Duodenal Sodium/Glucose Cotransporter 1 Expression Under Fasting Conditions Is Associated With Postload Hyperglycemia

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Context: Type 2 diabetes (T2DM) is associated with a higher intestinal expression of the glucose transporters sodium/glucose cotransporter 1 (SGLT-1) and glucose transporter 2 (GLUT-2). It is currently unsettled whether prediabetes conditions characterized by postprandial hyperglycemia, such as impaired glucose tolerance (IGT) and normal glucose tolerance (NGT) with 1-hour postload glucose ≥155 mg/dL (8.6 mmol/L) (NGT-1h-high) are associated with increased expression of these glucose carriers in the intestine.

Objective: We evaluated whether duodenal abundance of SGLT-1 and GLUT-2 is augmented in subjects with IGT and NGT-1h-high, in comparison with subjects with NGT and 1-hour postload glucose ˂155 mg/dL (NGT-1h-low).

Design: Cross-sectional.

Patients: A total of 54 individuals underwent an upper gastrointestinal endoscopy.

Main Outcome Measures: Duodenal SGLT-1 and GLUT-2 protein and messenger RNA levels were assessed by Western blot and reverse transcription polymerase chain reaction, respectively.

Results: Of the 54 subjects examined, 18 had NGT-1h-low, 12 had NGT-1h-high, 12 had IGT, and 12 had T2DM. Duodenal SGLT-1 protein and messenger RNA levels were significantly higher in individuals with NGT-1h-high, IGT, or T2DM in comparison with NGT-1h-low subjects. GLUT-2 abundance was higher in individuals with T2DM in comparison with NGT-1h-low subjects; no substantial increase in GLUT-2 expression was observed in NGT-1h-high or IGT individuals. Univariate correlations showed that duodenal SGLT-1 abundance was positively correlated with 1-hour postload plasma glucose levels ($r = 0.44$; $P = 0.003$) but not with fasting or 2-hour postload glucose levels.

Conclusions: Duodenal SGLT-1 expression is increased in individuals with 1-hour postload hyperglycemia or IGT, as well as in subjects with T2DM, and it positively correlates with early postload glucose excursion. (***J Clin Endocrinol Metab*** **102: 3979–3989, 2017)**

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Abbreviations: ANOVA, analysis of variance; BMI, body mass index; GLP-1, glucagon-like peptide 1; GLUT-2, glucose transporter 2; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HNF-1α, hepatocyte nuclear factor 1-alpha; IGT, impaired glucose tolerance; mRNA, messenger RNA; NGT, normal glucose tolerance; NGT-1h-high, NGT with 1-hour postload glucose ≥155 mg/dL; NGT-1h-low, NGT with 1-hour postload glucose <155 mg/dL; OGTT, oral glucose tolerance test; RT-PCR, reverse transcription polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SGLT-1, sodium/glucose cotransporter 1; T2DM, type 2 diabetes mellitus.

The prevalence and the incidence of type 2 diabetes mellitus (T2DM) has risen to epidemic proportions worldwide (1, 2). Because the progression of the disease can be prevented or delayed through lifestyle and pharmacological interventions (3–6), the early identification of individuals at higher risk to develop it represents a crucial step to most effectively target intervention. Impaired glucose tolerance (IGT) and impaired fasting glucose are "high-risk" states (also referred to as prediabetes) with annual diabetes conversion rates ranging from 3% to 11% per year (7). Recently, growing evidence has gathered suggesting that a 1-hour plasma glucose concentration during an oral glucose tolerance test (OGTT) ≥155 mg/dL (8.6 mmol/L) identifies individuals at increased risk to develop type 2 diabetes among those who have normal glucose tolerance (NGT) (8–11). The prevalence of 1-hour postload plasma glucose concentration ≥155 mg/dL among individuals with NGT ranges from 15.8% to 25.4% (8, 9, 12), suggesting that the exclusive use of impaired fasting glucose/IGT to identify individuals at high risk of type 2 diabetes may oversee a considerable proportion of individuals that will develop the disease. Previous studies aimed at characterizing the pathophysiological abnormalities causing 1-hour postload hyperglycemia have shown that individuals with 1-hour postload plasma glucose concentration ≥155 mg/dL (NGT-1h-high) exhibit reduced insulin sensitivity, decreased insulin clearance, and impaired β-cell function compared with NGT individuals with a 1-hour postload plasma glucose value <155 mg/dL (NGT-1h-low) (12–15). However, it is unclear whether these metabolic derangements are *bona fide* responsible for 1-hour postload hyperglycemia or rather represent secondary abnormalities resulting from elevated plasma glucose levels in the postprandial period.

Physiologically, increased glucose absorption in the proximal intestine would increase blood glucose concentrations during a meal or an OGTT. The absorption of dietary glucose is mediated by the sodium/glucose cotransporter 1 (SGLT-1), which transports the monosaccharide from the lumen of the intestine into the enterocytes (16, 17). Once accumulated in the intracellular compartment, glucose leaves enterocytes across the basolateral membrane via the glucose transporter 2 (GLUT-2) (18, 19). Studies in animal models have shown that experimentally induced diabetes is associated with enhanced activity and abundance of SGLT-1 and GLUT-2, resulting in increased intestinal glucose absorption and exacerbated postload blood glucose excursion (20–22). Accordingly, an enhanced glucose absorption associated to a higher expression of SGLT-1 and GLUT-2 has been observed in intestinal biopsies of subjects with type 2 diabetes (23).

No studies have been carried out to investigate whether intestinal expression of SGLT-1 and GLUT-2 is altered in prediabetes conditions characterized by postload hyperglycemia such as IGT or NGT-1h-high. We hypothesized that increased expression of intestinal SGLT-1 and GLUT-2 might contribute to the metabolic derangements of individuals with NGT-1h-high and IGT, resulting in augmented glucose absorption and postprandial hyperglycemia. To this aim, we assessed protein abundance of SGLT-1 and GLUT-2 in the duodenum of individuals with different states of glucose tolerance.

Materials and Methods

The study cohort includes 54 white subjects recruited at the Department of Medical and Surgical Sciences of the University Magna Graecia of Catanzaro. Exclusion criteria were: age <20 years, pregnancy, contraindication to endoscopy, history of autoimmune or malignant disease, end-stage renal disease, chronic gastrointestinal diseases such as celiac and Crohn disease, food allergy, a history of surgery of the stomach or small intestine, positivity for antibodies to hepatitis C virus or hepatitis B surface antigen, use of toxins or drugs, treatment with medications known to alter platelet aggregation or thrombus formation or medications known to influence glucose tolerance or intestinal expression of glucose carriers such as steroids, sartans, angiotensin-converting enzyme inhibitors, glucagon-like peptide 1 (GLP-1) analogs, and estroprogestins. Lactose intolerance was excluded by hydrogen breath test.

After a 12-hour fast, all individuals underwent anthropometrical evaluation including measurement of body mass index (BMI), waist circumference, and readings of clinic blood pressure. A 75-g OGTT was performed in nondiabetic individuals with 0-, 30-, 60-, 90-, and 120-minute sampling for plasma glucose and insulin assays. Individuals were classified as having NGT when fasting plasma glucose was <100 mg/dL and 2-hour postload glucose was <140 mg/dL, IGT when fasting plasma glucose was <126 mg/dL and 2-hour postload glucose was 140 to 199 mg/dL, and type 2 diabetes when fasting plasma glucose was ≥126 mg/dL and/or 2-hour postload glucose ≥200 mg/dL or glycated hemoglobin (HbA1c) ≥6.5%. Individuals with NGT were further divided into two subgroups (NGT-1h-low and NGT-1h-high) based upon their 1-hour plasma glucose concentration (below or above 155 mg/dL, respectively).

Duodenal biopsies

After 1 week from OGTT, all subjects underwent an upper gastrointestinal endoscopy. The endoscopy was performed after a 12-hour overnight fasting. Specimens of duodenal mucosa were collected from the second part of duodenum during the endoscopy. The duodenal biopsies were shown to be normal by histological examination. After removal, the biopsies were placed in a screw-capped cryotube, dropped immediately into liquid nitrogen, and stored at −80°C until use.

Additionally, some samples were fixed in 10% neutral-buffered formalin, paraffin embedded, and processed routinely. Four-micrometer-thick serial sections were obtained and stained with hematoxylin and eosin for microscopical examination.

The protocol was approved by the hospital ethical committee (Comitato Etico Azienda Ospedaliera "Mater Domini") and written informed consent was obtained from all participants in accordance with principles of the Declaration of Helsinki.

Western blot analysis

Specimens of duodenal mucosa obtained by all study participants were lysed in buffer containing 1% Triton X-100 and analyzed by Western blot. Forty-microgram protein samples were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransferred to nitrocellulose membranes (Whatman GmbH, Dassel, Germany). After a 2-hour blocking, the membranes were probed overnight at 4°C with antibodies (Abcam, Cambridge, MA) against SGLT-1 (dilution: 1:500), GLUT-2 (dilution 1:250), and hepatocyte nuclear factor 1-alpha (HNF-1α, dilution 1:500). Equal protein loading was confirmed by reblotting the membranes with monoclonal antibody against β-actin (Sigma-Aldrich, Milan, Italy; dilution 1:5000). Blots were visualized using appropriate peroxidase-conjugated secondary antibodies followed by enhanced chemiluminescence detection; band densities were quantified by densitometry.

Real-time reverse transcription quantitative polymerase chain reaction

Total RNA was extracted from duodenal biopsies of 24 study participants (seven subjects with NGT-1h-low, six with NGT-1h-high, six with IGT, and five with type 2 diabetes) for whom duodenal specimens were sufficient to allow RNA extraction. Extraction of total RNA was performed by using Trizol (Life Technologies, Gaithersburg, MD). Total RNA was reverse transcribed and analyzed by real-time reverse transcription quantitative polymerase chain reaction using a Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). Results were normalized to S18 messenger RNA (mRNA) levels and the relative gene expression data were analyzed according to the Livak method ($2^{-\Delta\Delta 2}$T)

Immunohistochemistry

For each case, 4-μm-thick serial sections were placed on poly-L-lysine–coated slides for immunohistochemical analysis. A standard protocol for immunohistochemistry using an automated immunostainer (Bond Max; Leica Biosystems, Melbourne, Australia) was performed using anti-SGLT-1 antibody with 1:100 dilution (Abcam). Detection of immunoreactivity was assessed using peroxidase/DAB. Negative control was processed by mixing the primary antibody anti–SGLT-1 with 20 μg/mL of the blocking (immunogen) peptide (SGLT-1 peptide, Abcam). The mixture of primary antibody and blocking peptide was incubated for 1 hour on a rotary shaker at room temperature before the application on automated immunostainer. Two pathologists (C.M. and G.D.), who had no knowledge of the clinical data, independently evaluated immunostaining. All samples were examined by using a Leica DMD 108 microscope (Leica Microsystems). Enterocytes showing brown staining were counted in 10 high-magnification random fields, and the degree of SGLT-1 immunostaining was expressed as SGLT-1 immunostaining score, measured by estimating the percentage of positive cells among the total number of cells. The cellular localization of the brown signal was specified, differentiating basolateral from apical signal.

Analytical determinations

Glucose, triglycerides, total cholesterol, and high-density lipoprotein (HDL) concentrations were determined by enzymatic methods (Roche, Basel, Switzerland). HbA1c was measured with high-performance liquid chromatography using a National Glycohemoglobin Standardization Program–certified automated analyzer (Adams HA-8160 HbA1C analyzer, Menarini, Italy). Plasma insulin levels were measured with a chemiluminescence-based assay (Immulite, Siemens, Italy).

Statistical analysis

Variables that were not normally distributed, including triglycerides, fasting insulin, 1- and 2-hour postload insulin, were natural log transformed for statistical analyses. Continuous variables are expressed as means ± standard deviation. Analysis of variance (ANOVA) was used to compare differences of continuous variables among groups. Differences in metabolic variables among groups were tested after adjusting for sex and age using a general linear model with *post hoc* Fisher least significant difference correction for pairwise comparisons. Categorical variables were compared by χ^2 test. Relationships between variables were determined by Pearson correlation coefficient (r). Considering that previous studies have reported a 30% increase in duodenal SGLT-1 expression of subjects with metabolic abnormalities (23, 24), we calculated that 12 subjects for each group had 92% power to detect a 30% difference in duodenal abundance of glucose transporters with a level of significance of 5% using the power calculation available at http://www.statisticalsolutions.net/pss_calc.php. A P value ≤ 0.05 was considered statistically significant. All analyses were performed using SPSS software program, version 17.0, for Windows.

Results

A total of 54 individuals were evaluated, of which 30 had NGT, 12 had IGT, and 12 had type 2 diabetes. Of this latter group, five individuals were newly diagnosed by an OGTT, whereas seven had a previous diagnosis of diabetes and were treated with metformin alone. NGT subjects were divided into two subgroups: 18 subjects with NGT-1h-low and 12 with NGT-1h-high. Ten of the 12 individuals with IGT and all five patients with OGTT-diagnosed type 2 diabetes had NGT-1h-high.

Anthropometric and biochemical characteristics of the study participants are summarized in Table 1. The mean age of the entire study sample was 50 ± 10 years, 29 (53.7%) were male, and mean BMI was 31.2 ± 6.9 kg/m^2. Individuals with diabetes were significantly older compared with NGT-1h-low and NGT-1h-high groups. No substantial differences in sex, BMI, waist circumference, and blood pressure were observed among the four groups. After adjusting for age and sex, individuals with diabetes displayed lower levels of HDL in comparison with the NGT-1h-low group.

We assessed SGTL-1 and GLUT-2 protein levels by Western blot in total duodenal lysates obtained from all

Table 1. Anthropometric and Metabolic Characteristics of the Study Subjects Stratified According to Glucose Tolerance

	NGT				
Variables	**NGT-1h-low**	**NGT-1h-high**	**IGT**	**T2DM**	***P***
Number (male/female)	18 (10/8)	12 (8/4)	12 (3/9)	12 (8/4)	0.131
Age, y	43 ± 8	50 ± 7	51 ± 13	59 ± 9	0.001
BMI, kg/m^2	30 ± 7.7	32.9 ± 5.6	31.5 ± 9.5	31.3 ± 4.6	0.430
Waist circumference, cm	101 ± 13.6	109.8 ± 10.6	106 ± 15	109 ± 11	0.09
Systolic blood pressure, mm Hg	121 ± 14	123 ± 20	120 ± 13	124 ± 17	0.970
Diastolic blood pressure, mm Hg	76 ± 10	75 ± 12	75 ± 7	74 ± 10	0.838
Total cholesterol, mg/dL	177 ± 30	193 ± 51	200 ± 24	178 ± 51	0.294
HDL, mg/dL	55 ± 14	48 ± 8	54 ± 17	44 ± 11	0.05
Triglycerides, mg/dL	93 ± 50	100 ± 35	138 ± 78	141 ± 84	0.07
HbA1c, % (mmol/mol)	5.3 ± 0.2 (34)	5.5 ± 0.3 (37)	5.6 ± 0.3 (38)	6.7 ± 0.9 (45)	<0.0001
Fasting glucose, mg/dL	87 ± 7	87 ± 6	92 ± 14	113 ± 18	<0.0001
1-h glucose, mg/dL	120 ± 26	182 ± 10	176 ± 20	223 ± 23[a]	<0.0001
2-h glucose, mg/dL	95 ± 25	118 ± 18	158 ± 18	218 ± 23[a]	<0.0001
Fasting insulin, μU/mL	11 ± 8	14 ± 6	16.0 ± 8	39 ± 25	0.001
1-h insulin, μU/mL	119 ± 72	169 ± 79	162 ± 103	268 ± 194[a]	0.406
2-h insulin, μU/mL	55 ± 50	110 ± 47	199 ± 137	391 ± 339[a]	0.001

Data are means ± standard deviation. Fasting, 1-h and 2-h insulin, and triglyceride levels were log transformed for statistical analysis, but values in the table represent back transformation to the original scale. Comparisons among the four groups were performed using a general linear model. *P* values refer to results after analyses with adjustment for age and sex. Categorical variables were compared by χ^2 test.

[a]Data referred to five patients with newly diagnosed T2DM.

participants, who were stratified according to the dysglycemic state (Fig. 1A and 1B). SGTL-1 abundance was significantly higher in individuals with NGT-1h-high (+42%, $P \leq 0.0001$), IGT (+28%, $P = 0.05$), or type 2 diabetes (+31%, $P = 0.05$) compared with NGT-1h-low individuals after adjusting for age and sex. No differences were observed among NGT-1h-high, IGT, or diabetic individuals (Fig. 1A). As compared with NGT-1h-low individuals, GLUT-2 abundance was significantly higher in individuals with type 2 diabetes (+63%, $P = 0.001$), but no substantial changes were observed in NGT-1h-high or IGT individuals (Fig. 1B). Moreover, no important differences in SGLT-1 and GLUT-2 protein levels were found between subjects having newly diagnosed type 2 diabetes and those with a previous diagnosis of diabetes (Supplemental Fig. 1).

Duodenal mRNA levels of SGLT-1 and GLUT-2 were assessed by reverse transcription polymerase chain reaction (RT-PCR) (Fig. 2). We found that subjects with NGT-1h-high, IGT, and type 2 diabetes had significantly higher levels of SGLT-1 mRNA in comparison with NGT-1h-low individuals ($P = 0.02$). No differences were observed among NGT-1h-high, IGT, or type 2 diabetic individuals (Fig. 2A). A significant 3.6-fold increase in GLUT-2 mRNA levels was observed in subjects with type 2 diabetes compared with the NGT-1h-low ($P = 0.01$), but not the NGT-1h-high and IGT groups (Fig. 2B).

Next, we assessed protein levels of HNF-1α, a known transcription factor essential for duodenal expression of SGLT-1 (25, 26). We found that duodenal HNF-1α abundance was significantly higher in subjects with NGT-1h-high (+30%, $P = 0.01$), IGT (+29%, $P = 0.05$), and type 2 diabetes (+43%, $P = 0.01$) in comparison with NGT-1h-low individuals even after adjusting for age and sex (Fig. 3).

Furthermore, we evaluated the localization of SGLT-1 in duodenal biopsies (Fig. 4; additional images at larger magnifications in Supplemental Fig. 2). As expected, SGLT-1 was localized in the apical membrane, whereas it was not detected in the basolateral plasma membrane (Fig. 4A; Supplemental Fig. 2). Because duodenal mucosa biopsies were obtained after 12 hours of fasting, a large fraction of SGLT-1 was localized at intracellular compartment (27) (Fig. 4A; Supplemental Fig. 2). Accordingly with Western blot analysis, abundance of SGLT-1 assessed by SGLT-1 immunostaining score was increased in subjects with NGT-1h-high, IGT, and type 2 diabetes in comparison with NGT 1h-low individuals ($P \leq 0.01$; Fig. 4A and 4B). Specificity of anti–SGLT-1 antibody was confirmed by the elimination of immunohistochemical signal by preincubation of the antibody with SGLT-1 peptide as shown in Supplemental Fig. 3.

Relationships between intestinal glucose transporters and glucose levels during OGTT

Univariate correlations revealed a significant relationship between duodenal abundance of SGLT-1 and 1-hour postload plasma glucose levels ($r = 0.44$;

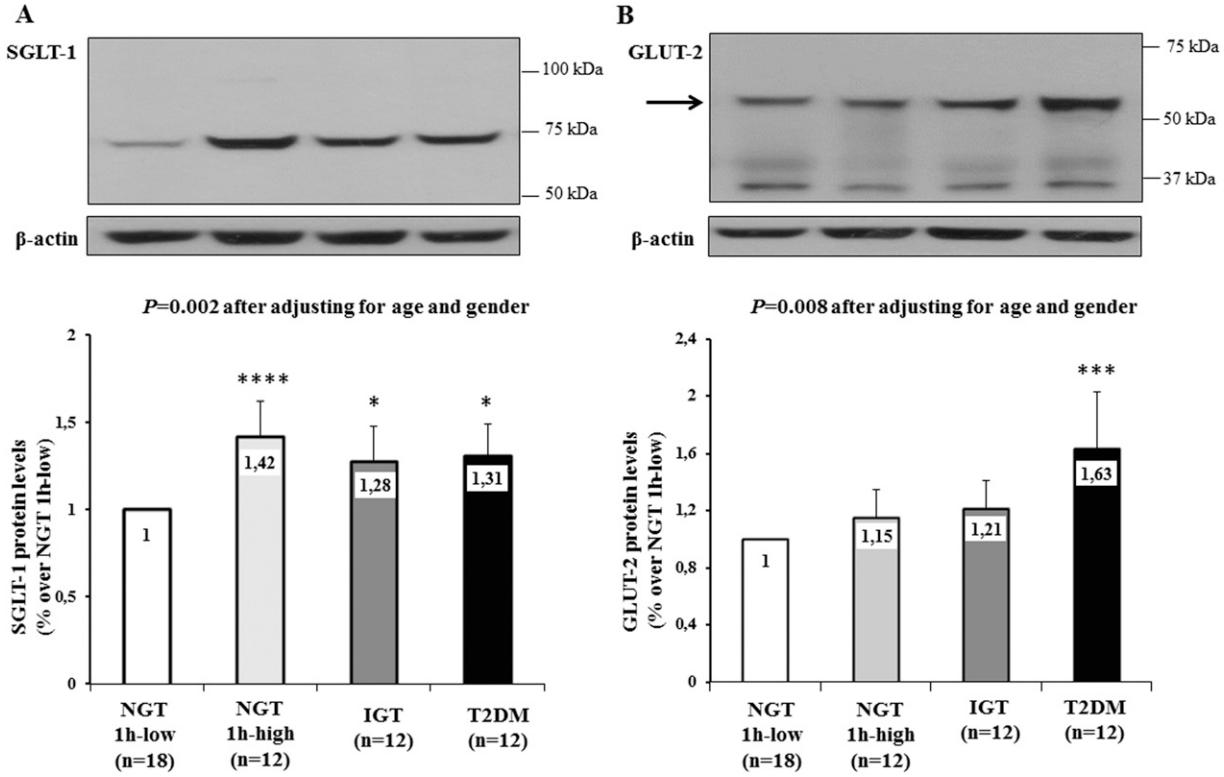

Figure 1. Immunoblot and quantification of (A) SGTL-1 and (B) GLUT-2 in the duodenal mucosa of fasted individuals with NGT-1h-low, NGT-1h-high, IGT, and T2DM. Equal amounts of tissue lysates were resolved by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were then incubated with antibodies against SGLT-1 (predicted molecular weight, 73 kDa) and GLUT-2 (predicted molecular weight, 57 kDa). Blots were visualized using peroxidase-conjugated secondary antibodies followed by enhanced chemiluminescence. Band densities were quantified by densitometry. Loading was normalized using anti-β-actin antibody. Representative immunoblots of one participant from each of the four study groups are shown; data summarized in graphs are means of densitometry of SGLT-1 and GLUT-2. *P* values above each graph refer to multiple comparison vs NGT-1h-low group performed by analysis of covariance. $^*P \leq 0.05$, $^{***}P \leq 0.001$, $^{****}P \leq 0.0001$ vs NGT-1h-low group.

$P = 0.003$); by contrast, no significant correlations were observed between duodenal SGLT-1 abundance and fasting ($r = 0.20$; $P = 0.16$) or 2-hour postload ($r = 0.14$; $P = 0.36$) plasma glucose levels (Fig. 5A–5C). Duodenal abundance of GLUT-2 was significantly correlated with fasting ($r = 0.48$; $P = 0.001$) and 1- ($r = 0.43$; $P = 0.008$) and 2-hour postload ($r = 0.31$; $P = 0.05$) glucose levels. Similar results were observed for insulin levels.

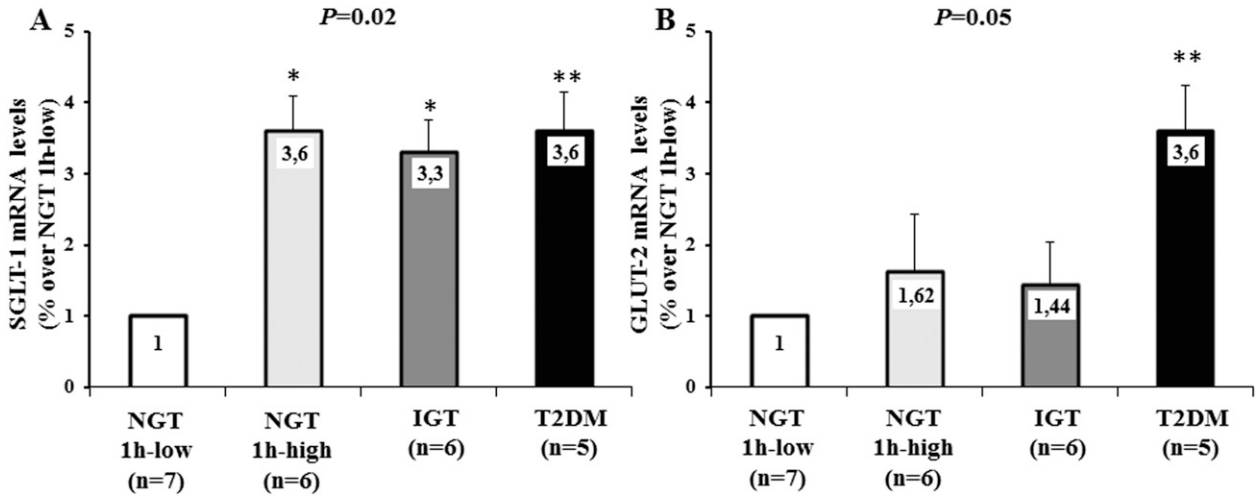

Figure 2. Duodenal expression of (A) SGLT-1 and (B) GLUT-2 in subjects with NGT-1h-low, NGT-1h-high, IGT, and T2DM. mRNA levels of SGLT-1 and GLUT-2 were assessed by RT-PCR. *P* values above each graph refer to multiple comparison vs NGT-1h-low group performed by ANOVA. $^*P \leq 0.05$, $^{**}P \leq 0.01$ vs NGT-1h-low group.

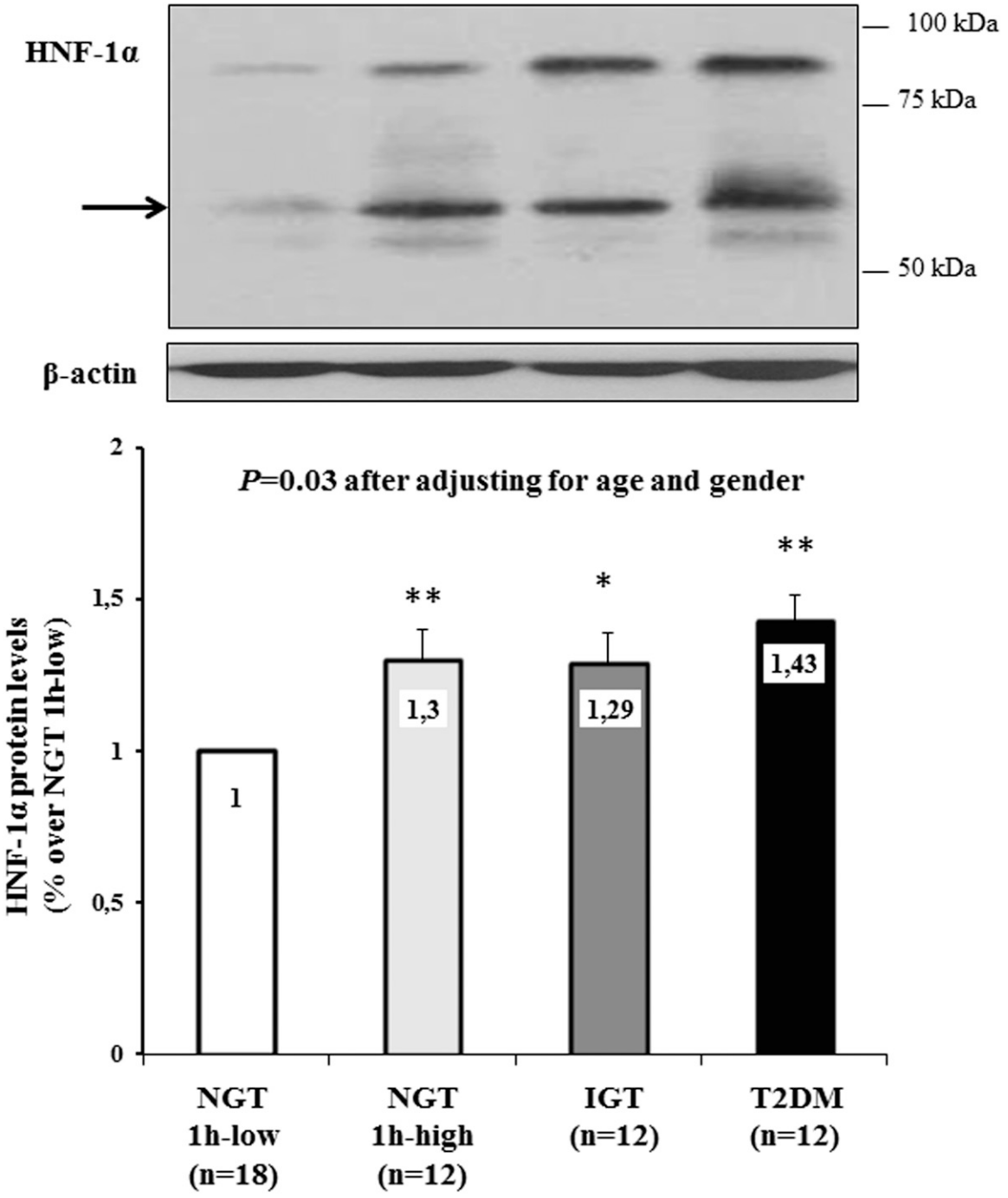

Figure 3. Duodenal protein levels of HNF-1α in NGT-1h-low, NGT-1h-high, IGT, and T2DM groups. Equal amounts of tissue lysates were resolved by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were incubated with antibody against HNF-1α (predicted molecular weight, 67 kDa). Blots were visualized using peroxidase-conjugated secondary antibodies followed by enhanced chemiluminescence. Band densities were quantified by densitometry. Loading was normalized using anti-β-actin antibody. Representative immunoblots of one participant from each of the four study groups are shown; data summarized in the graph are means of HNF-1α densitometry. *P* values reported above the graph refers to multiple comparison vs NGT-1h-low group performed by analysis of covariance. $*P \leq 0.05$, $**P \leq 0.01$ vs NGT-1h-low group.

Duodenal protein levels of SGLT-1 were positively associated with 1-hour postload plasma insulin levels ($r = 0.41$; $P = 0.04$), whereas no significant association between duodenal SGLT-1 levels and fasting ($r = 0.21$; $P = 0.19$) or 2-hour postload ($r = 0.31$; $P = 0.08$) insulin concentrations was found. Duodenal abundance of GLUT-2 was significantly correlated with fasting ($r = 0.30$; $P = 0.05$) and 1- ($r = 0.37$; $P = 0.05$) and 2-hour postload ($r = 0.44$; $P = 0.02$) insulin levels.

Discussion

This study demonstrates that individuals with NGT-1h-high exhibit an important increase in duodenal abundance

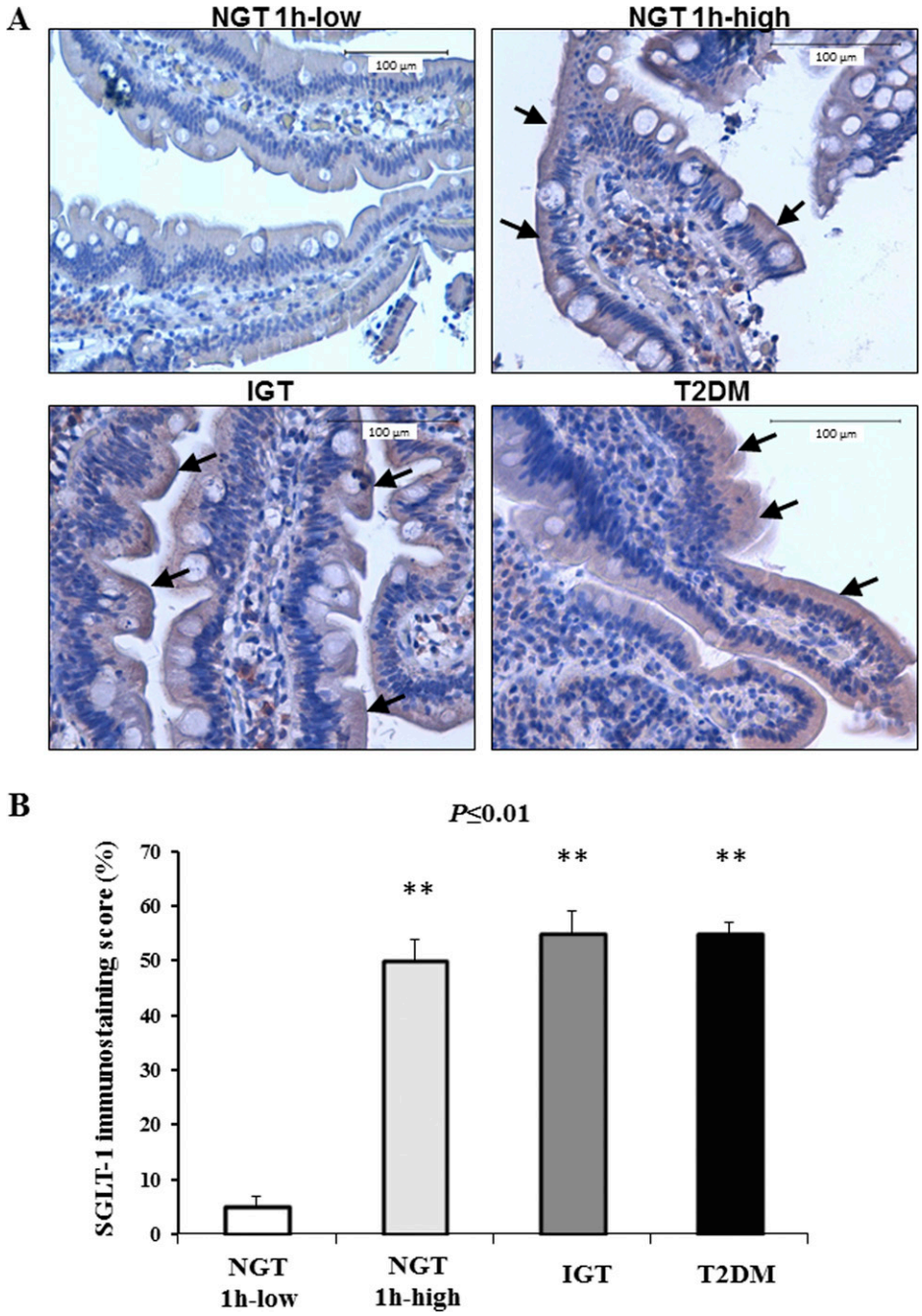

Figure 4. Duodenal localization of SGLT-1 in subjects with NGT-1h-low, NGT-1h-high, IGT, and T2DM. (A) Representative images of SGLT-1 immunostaining (brown signal) for each group (40×). The apical staining (marked by black arrows) is evident with a lower SGLT-1 signal in NGT-1h-low compared with the other groups (intensity of brown signal is higher in NGT-1h-high, IGT, and T2DM subjects in comparison with NGT-1h-low group). (B) SGLT-1 immunostaining score, evaluated estimating the percentage of positive cells among the total number of cells in 10 high-magnification random fields. Subjects for each group = 4. *P* values reported above the graph refers to multiple comparison vs NGT-1h-low group performed by ANOVA. $^{**}P \leq 0.01$ vs NGT-1h-low group.

of SGLT-1 compared with subjects with NGT-1h-low. Interestingly, the increased abundance of SGLT-1 observed in individuals with NGT-1h-high was similar to that observed in subjects with IGT or early type 2 diabetes. It is noteworthy that virtually all the individuals with IGT or newly diagnosed diabetes had an NGT-1h-high value according to previous reports (12). The increased duodenal protein levels of SGLT-1 found in individuals with NGT-1h-high, IGT, and type 2 diabetes were accompanied by higher levels of SGLT-1 mRNA, suggesting that augmented abundance of SGLT-1 in proximal intestine was due to an increase in the transcription of the SGLT-1 gene rather than to post-translational processing of SGLT-1 protein. Accordingly, protein levels of HNF-1α, a transcription factor known to regulate the expression of SGLT-1 (25, 26), were increased

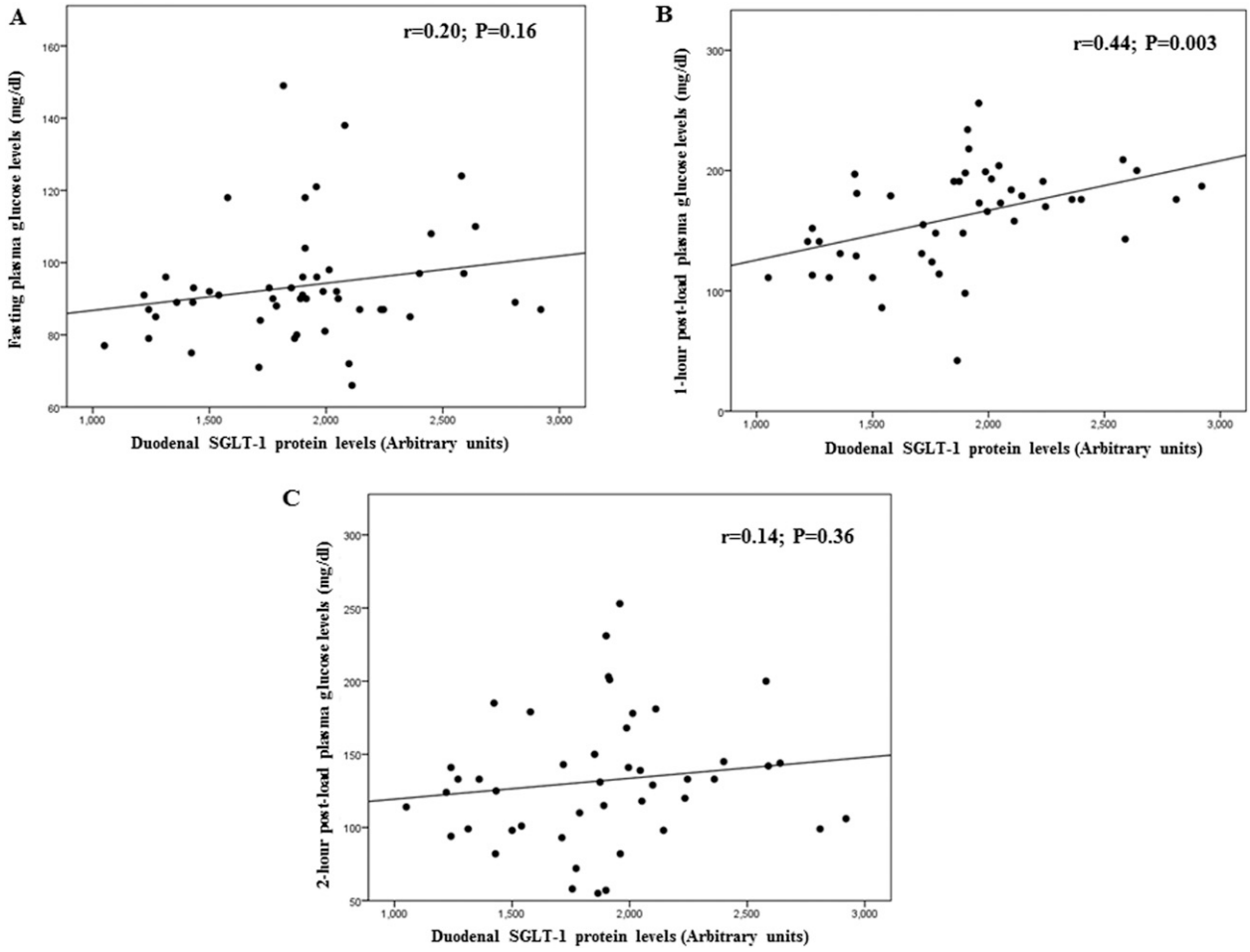

Figure 5. Relationships between (A) duodenal SGTL-1 abundance and fasting, (B) 1-hour postload, and (C) 2-hour postload plasma glucose.

in individuals with NGT-1h-high, IGT, and type 2 diabetes compared with the NGT-1h-low group. We also found a substantial direct relationship between 1-hour postload plasma glucose levels and duodenal SGTL-1 protein levels. In contrast, no relationship was observed between fasting or 2-hour postload plasma glucose concentrations and SGTL-1 levels, suggesting that early postprandial glucose is highly dependent on SGTL-1 duodenal abundance. According to previous studies (21, 23, 28), we observed a substantially increased expression of GLUT-2 in the duodenum of diabetic patients, but not in dysglycemic prediabetes conditions.

Because increased expression of SGLT-1 has been shown to be associated with accelerated glucose absorption (17), the present observations suggest that dysregulated glucose absorption in the proximal gut resulting from up-regulation of SGTL-1 in individuals at risk to develop type 2 diabetes, such as those with NGT-1h-high or IGT, may play an important pathophysiological role in causing these dysglycemic conditions. In this respect, it is important to note that postprandial hyperglycemia has been shown to be related to a high risk of developing type 2 diabetes (7–11).

A body of evidence has suggested that consumption of a diet rich in glucose leads to an increase in intestinal glucose transport and intestinal expression of SGLT-1 (29–31). Likewise, consumption of a high-fructose diet also increased SGLT-1 expression and function, although fructose is not itself a substrate of SGLT-1 (30). Interestingly, we have recently reported that individuals with NGT-1h-high exhibit higher dietary intake of oligosaccharides and fructose compared with individuals with NGT-1h-low (32). These data provide a plausible explanation for the increased abundance of intestinal SGLT-1 observed in individuals with NGT-1h-high. This association has a clinical relevance that needs to be considered. Given the major role of food nutrients including glucose and fructose in regulation of intestinal SGLT-1, in high-risk subjects, such as those with NGT-1h-high or IGT, it is reasonable to consider dietary interventions aimed at inhibiting glucose uptake by modulating the expression of the SGLT-1 transporter. In this regard, it has been shown that the phenolic compounds in apple juice are able to delay intestinal glucose uptake (33), and an apple extract containing phlorizin, quercetin, kaempferol, phloretin, and chologenic acid

inhibits human SGLT-1 expressed in oocytes and reduces postload glucose responses in healthy volunteers (34).

Prevention or delay of type 2 diabetes relies chiefly on reduction of dietary intake and on the use of glucose-lowering agents (3–6). The results of the current study highlight the potential for therapeutic intervention in type 2 diabetes and prediabetes conditions with pharmaceutical agents able to reduce the abundance and/or the activity of SGLT-1 transporter in the intestinal mucosa to counteract postprandial hyperglycemia. Remarkably, several studies have reported that subjects with NGT-1h-high exhibit a worse cardiometabolic risk profile (11–15, 35), and elevated 1-hour postload plasma glucose levels have been shown to be associated not only with an increased risk to develop diabetes (7–11), but also with a higher total mortality in nondiabetic population (36). This evidence supports the hypothesis that pharmaceutical agents able to affect postprandial glucose rise would be beneficial in reducing progression to overt diabetes and the associated cardiovascular risk in subjects with prediabetes conditions characterized by postprandial hyperglycemia such as IGT and NGT-1h-high. Dual inhibitors of SGLT-1 and SGLT-2 have been recently developed (37–40) and shown to significantly improve glycemic control and decrease blood glucose excursions after an OGTT, suggesting that such inhibitors may be effective treatments to prevent or delay onset of overt type 2 diabetes in high-risk individuals including IGT and NGT-1h-high.

The current study has several strengths, including the matching of the four groups for sex and adiposity variables; the detailed anthropometric and biochemical data collected according to a standardized protocol by trained staff; the assay of metabolites in fresh blood samples rather than in stored samples; the exclusion of confounding conditions characterized by intestinal damage including heavy drinking, history of autoimmune or malignant disease, chronic gastrointestinal diseases, food allergy, lactose intolerance, treatment with medications known to influence glucose tolerance and/or duodenal abundance of the glucose carriers; and history of abdominal surgery. However, in interpreting the present observations, some limitations should be recognized. The sample size was relatively small although power calculations allow us to exclude type I error. Dietary intakes potentially affecting intestinal transporters expression were not recorded. However, in a larger sample, we had previously carefully characterized habitual dietary intake of individuals with NGT-1h-high (32). Next, the evaluation of OGTT was performed once, and this may have introduced some inaccuracy in the classification of individuals into the dysglycemic categories. Moreover, there was an age difference between the diabetic group and the control group. Although the effect of aging on the expression of intestinal glucose transporters is unclear, the observed relationships were not affected by adjustment for age. Additionally, gastric emptying and glucose kinetics in response to an OGTT were not measured, although these phenomena would be of considerable interest in regulating postload glucose levels. Next, considering that a cross-talk among SGLT-1, GLP-1, and peptide YY has been described (17, 38, 40), another limitation of the current study is represented by the lack of plasma GLP-1 and protein YY level measurements. Finally, the demonstration of relationships does not establish causality, and other factors might be responsible for elevation of plasma glucose at 1 hour during OGTT.

In conclusion, we have shown that individuals with NGT-1h-high have an increased abundance of duodenal SGTL-1 similar to that observed in subjects with IGT or type 2 diabetes. Importantly, there was a strong direct relationship between blood glucose at 1 hour during an OGTT and SGTL-1 duodenal abundance. In contrast, no relationship was observed between fasting glycemia or blood glucose at 2 hours during an OGTT and duodenal SGTL-1 abundance, suggesting that early postprandial glucose is highly dependent on SGTL-1 duodenal abundance. Although the current findings support the idea that elevated 1-hour postload plasma glucose may underlay increased abundance of duodenal SGTL-1, further investigation, including the use of specific inhibitors of SGTL-1, is required.

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