Genetic variant in the glucose transporter type 2 is associated with higher intakes of sugars in two distinct populations

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Eny KM, Wolever TM, Fontaine-Bisson B, El-Sohemy A. Genetic variant in the glucose transporter type 2 is associated with higher intakes of sugars in two distinct populations. Physiol Genomics 33: 355–360, 2008. First published March 18, 2008; doi:10.1152/physiolgenomics.00148.2007.—Glucose sensing in the brain has been proposed to be involved in regulating food intake, but the mechanism is not known. Glucose transporter type 2 (GLUT2)-null mice fail to control their food intake in response to glucose, suggesting a potential role for this transporter as a glucose sensor in the brain. Here we show that individuals with a genetic variation in GLUT2 (Thr110Ile) have a higher daily intake of sugars in two distinct populations. In the first population, compared with individuals with the Thr/Thr genotype, carriers of the Ile allele had a significantly higher intake of sugars as assessed from 3-day food records administered on two separate visits (visit 1: 112 ± 9 vs. 86 ± 4 g/day, P = 0.01; visit 2: 111 ± 8 vs. 82 ± 4 g/day, P = 0.003), demonstrating within-population reproducibility. In a second population, carriers of the Ile allele also reported consuming a significantly greater intake of sugars (131 ± 5 vs. 115 ± 3 g/day, P = 0.007) over a 1-mo period as measured from a food frequency questionnaire. GLUT2 genotypes were not associated with fat, protein, or alcohol intake in either population. These observations were consistent across older and younger adults as well as among subjects with early Type 2 diabetes and healthy individuals. Taken together, our findings show that a genetic variation in GLUT2 is associated with habitual consumption of sugars, suggesting an underlying glucose-sensing mechanism that regulates food intake.

Glucose-sensing mechanisms have been described to be similar to glucose sensing in the pancreatic β-cell (25). GLUT2-null mice, which express a GLUT1 transgene in their β-cells using the rat insulin promoter (ripglut1/;glut2−/− mice), have been found to consume more food than their wild-type littermates (7). In response to intracerebroventricular administration of glucose, wild-type mice decrease their food intake appropriately, whereas GLUT2-null mice do not alter their food intake (7). The impaired regulation of food intake among GLUT2-null mice corresponded to abnormal regulation of neuropeptide Y (NPY) and proopiomelanocortin (POMC) gene expression, important hypothalamic neuropeptides that are orexigenic and anorexigenic, respectively (7).

In accordance with the role of GLUT2 in glucose-induced insulin secretion, a common single nucleotide polymorphism (rs5400) in the GLUT2 gene (SLC2a2) resulting in a threonine to isoleucine amino acid substitution at codon 110 (Thr110Ile) has been associated with risk of Type 2 diabetes (8, 17, 18, 37). However, the role of GLUT2 in food intake regulation in humans is not known. Since glucose sensing in the brain has been described to be similar to glucose sensing in the pancreatic β-cell (25), we investigated whether the Thr110Ile polymorphism is associated with differences in the consumption of dietary sugars or other available carbohydrates that affect blood glucose concentrations. Because of the importance of replicating genotype-phenotype associations (11), we compared measurements repeated within a population and between two distinct populations using two different methods of dietary assessment.

MATERIALS AND METHODS

Population 1. For the first study population, we used baseline data collected on participants of the Canadian trial of Carbohydrates in Diabetes multicenter intervention study, described in detail elsewhere (12). Subjects were recruited from five centers across Canada (Edmonton, London, Toronto, Montreal, and Sherbrooke) over a 1 yr period (2002–2003). Subjects included 127 men (n = 60) and women (n = 67) who had early Type 2 diabetes, with near-normal HbA1c (6.2 ± 0.6%), and who were considered not to require medications. Therefore, subjects were instructed to follow the Canadian Diabetes...
Association dietary guidelines and were excluded if they were using any hypoglycemic, antihyperglycemic, or oral steroid drugs or experienced a major cardiovascular event or surgery in the past 6 mo. Of the 127 subjects, one subject’s genotype remained undetermined, four subjects had incomplete baseline dietary data, and 22 individuals had missing data on potential confounders, leaving 100 subjects for the final analyses. According to two sets of 3-day food records, all subjects reported consuming between 800 and 3,500 kcal/day for women and 800 and 4,000 kcal/day among men, and, therefore, no exclusions were made for potential under- or over-reporting. The study consisted of men (n = 50) and women (n = 50), between the ages of 42–75 yr, with an average body mass index (BMI) of 30.7 ± 4.2 kg/m² (mean ± SD). All subjects were diagnosed according to the Canadian Diabetes Association criteria (28). The study protocol was approved by the ethics review boards of each participating institution, and informed consent was obtained from all subjects.

Population 1. This population is part of the Toronto Nutrigenomics and Health Study, which is a cross-sectional examination of young men and women between 20 and 29 yr of age recruited from the University of Toronto campus. Subjects included 720 free-living young men (n = 224) and women (n = 496) with an average BMI of 22.5 ± 3.3 kg/m² (mean ± SD). Participants were recruited between September 2004 and May 2007, and women who were pregnant or breast feeding were excluded from the study. For the current analyses we also excluded subjects who may have underreported (<800 kcal/day) or overreported (>3,500 kcal/day female, >4,000 kcal/day male) their energy intakes (n = 53) or reported following a special diet that restricted carbohydrates, fat, or calories (n = 36). Smokers (n = 43) were also excluded from the analysis since smoking status has been associated with carbohydrate intake patterns (29). One subject who had Type 1 diabetes was excluded from the analysis so we could examine the association in a diabetes-free population. The final sample size consisted of 182 men and 405 women. The study was approved by the Research Ethics Board at the University of Toronto, and informed consent was obtained from all subjects.

Dietary assessment. To assess habitual intake of food and beverages we used two different methods of dietary assessment: 3-day food records with population 1 and a food frequency questionnaire (FFQ) with population 2. For population 1, each subject was instructed by each center’s research dietitian on how to complete a 3-day food record, including 2 weekdays and 1 weekend day (visit 1). To determine the reproducibility within this population we examined a second set of 3-day food records that were collected 2 wk later (visit 2). Each food record was coded by a dietitian using an in-house computer program, which is based on the Canadian Condensed Nutrient File (1). Nutrient intakes were averaged over the 3 days for each set of 3-day food records.

In population 2, each subject completed a 196-item self-administered FFQ to assess habitual food intake over the past month. To capture Canadian dietary habits, the FFQ was modified from the Willett questionnaire (15), with the addition of 26 food items: six fruits; seven vegetables; six cereals, breads; four beverages; and three tree nuts. The final FFQ included 12 items on vitamins and other dietary supplements and 184 items on foods and beverages. Additional modifications to the questionnaire included prompts for certain food items to clarify sugar content, whole grain content, and beverage serving size. To improve the measurement of self-reported food intake, each subject was given instructions on how to complete the FFQ using visual aids. In addition to total sugars (defined as mono- and disaccharides), the nutrient database for the FFQ also provided information on intake of sucrose, maltose, lactose, fructose, and glucose. To examine the type of foods consumed contributing to sugar, consumption of daily servings from specific food groups containing sugars were compared. Each food item response was first converted into daily servings and subsequently summed within its respective food group. Total fruit, which included fruit juice and fruit, and dairy products corresponded to the original sections of food groups in the FFQ. Sweets included chocolates, candy, jams, and baked goods. Sweetened beverages included carbonated soft drinks, fruit drinks (not fruit juice), and sport drinks.

Anthropometrics and physical activity. Anthropometric measurements including height, weight, and waist circumference were measured, and BMI (kg/m²) was calculated. Modifiable activity was measured by questionnaire and expressed as metabolic equivalent (MET) hours per week, which represents both leisure and occupational activity, not including sedentary hours of sleeping or sitting. One MET is equal to 1 kcal expended per kg body wt per hour sitting at rest (2).

Laboratory analyses. Each subject had venous blood drawn after a 12-h overnight fast to measure glucose and insulin concentrations using standard laboratory procedures.

Genotyping. DNA was isolated from whole blood using the GenomicPrep Blood DNA Isolation kit (Amersham Pharmacia Biotech, Piscataway, NJ). The Thr110Ile polymorphism (rs5400) was detected by using a TaqMan allelic discrimination assay (ABI no. 5304218G, 10) from Applied Biosystems (Foster City, CA), with real-time PCR on an ABI 7000 Sequence Detection System. PCR conditions were 95°C for 10 min and 40 cycles of 95°C for 5 s and 60°C for 1 min.

Statistical analysis. All statistical analyses were performed using SAS Statistical Analysis Software (version 9.1; SAS Institute, Cary, NC). The χ²-test with 1 degree of freedom was used to determine if genotypes were in Hardy-Weinberg equilibrium. Unpaired t-tests assuming unequal variances were used to compare subject characteristics and dietary intakes between genotypes, and Wilcoxon tests were used if variables were skewed. The χ²-test was used for categorical variables. Multiple linear regression adjusted for age, sex, BMI, and physical activity as continuous variables was used to test for differences in nutrients and food groups consumed. Skewed variables were log or square root transformed. The GENMOD procedure using the normal distribution, log link function was used for the food sources of sugars analysis on untransformed data when the error term of the model was not normally distributed. All P values from nonparametric tests conducted for skewed variables are presented in the tables; however, the means ± SE or SD are displayed from parametric tests conducted to facilitate interpretation. Significant P values are two-sided and <0.05.

RESULTS

Population 1. The genotype distribution of the study population did not deviate from Hardy-Weinberg equilibrium (P = 0.32) and consisted of 80 individuals with the Thr/Thr genotype, 17 heterozygotes, and 3 individuals with the Ile/Ile genotype. Carriers of the Ile allele were combined for all analyses. None of the subject characteristics differed between the two genotype groups (Table 1).

To determine the difference between subjects homozygous for the Thr allele and carriers of the Ile allele we compared dietary intakes from two sets of 3-day food records collected 2 wk apart, which enables us to assess within-population reproducibility. All analyses were adjusted for age, sex, BMI, and physical activity. Compared with subjects homozygous for the Thr allele, carriers of the Ile allele consistently reported consuming a greater amount of sugars on visit 1 (112 ± 9 vs. 86 ± 4 g/day, P = 0.01) and visit 2 (111 ± 8 vs. 82 ± 4 g/day, P = 0.003) (Table 2). Ile carriers also consumed more available carbohydrates on both visits (visit 1: 246 ± 14 vs. 203 ± 7 g/day, P = 0.009; and visit 2: 255 ± 14 vs. 202 ± 7 g/day, P = 0.001) compared with individuals homozygous for the Thr allele. However, this was largely attributed to the greater intake in sugars since the difference in starch intake was not signifi-
The higher energy intake observed on visit 1 (134 ± 9 vs. 117 ± 5 g/day, P = 0.10) but was higher among Ile carriers on visit 2 (144 ± 10 vs. 120 ± 5 g/day, P = 0.03). Intake of protein, fat, and alcohol did not differ between the two groups at either visit (Table 2).

The higher energy intake observed on visit 1 (2,111 ± 110 vs. 1,843 ± 55 kcal/day, P = 0.03) can be attributed to the higher consumption of sugars. Similar results were observed for unadjusted analyses (data not shown).

Population 2. To assess the between-population reproducibility of our results we examined a second population. The genotype distribution in this population was also in Hardy-Weinberg equilibrium (P = 0.68) and consisted of 478 individuals with the Thr/Thr genotype, 102 heterozygotes, and 7 individuals with the Ile/Ile genotype. Table 1 shows the comparison of subject characteristics between genotypes. Compared with individuals with the Thr/Thr genotype, carriers of the Ile allele had a higher BMI (P = 0.02). Adjusting for age, sex, and physical activity did not materially alter this result (data not shown).

For this population, we assessed dietary intake using a FFQ and compared individuals homozygous for the Thr allele with carriers of the Ile allele after adjusting for age, sex, BMI, and physical activity. Consistent with the first population, only intake of sugars was greater among carriers of the Ile allele compared with those with the Thr/Thr genotype (131 ± 5 vs. 115 ± 3 g/day, P = 0.007). The FFQ nutrient database enabled a further analysis of the types of sugars consumed. As shown in Table 3, we found that carriers of the Ile allele consumed more sucrose (55 ± 3 vs. 47 ± 1 g/day, P = 0.01), fructose (28.0 ± 1.3 vs. 25.4 ± 0.7 g/day, P = 0.04), and glucose (26.0 ± 1.2 vs. 23.7 ± 0.6 g/day, P = 0.03) than subjects who

Table 1. Comparison of subject characteristics by GLUT2 genotype for population 1 and population 2

<table>
<thead>
<tr>
<th>n</th>
<th>Age</th>
<th>Sex %Female</th>
<th>Weight, kg</th>
<th>Height, cm</th>
<th>BMI, kg/m²</th>
<th>Waist circumference, cm</th>
<th>Physical activity, MET-h/wk</th>
<th>Fasting glucose, mmol/l*</th>
<th>Fasting insulin, pmol/l†</th>
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<td>Population 1</td>
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<tr>
<td>Thr/Thr</td>
<td>152</td>
<td>54.6 ± 9.1</td>
<td>84 ± 14</td>
<td>166 ± 16</td>
<td>30.5 ± 4.1</td>
<td>101 ± 12</td>
<td>2.5 ± 1.8</td>
<td>7.5 ± 1.1</td>
<td>56.2 ± 40.6</td>
</tr>
<tr>
<td>Thr/Ile</td>
<td>100</td>
<td>57.7 ± 7.3</td>
<td>88 ± 13</td>
<td>168 ± 9</td>
<td>31.2 ± 4.4</td>
<td>104 ± 11</td>
<td>2.7 ± 1.5</td>
<td>7.5 ± 1.4</td>
<td>63.8 ± 38.4</td>
</tr>
<tr>
<td>Ile/Ile</td>
<td>37</td>
<td>53.1 ± 7.6</td>
<td>106 ± 14</td>
<td>166 ± 12</td>
<td>35.5 ± 5.0</td>
<td>23.2 ± 3.8</td>
<td>3.5 ± 2.7</td>
<td>8.1 ± 2.1</td>
<td>5.3 ± 2.0</td>
</tr>
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Table 2. Comparison between individuals homozygous for the Thr allele and carriers of the Ile allele for 3-day average daily intakes of macronutrients for population 1 on visit 1 and visit 2 taken 2 wk apart

<table>
<thead>
<tr>
<th>Visit</th>
<th>n</th>
<th>Calories, kcal/day</th>
<th>Protein, g/day</th>
<th>Fat, g/day</th>
<th>Total carbohydrate, g/day</th>
<th>Fiber, g/day</th>
<th>Available carbohydrate, g/day</th>
<th>Starch, g/day</th>
<th>Sugars, g/day</th>
<th>Cholesterol, mg/day</th>
<th>Alcohol, g/day</th>
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<tr>
<td>Thr/Thr</td>
<td>80</td>
<td>1,843 ± 55</td>
<td>91 ± 3</td>
<td>70 ± 3</td>
<td>224 ± 8</td>
<td>20.6 ± 0.9</td>
<td>203 ± 7</td>
<td>117 ± 5</td>
<td>86 ± 4</td>
<td>293 ± 13</td>
<td>5.0 ± 1.1</td>
</tr>
<tr>
<td>Thr/Ile</td>
<td>20</td>
<td>2,111 ± 110</td>
<td>92 ± 2</td>
<td>73 ± 2</td>
<td>271 ± 15</td>
<td>24 ± 0.9</td>
<td>247 ± 4</td>
<td>120 ± 5</td>
<td>112 ± 9</td>
<td>302 ± 13</td>
<td>6.0 ± 1.0</td>
</tr>
<tr>
<td>Ile/Ile</td>
<td>20</td>
<td>2,082 ± 101</td>
<td>95 ± 5</td>
<td>76 ± 3</td>
<td>280 ± 16</td>
<td>25 ± 1.0</td>
<td>241 ± 4</td>
<td>134 ± 9</td>
<td>118 ± 8</td>
<td>307 ± 30</td>
<td>5.3 ± 2.0</td>
</tr>
</tbody>
</table>

Table 3. Comparison between individuals homozygous for the Thr allele and carriers of the Ile allele for 1-mo average daily intakes of macronutrients for population 2

<table>
<thead>
<tr>
<th>Thr/Thr</th>
<th>478</th>
<th>Calories, kcal/day</th>
<th>Protein, g/day</th>
<th>Fat, g/day</th>
<th>Total carbohydrate, g/day</th>
<th>Fiber, g/day</th>
<th>Available carbohydrate, g/day</th>
<th>Starch, g/day</th>
<th>Sugars, g/day</th>
<th>Cholesterol, mg/day</th>
<th>Alcohol, g/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thr/Ile + Ile/Ile</td>
<td>109</td>
<td>2,082 ± 61</td>
<td>86 ± 2</td>
<td>66 ± 1</td>
<td>279 ± 9</td>
<td>22.0 ± 0.9</td>
<td>257 ± 8</td>
<td>125 ± 5</td>
<td>113 ± 5</td>
<td>252 ± 12</td>
<td>6.4 ± 0.7</td>
</tr>
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Values shown are means ± SE adjusted for age, sex, BMI, and physical activity. P values from log transformed analyses are displayed for fiber, sugars, and alcohol and on both visits.
GLUT2-null mice (7). Compared with wild-type mice, GLUT2-sensing by GLUT2 has been shown to regulate food intake in individual differences in cravings for sugars. These observations in mice suggest that GLUT2 acts as a glucose sensor that regulates neuropeptides involved in food intake regulation.

Given that GLUT2-dependent glucose sensors have been identified in the portal vein (10) and brain (24), our observations may reflect glucose sensing in either or both of these regions (25). In the human brain, GLUT2 mRNA and protein have been localized in the hypothalamus (34). Analyses from rodents have further identified the expression of GLUT2 in specific nuclei of the hypothalamus involved in glucose sensing such as the paraventricular nucleus, lateral hypothalamic area, and arcuate nucleus, as well as the nucleus tractus solitarius of the brainstem (5, 6, 19). Despite the functional evidence (7) and tissue distribution of GLUT2 (5, 6, 19, 34) that implicate this transporter in glucose sensing in the brain, physiological evidence from human studies has been lacking. One concern related to the physiological relevance of GLUT2 in the brain has been whether GLUT2 senses glucose in these regions due to its high Km and the lower glucose concentrations found in the brain. However, GLUT2 mRNA has been detected in the area postrema (21), which is one of the eight circumventricular organs that lacks a blood brain barrier (13). Thus, in regions such as the area postrema, GLUT2 can be exposed to physiological levels of circulating glucose.

Although genetic association studies have linked the Thr110Ile polymorphism to risk of Type 2 diabetes (8, 17, 37) the functional properties of this polymorphism have not been fully characterized. Because this polymorphism is in strong linkage disequilibrium with two polymorphisms in the promoter region of the GLUT2 gene (18, 30), the functional consequence could be related to differences in either protein function or levels. Although Thr and Ile alleles reportedly did not differ in transporter activity in cultured oocytes (31), this may have been due to the limitation of the model system used. Given the observations in GLUT2-null mice and that Ile carriers consumed a greater amount of sugars as measured over a 1-mo period. The robustness of these findings suggests that GLUT2 is involved in glucose sensing to affect food intake in humans and may explain individual differences in cravings for sugars.

Dairy products 1.58±0.06 1.82±0.13 0.07
Total fruit 2.65±0.10 2.64±0.19 0.90
Fruit juice 0.70±0.05 0.89±0.09 0.12
Fruit 1.95±0.08 1.75±0.16 0.32
Sweetened beverages 0.34±0.02 0.49±0.05 0.0004
Sweets 1.08±0.05 1.45±0.10 0.002

These observations in mice suggest that GLUT2 acts as a glucose sensor that regulates neuropeptides involved in food intake regulation.

DISCUSSION

The results of the present study demonstrate that a genetic polymorphism of GLUT2 is associated with differences in the habitual consumption of sugars both within and between two distinct populations, using two types of dietary assessment tools that cover different durations. In a population of older adults with early Type 2 diabetes, the consumption of sugars increased by 10.220.32.246 on June 20, 2017 http://physiolgenomics.physiology.org/ Downloaded from http://physiolgenomics.physiology.org/ by 10.220.22.246 on June 20, 2017
and is not confounded by differences in circulating insulin or leptin, which are additional metabolic inputs that converge on the glucose-sensing cells in the brain (25).

Previous studies relating GLUT2 genotypes with risk of Type 2 diabetes have yielded conflicting results (8, 16–18, 30, 37), which may be due to failure to account for environmental factors such as diet. Since quality of dietary carbohydrates has been associated with diabetes risk (22, 38), and individuals with the Ile allele consumed a greater amount of sugars in the present study, differences in dietary selection of foods high in sugars might confound gene-disease association studies involving GLUT2 (8, 16–18, 30, 37). Among the second population we examined, we assessed the types of food groups contributing to the higher consumption of sugars. We observed that carriers of the Ile allele consumed a diet higher in sugars from sweets, such as baked goods and chocolate, and sweetened beverages, rather than selecting food from other sources such as fruit, which may have been related to the food available and dietary practices of this population of young adults. Therefore, the type of dietary carbohydrate consumed should be examined in future gene-disease association studies of GLUT2 genotype, especially given that different factors may influence the source of dietary sugar such as availability, cultural practices, and cost.

In conclusion, we found that a polymorphism in the GLUT2 gene is associated with a higher intake of sugars within and between two distinct populations using two different methods of dietary assessment. These observations suggest that GLUT2 plays a role in glucose sensing to affect food intake in humans and may explain individual differences in preference for foods high in sugars. GLUT2 may, therefore, be a candidate susceptibility gene for disorders affecting food intake.

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GRANTS

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