
<th>Randomized high-protein diets (n = 197)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake, kcal/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2,282 (796)</td>
<td>2,160 (708)</td>
</tr>
<tr>
<td>Week 4</td>
<td>1,451 (534)</td>
<td>1,489 (523)</td>
</tr>
<tr>
<td>Week 26</td>
<td>1,637 (620)</td>
<td>1,586 (554)</td>
</tr>
<tr>
<td>Protein, E%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>17.4 (3.9)</td>
<td>16.7 (3.8)</td>
</tr>
<tr>
<td>Week 4</td>
<td>17.9 (4.8)</td>
<td>21.9 (5.0)</td>
</tr>
<tr>
<td>Week 26</td>
<td>17.5 (4.8)</td>
<td>21.7 (5.0)</td>
</tr>
<tr>
<td>Carbohydrates, E%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>42.8 (7.7)</td>
<td>44.1 (7.8)</td>
</tr>
<tr>
<td>Week 4</td>
<td>51.3 (10.9)</td>
<td>45.7 (7.9)</td>
</tr>
<tr>
<td>Week 26</td>
<td>50.6 (9.3)</td>
<td>45.1 (7.3)</td>
</tr>
<tr>
<td>Fat, E%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>38.0 (7.1)</td>
<td>36.7 (7.2)</td>
</tr>
<tr>
<td>Week 4</td>
<td>29.4 (9.2)</td>
<td>31.0 (8.2)</td>
</tr>
<tr>
<td>Week 26</td>
<td>31.4 (8.6)</td>
<td>31.7 (8.0)</td>
</tr>
</tbody>
</table>

E% = Energy-percent.

Participants were included if they completed the LCD phase and reached the target of at least 8% weight loss, and if genotype and dietary intake data were available. Out of the 358 participants included in the analysis, 74 were not included in the week 26 analysis due to dropout or missing dietary intake data. Alcohol intake was 2.2 E% (SD = 3.5) at baseline and lower during the intervention.
dietary macronutrients and have found contradictive results [13–20]. Three cross-sectional or longitudinal studies reported an interaction between FTO and total or saturated fat on BMI [14, 15] or waist circumference [16], while another cross-sectional study [17] and two randomized trials did not support these findings [13, 18]. Dietary protein and FTO interacted with changes in body composition in a 2-year randomized trial [18], but no interaction was found for weight maintenance in the DiOGenes study [19], for weight changes in a longitudinal study [20], or for BMI in two cross-sectional studies [14, 17]. These inconsistencies for FTO-macronutrient interactions in obesity could be accounted for by differences in study designs and small sample sizes for gene-diet effects, similar to the TFAP2B-macronutrient interaction.

This study includes the use of a large randomized study with dietary regimens highly relevant to test for their interaction with TFAP2B in relation to weight changes. The study had a 29% dropout rate [6], which is comparable to that in other dietary intervention trials [21], but it had also incomplete genotyping. Whilst unsuccessful genotyping in 15% of participants reduced the sample size and statistical power, it is most likely to have been randomly distributed among participants and thus should not have caused any bias. Fat mass and fat-free mass had additional missing samples, which, together with potential misclassification of these variables in most centers due to their use of bioelectrical impedance analysis rather than dual energy X-ray absorptiometry to assess body composition, might have contributed to the null results for fat mass and fat-free mass despite the rs987237-macronutrient interaction with weight. However, a strong interaction with weight, but not waist, was also shown in the NUGENOB trial (though in the opposite direction to the results from this study). Participant characteristics in the two studies were very similar in terms of demographics, age, BMI, and baseline dietary intake [4]. The results for TFAP2B-macronutrient interactions in the two studies may therefore be attributed to the specific designs and dietary regimens used or to chance findings.

In conclusion, this study showed a borderline significant interaction between TFAP2B rs987237 and dietary protein/carbohydrates in relation to weight maintenance after weight loss in individuals with obesity. These results differed in direction compared to our recently reported interaction between TFAP2B rs987237 and dietary fat/carbohydrates and weight loss. However, the two studies differed in their design and dietary regimen. Combined, the results from these two studies indicate that TFAP2B might interact with dietary macronutrients in relation to weight changes, but the specific mode of interaction and the physiological mechanisms remain to be clarified.

Acknowledgements

The DiOGenes project was supported by the European Commission Food Quality and Safety Priority of the Sixth Framework Program (FP6-2005-513946), and the present study was further supported by the Nordea Foundation, as part of the OPUS project (Optimal well-being, development and health for Danish children through a healthy New Nordic Diet), and the Danish Strategic Research Council (GENDINOB project; to T.S. and L.A.).

Table 3. Main effects of TFAP2B rs987237 and interactions with diet in relation to changes in weight, waist circumference, fat mass, and fat-free mass during the 6-month weight maintenance phase

<table>
<thead>
<tr>
<th>End point</th>
<th>n</th>
<th>Main effect</th>
<th>p</th>
<th>SNP-dietary protein</th>
<th>p</th>
<th>SNP-GI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Weight</td>
<td>468</td>
<td>0.09 (–0.76; 0.93)</td>
<td>0.8</td>
<td>1.84 (0.02; 3.67)</td>
<td>0.047</td>
<td>0.80 (–1.04; 2.65)</td>
<td>0.4</td>
</tr>
<tr>
<td>Δ WC</td>
<td>442</td>
<td>–0.55 (–1.57; 0.47)</td>
<td>0.3</td>
<td>2.17 (–0.06; 4.40)</td>
<td>0.06</td>
<td>–0.83 (–3.06; 1.40)</td>
<td>0.5</td>
</tr>
<tr>
<td>Δ FM</td>
<td>372</td>
<td>–0.25 (–1.01; 0.51)</td>
<td>0.5</td>
<td>1.37 (–0.31; 3.05)</td>
<td>0.1</td>
<td>0.87 (–0.82; 2.56)</td>
<td>0.3</td>
</tr>
<tr>
<td>Δ FFM</td>
<td>372</td>
<td>0.22 (–0.29; 0.72)</td>
<td>0.4</td>
<td>0.08 (–1.04; 1.19)</td>
<td>0.9</td>
<td>–0.18 (–1.31; 0.95)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

WC = Waist circumference; FM = fat mass; FFM = fat-free mass.

β (95% CI) per G allele derived from linear regression using an additive genetic model, adjusted for sex, family structure, intervention center, baseline age, baseline BMI, weight change during the LCD phase, length of the LCD phase (linear and quadratic), and measures of the outcome variable at start of the intervention.

β (95% CI) per G allele on a high-protein versus low-protein diet or high-GI versus low-GI diet derived from linear regression using the rs987237 main effects model with additional inclusion of 3 dietary indicator variables for dietary protein, GI, and control group, and interaction terms for rs987237 and each dietary indicator variable.

DOI: 10.1159/000353591 Hum Hered 2013;75:213–219
References


