Expression of monosaccharide transporters in intestine of diabetic humans

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Received 13 July 2001; accepted in final form 2 October 2001

Dyer, J., I. S. Wood, A. Palejwala, A. Ellis, and S. P. Shirazi-Beechey. Expression of monosaccharide transporters in intestine of diabetic humans. Am J Physiol Gastrointest Liver Physiol 282: G241–G248, 2002.—Noninsulin-dependent diabetes mellitus (NIDDM) is an increasingly common disease, which brings a number of life-threatening complications. In rats with experimentally induced diabetes, there is an increase in the capacity of the intestine to absorb monosaccharides. We have examined the activity and the expression of monosaccharide transporters in the intestine of patients suffering from NIDDM. Na+/dependent d-glucose transport was 3.3-fold higher in brush-border membrane (BBM) vesicles isolated from duodenal biopsies of NIDDM patients compared with healthy controls. Western analysis indicated that SGLT1 and GLUT5 protein levels were also 4.3- and 4.1-fold higher in diabetic patients. This was associated with threefold increases in SGLT1 and GLUT5 mRNA measured by Northern blotting. GLUT2 mRNA levels were also increased threefold in the intestine of diabetic patients. Analysis of other BBM proteins indicated that the activity and abundance of sucrase and lactase were increased by 1.5- to 2-fold and the level of the structural proteins villin and β-actin was enhanced 2-fold in diabetic patients compared with controls. The increase in the capacity of the intestine to absorb monosaccharides in human NIDDM is due to a combination of intestinal structural change with a specific increase in the expression of the monosaccharide transporters SGLT1, GLUT5, and GLUT2.

noninsulin-dependent diabetes mellitus; SGLT1; GLUT5; GLUT2; human intestine

TYPE II OR NONINSULIN-DEPENDENT diabetes mellitus (NIDDM) is one of the most prevalent diseases affecting individuals in the industrialized world. The occurrence of this disease is also increasing in people from developing countries exposed to a Westernized lifestyle (7). Most patients with diabetes are at the risk of long-term complications such as vision loss, renal failure, nerve damage, and heart disease. NIDDM is currently estimated to affect 100 million people worldwide, and this is predicted to increase to 200 million by 2010 (1, 40). NIDDM is most prevalent in populations that consume, in excess, Western-type diets. In these diets, glucose represents a large proportion of dietary carbohydrate and is thought to contribute to the increase in hyperinsulinism and insulin resistance seen in these populations (28). Management of diabetes at present relies mainly on reduction of dietary carbohydrate intake and the use of hypoglycemic agents to lower the level of circulating glucose. Control over the intestinal absorption of products of carbohydrate digestion would help to improve levels of glucose in the bloodstream and reduce complications.

Dietary carbohydrates are digested in the gut, through the action of pancreatic α-amylase and the intestinal brush-border membrane (BBM) disaccharidases (lactase, maltase, and sucrase), into the monosaccharides d-glucose, d-galactose, and d-fructose. d-Glucose and d-galactose are transported from the lumen of the intestine, across the BBM by the Na+/glucose cotransporter SGLT1 (30). Fructose is transported from the intestinal lumen into the enterocytes by the fructose transporter GLUT5 (3). These monosaccharides, once accumulated in the enterocytes, exit the cell across the basolateral membrane (BLM) by the Na+/independent monosaccharide transporter GLUT2 (30, 35).

It has been shown in rats with experimentally induced diabetes that the capacity of the small intestine to absorb d-glucose increases. This increase is due mainly to enhanced activity and abundance of SGLT1 and GLUT2 (2, 11, 13). It has also been proposed that GLUT2 is “trafficked” to the BBM of rat jejunum and the levels of the luminal membrane GLUT2 are increased in streptozotocin-induced diabetic rat intestine (20). No studies have been carried out to investigate whether the capacity of the human intestine to absorb sugars is affected in type II diabetes. The ability of the intestine to increase monosaccharide absorption in diabetes can further complicate the pathophysiology of this disease.

In this study, we assess the activity and expression of SGLT1, GLUT5, disaccharidases (sucrase and lactase), and the structural proteins villin and β-actin and GLUT2 expression in the duodenum of healthy individuals and patients with NIDDM. We show that in the intestine of diabetic patients there is a specific increase
METHODS

Subjects. Male and female human subjects with an average age of 58 ± 6.4 (range 26–86, n = 27) and 76 ± 3.5 (range 42–88, n = 25) years, for control and diabetic groups, respectively, participated in this study. Type II diabetes was assessed by the determination of fasting blood glucose levels being >5.5 mM and/or random blood glucose concentrations >10.5 mM. Control individuals consumed normal diets whereas diabetic patients were on reduced carbohydrate diets. In addition, the diabetic patients were on the following medication: 15 patients were taking antidiabetic sulfonlurea drugs (either gliclazide or glibenclamide) and of these 8 were also taking metformin. Five patients were on no antidiabetic medication, and no information is available for the remaining five patients.

Removal and storage of intestinal biopsies and resections. Biopsy samples were removed, from the second part of the duodenum of control subjects and diabetic patients, during endoscopy for investigation of possible gastrointestinal disease. All samples were removed routinely at a morning clinic between 9 and 11 AM. The duodenal biopsies from both groups were shown to be normal by histological examination. After removal, the biopsies were placed either in a labeled screw-capped cryotube and dropped immediately into liquid nitrogen or in a tube containing RNAlater (Ambion) solution. Subsequently, all samples were stored at −80°C until use. The former samples were used for the isolation of BBM vesicles (BBMV) and the latter for RNA isolation.

Sections of histologically normal human small intestine were obtained during the course of gastrointestinal surgery. After removal, the sections were cut open longitudinally and flushed immediately with ice-cold 0.9% (wt/vol) NaCl, pH 7.4, and then wrapped in aluminum foil before immediate freezing in liquid nitrogen. The frozen tissue was stored subsequently at −80°C until use.

The approval of the ethics committee of Royal Liverpool and Broadgreen University Hospitals was obtained for the work in this study. Informed written consent was secured from individuals before tissue removal.

Preparation of BBMV. BBMV were prepared from either intestinal sections or biopsy samples using a combination of cation precipitation and differential centrifugation as described previously (31). The final purified BBMV were suspended in a buffer containing 300 mM mannitol, 20 mM HEPES-Tris, pH 7.4, and 0.1 mM MgSO₄. BBMV were then stored in liquid nitrogen until use. The plasma membrane origin of the BBMV was assessed by determination of the enrichment of the activity and the abundance of the marker proteins of the brush-border membrane. BBMV purity was determined by assessing the levels of marker proteins characteristic of basolateral and organelle membranes (31).

Preparation of BLM vesicles. BLM vesicles (BLMV) were prepared from resected human small intestine by differential centrifugation followed by density gradient centrifugation, as described previously (10). Briefly, frozen sections of intestine were thawed in physiological saline containing 0.1 mM di-thiothreitol (DTT) and 0.2 mM phenylmethylsulfonyl fluoride (PMSF). The tissue was blotted with paper towels to remove excess fluid and mucus, and the mucosa was scraped off and placed in a buffer containing 12.5 mM NaCl, 20 mM Tris·HCl, pH 8.0, 0.1 mM DTT, and 0.2 mM PMSF. The mucosal scrapings were homogenized using a Ystral polytron at setting 5 for 2 min and filtered through nylon gauze to remove mucus. BLMV were isolated according to the protocol described for rabbit intestine, without modification (10). The resulting BLMV were enriched 11-fold in the basolateral marker Na⁺/K⁺-ATPase and showed no enrichment of markers associated with either the BBM or intracellular organelles (10, 31).

The protein concentration in the plasma membrane vesicles was estimated by its ability to bind Coomassie blue according to the Bio-Rad assay technique (34). Bovine γ-globulin was used as the standard.

Measurement of monosaccharide transport activity. To assess the activity of SGLT1, the initial rate of 0.1 mM D-glucose transport in BBMV was measured at 37°C in the presence of NaSCN, using the rapid filtration stop technique, as described previously (31). The uptake was initiated by the addition of 100 μl of incubation medium containing 100 mM NaSCN, 100 mM mannitol, 20 mM HEPES-Tris, pH 7.4, 0.1 mM MgSO₄, 0.02% (wt/vol) NaSCN, and 0.1 mM β-(U-14C)glucose, to 20 μg BBMV protein. After 3 s, 1 ml of ice-cold stop buffer, containing 150 mM KCl, 20 mM HEPES-Tris, pH 7.4, 0.1 mM MgSO₄, 0.02% (wt/vol) NaSCN, and 0.05 mM chlorazin (specific competitive inhibitor of SGLT1), was added. A 0.9-ml portion of the reaction mixture was removed and filtered, under vacuum, through a 0.22-μm pore cellulose acetate/nitrate filter (GSTF02500; Millipore). The filter was then washed with 5 × 1 ml stop buffer and the radioactivity retained on the filter was measured using a scintillation counter (Beckman Coulter). The uptake at time 0 was measured by adding the stop buffer before adding the incubation medium. All time points were assayed in triplicate. To assess the activity of any facilitative glucose transporter, the initial rate of uptake of 1 mM 2-deoxy-D-glucopyranosone, a specific substrate of Na⁺-independent D-glucose transporter isoforms, was determined at 37°C in incubation medium consisting of 300 mM mannitol, 20 mM HEPES-Tris, pH 7.4, 0.1 mM MgSO₄, and 0.02% (wt/vol) NaSCN in the presence and absence of 50 μM cytochalasin B, as described previously (27). After 3 s, the reaction was stopped by the addition of 1 ml of ice-cold stop buffer containing 300 mM mannitol, 20 mM HEPES-Tris, pH 7.4, 0.1 mM MgSO₄, 0.02% (wt/vol) NaSCN, and 0.1 mM cytochalasin B. A 0.9-ml aliquot of the reaction mixture was removed and treated as above.

Measurement of disaccharide transport activity. The specific activities of sucrose and lactase were measured, using either sucrose or lactose as substrate, as described previously (31–33). The concentration of the released glucose was determined using a commercially available kit (Boehringer-Mannheim). Lactase was assayed in the presence of 0.2 mM p-chloromercuribenzoate to inhibit any potential lysosomal β-galactosidase activity.

Quantitative immunodetection of SGLT1. The abundance of SGLT1 protein was measured by quantitative Western blotting as described previously (9, 12). The protein contents of BBMV were separated on an 8% polyacrylamide gel containing 0.1% (wt/vol) SDS and electrotransferred to polyvinylidene difluoride (PVDF) membrane (Immun-Blot PVDF, Bio-Rad). Prestained molecular weight markers (SeeBlue, Novex) permitted quantitative estimation of the efficiency of transfer.

A standard calibration curve (0.01–6 pmol) was constructed by slot blotting the synthetic peptide, amino acids 402–420 of the SGLT1 sequence, to which the antibody was raised, onto PVDF membrane, and this was probed concurrently with the BBMV samples. The specific immunoreactive band was blocked when antibodies were preincubated with the immunizing peptide. The membranes were developed using the enhanced chemiluminescence system (Amersham,
Little Chalfont, UK) and exposed to film (BioMax MS, Kodak).

The intensity of the immunoreactive bands detected in the BBMV and the peptide standard samples was quantified using scanning densitometry (Phoretix 1D, Nonlinear Dynamics), and the abundance of SGLT1 protein per milligram of BBM protein was calculated from the peptide standard curve.

Western blot analyses of GLUT5, disaccharidases, and structural proteins. The PVDF membrane used for Western blotting (see above) was stripped of SGLT1 antibody by washing three times, 10 min for each wash, in an acidic buffer (137 mM NaCl and 20 mM glycine-Cl, pH 2.5). The membrane was rinsed in Tris-buffered saline containing 0.05% (vol/vol) Tween 20 (TBTS), and the samples were then blotted for GLUT5 using an antibody raised against the COOH terminus of human GLUT5 at 1:1,000 dilution in TTBS (Sigma, Poole, UK). The stripping procedure was repeated after each blot.

RNA isolation. Total RNA was isolated from each individual biopsy using the RNeasy kit (Qiagen). RNA was quantified spectrophotometrically, and the quality was assessed by denaturing agarose gel electrophoresis (39). RNA yields were 16.3 ± 1.3 μg/biopsy (n = 14) from control subjects and 15.6 ± 1.4 μg/biopsy (n = 14) from diabetic patients.

Northern blot analysis. RNA samples, 5 μg per lane, were separated on 1% agarose gels containing 0.66 M formaldehyde and transferred to a nylon membrane (Hybond N, Amersham). RNA integrity and equality of loading were determined by methylene blue staining. The nylon membrane was prehybridized for 3 h at 42°C in a buffer containing 50% (vol/vol) deionized formamide, 5 × SSC, 6 × Denhardt’s reagent, 0.2% (wt/vol) SDS, 10% (wt/vol) dextran sulfate, 2.5 mM sodium pyrophosphate, 25 mM MES, pH 6.5, and 0.01% (vol/vol) antifom B (Sigma). cDNA probes (25 ng) generated by PCR were labeled with [α-32P]dCTP by random priming and hybridized with the membrane for 16 h at 42°C. Washes were performed at 55°C for 15 min, once with 5 × SSC, 0.5% (wt/vol) SDS, and 0.25% (wt/vol) sarkosyl and three times with 0.1 × SSC and 0.1% (wt/vol) SDS. The membrane was exposed to X-ray film (BioMax MS, Kodak), with a single intensifying screen, at −80°C for 6 h. The intensity of the transcripts was quantified using scanning densitometry (Phoretix 1D Quantifier, Nonlinear Dynamics).

Results

Duodenal biopsies removed from both control subjects and diabetic patients were of similar weight (5.4 ± 1.1 and 5.2 ± 1.5 mg, respectively). This was reflected in the values for total protein recovered from each biopsy, which were 1.25 ± 0.4 and 1.1 ± 0.3 mg for control subjects and diabetic patients, respectively.

Monosaccharide transport. The results for the initial rates of d-glucose transport by the isolated BBMV are presented in Fig. 1. Initial rates of d-glucose uptake were 26.9 ± 2.9 and 85.1 ± 16.3 pmol·s−1·mg−1 in control and diabetic BBMV, respectively. The initial rate of d-glucose uptake was 3.3-fold higher (P < 0.05) in BBMV isolated from biopsies from diabetic patients vs. those from controls.

It has been proposed (19, 20) that GLUT2, a BLM monosaccharide transporter, is present on the luminal membrane of rat jejunal enterocytes and that the activity and abundance of this protein are enhanced in the intestines of rats with streptozotocin-induced diabetes. To assess the potential presence of GLUT2 in BBMV isolated from control subjects and diabetic patients, the uptake of 1 mM 2-deoxy-d-glucose was measured in the presence and absence of 50 μM cytochalasin B. There was no cytochalasin B-sensitive

![Initial rate of d-glucose uptake in brush-border membrane vesicles (BBMV) prepared from duodenal biopsies of control subjects and diabetic patients. The initial rate of 0.1 mM d-glucose transport in BBMV was measured at 37°C in the presence of NaSCN, using the rapid filtration stop technique, as described previously (Ref. 31). Values are means ± SE of 8 experiments carried out in triplicate (P = 0.012).](http://ajpgi.org/)

Fig. 1. Initial rate of d-glucose uptake in brush-border membrane vesicles (BBMV) prepared from duodenal biopsies of control subjects and diabetic patients. The initial rate of 0.1 mM d-glucose transport in BBMV was measured at 37°C in the presence of NaSCN, using the rapid filtration stop technique, as described previously (Ref. 31). Values are means ± SE of 8 experiments carried out in triplicate (P = 0.012).

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Determination of abundance of monosaccharide transport proteins. To determine whether the increase in SGLT1 activity seen in the diabetic samples was due to an increase in the levels of SGLT1 protein, we performed Western blot analysis. As depicted in Fig. 2, the antibody recognizes a single protein of 78 kDa (12), corresponding to SGLT1, in the samples from both control subjects and diabetic patients. This band was specifically blocked by preabsorbing the primary antibody with the immunizing peptide (data not shown). A 4.3-fold increase \( (P < 0.01) \) in the abundance of SGLT1 in the BBMV isolated from diabetic patients \( (85.2 \pm 13.4 \text{ pmol/mg protein}, n = 6) \) compared with controls \( (20.0 \pm 5.6 \text{ pmol/mg protein}, n = 6) \) was determined by quantitative Western blotting, using the synthetic peptide to which the SGLT1 antibody was raised as a standard \( (9) \). The abundance of the fructose transporter GLUT5 was also determined in the same population of BBMV by Western blotting. The results (Fig. 2) indicate that the level of the fructose transporter GLUT5 is also increased significantly, being 4.1-fold higher \( (P < 0.01) \) in diabetic patients compared with control subjects.

Western blot analysis, with an antibody raised against the COOH-terminal region of human GLUT2 \( (37) \), did not cross-react with any proteins in the BBMV from either control subjects or diabetic patients. However, the antibody reacted with a protein of 55 kDa in BLMV isolated from resected pieces of human small intestine. There was no cross-reaction of GLUT2 antibody with any proteins in BBMV isolated from the same resected segments of human intestine (Fig. 3). The latter data indicate that GLUT2 is not expressed on the luminal membrane of intestine from healthy and/or diabetic humans. The data also show that the BBMV isolated from the human intestine are free from any BLM contamination.

Abundance and activities of disaccharidases in intestinal BBMV from diabetic patients and control subjects. Having demonstrated that there is a significant increase in the levels of SGLT1 and GLUT5 proteins in the intestine of diabetic patients compared with the intestine of healthy individuals, we sought to determine whether this increase is specific or the result of a generalized change in the levels of BBMV proteins. Therefore, the levels of the disaccharidases lactase and sucrase were measured in the same BBMV populations, and the results are presented in Fig. 4. As assessed by densitometric analyses (Fig. 4), sucrase levels \( (277.8 \pm 26.4 \text{ in control subjects vs. } 391 \pm 42.9 \text{ in diabetic patients}) \) increased by 1.4-fold \( (P < 0.1) \) and lactase abundance \( (195.1 \pm 34.8 \text{ in control subjects vs. } 294.9 \pm 43.2 \text{ in diabetic patients}) \) was enhanced 1.5-fold \( (P < 0.05) \). Sucrease and lactase activities were measured in the same BBMV populations and were shown to be increased in the BBMV of diabetic patients by 2.5- and 2.2-fold, respectively, compared with controls (Table 1), indicating that the increase in the

![Fig. 2. Western blot analysis of SGLT1 and GLUT5 expression in BBMV isolated from duodenal biopsies of control subjects (C) and diabetic patients (D). BBMV proteins (7 μg/lane) were separated on 8% (wt/vol) polyacrylamide gels containing 0.1% (wt/vol) SDS and then electrotransferred to polyvinylidene difluoride (PVDF) membrane (Immun-Blot PVDF, Bio-Rad). SGLT1 was quantified as described previously (Ref. 9). The intensity of the immunoreactive bands detected in BBMV from the control and diabetic samples was quantified using scanning densitometry (Phoretix). For SGLT1, values are means ± SE given as pmol SGLT1 protein/mg BBMV protein \( (n = 6) \). For GLUT5, values are means ± SE given as densitometric abundance \( (n = 6) \). Mm, molecular mass.](image1)

![Fig. 3. Western blot analysis of GLUT2 expression in basolateral membrane vesicles (BLMV) and BBMV isolated from human small intestinal resections and biopsies. BBMV and BLMV proteins (10 μg/lane) were separated on 8% (wt/vol) polyacrylamide gels containing 0.1% (wt/vol) SDS and then electrotransferred to PVDF membrane (Immun-Blot PVDF, Bio-Rad). Membranes were probed with a polyclonal antibody raised against human GLUT2. Lane 1, BBMV isolated from a resected piece of human proximal small intestine; lane 2, BLMV isolated from a section of the same tissue as lane 1; lane 3, BBMV isolated from duodenal biopsies of control subjects, see Fig. 2 also; lane 4, BBMV isolated from duodenal biopsies of diabetic patients, see Fig. 2 also.](image2)

![Fig. 4. Western blot analysis of disaccharidase expression in BBMV isolated from duodenal biopsies of control subjects (C) and diabetic patients (D). The PVDF membrane used in Fig. 2 was stripped and reprobed with monoclonal antibodies to sucrase and then lactase, as described in METHODS. The intensity of the immunoreactive bands detected in BBMV from the control and diabetic samples was quantified using scanning densitometry (Phoretix). Values are means ± SE given as densitometric abundance \( (n = 6) \).](image3)
abundance of these disaccharidases is due to an enhanced functional protein. There was no difference in the enrichment or recovery values between control and diabetic groups, indicating that the differences in activity and abundance are not due to variation in the purity of the BBMV.

The 1.4- to 1.5-fold increase in the levels of disaccharidases indicates that enhancement in the levels of BBM proteins could be, in part, due to a generalized increase in protein levels in the intestine of diabetic patients. It is noteworthy, however, that this increase is half that of the overall increase in SGLT1 and GLUT5 abundance detected in the BBMV isolated from the intestine of diabetic patients.

Abundance of structural proteins β-actin and villin in intestinal BBMV from diabetic patients and control subjects. To determine whether the changes in the BBM protein levels in the intestine of diabetic patients are related to any modification in the intestinal structure, we measured the abundance of the structural proteins β-actin and villin. The results are given in Fig. 5. The abundance of β-actin was 247.9 ± 80.4 U in BBMV isolated from the intestine of control subjects and 515.8 ± 102.6 U in diabetic patients, representing a 2.1-fold increase (P < 0.005) in β-actin in the BBMV samples from the diabetic patients. A similar 2.2-fold increase (P < 0.1) was seen in villin levels; the abundance was 26.2 ± 4.2 vs. 58.9 ± 11.6 U in control subjects vs. diabetic patients.

Northern blot analysis. To determine if changes in the monosaccharide transporter protein levels correlated with any alterations in levels of the corresponding mRNA, we performed Northern blotting of RNA samples isolated from control and diabetic biopsies. The results are presented in Fig. 6. RNA loading, as assessed by 18S rRNA, was similar in all samples and indicated that there was no significant difference in the levels of 18S rRNA between diabetic and control samples. To correct for negligible variations in loading, we normalized all densitometry values to 18S rRNA signals.

The human SGLT1 cDNA probe hybridized to three transcripts in all samples, a major transcript of ~4.8 kb and two minor transcripts of 2.6 and 2 kb, consistent with previous reports (18). Densitometric analysis indicated a significant 3.1-fold increase (P < 0.05) in total transcript abundance in the RNA samples isolated from the intestine of diabetic patients. The human GLUT5 cDNA probe identified three transcripts (4) of ~3.8, 2, and 1.5 kb in both control and diabetic RNA samples. GLUT5 mRNA levels were threefold higher (P < 0.05) in diabetic samples compared with controls. The human GLUT2 cDNA probe hybridized to three transcripts of 5.4, 3.4, and 2.9 kb, consistent with previous reports (4). GLUT2 mRNA levels were threefold higher (P < 0.05) in diabetic samples compared with controls. There was no significant difference in the levels of β-actin mRNA (55 ± 5 vs. 56 ± 5 arbitrary units for control subjects vs. diabetic patients, respectively) between the two groups.

DISCUSSION

NIDDM or type II diabetes is a heterogeneous disorder caused by a combination of genetic predisposition and environmental factors (16). One factor that appears to influence the risk of developing NIDDM is the excessive consumption of diets containing high levels of carbohydrates. A better understanding of the mechanisms involved in the absorption of monosaccharides in human small intestine and ways in which they may be controlled in diabetic patients may contribute to a better management of the disease. Using a technique developed in our laboratory, we isolated purified BBMV from intestinal biopsies of control and diabetic individuals. BBMV prepared using this technique originate from villus enterocytes and are free from significant contamination by basolateral and organelle membranes (31). We investigated, for the first time, levels of

<table>
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<tr>
<th>Specific activity</th>
<th>Enrichment</th>
<th>Recovery</th>
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<tr>
<td><strong>Sucrase</strong></td>
<td></td>
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<tr>
<td>Control</td>
<td>0.078 ± 0.005</td>
<td>7.8 ± 0.5</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.274 ± 0.046</td>
<td>7.9 ± 1.2</td>
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<td><strong>Lactase</strong></td>
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<tr>
<td>Control</td>
<td>0.072 ± 0.013</td>
<td>8.8 ± 1.6</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.155 ± 0.033</td>
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Values are means ± SE of experiments carried out in duplicate. Results are given as μmol·min⁻¹·mg protein⁻¹, except for recovery, which is given in %. For control sucrase activity, n = 5 and for control lactase activity, n = 8. For diabetic groups, n = 6. The specific activities of sucrase and lactase were measured in brush-border membrane vesicles (BBMV), as described previously (Ref. 31). Glucose concentrations are given in %. For control sucrase activity, the purity of the BBMV.
expression of monosaccharide transporters in the intestine of humans suffering from NIDDM.

The abundance of SGLT1 is increased significantly (4.3-fold) in the BBMV prepared from duodenal biopsies of patients with NIDDM compared with healthy controls. The abundance of the fructose transporter GLUT5 is also increased more than fourfold in the NIDDM intestinal BBMV. It appears therefore that the absorption of monosaccharides is enhanced in the intestine of individuals with diabetes. Northern analyses, using cDNA probes derived from the human SGLT1, GLUT2 and GLUT5 coding regions, revealed that in the intestine of diabetic patients there are similar threefold increases in SGLT1, GLUT5, and GLUT2 mRNA levels when normalized against 18S rRNA signals. The similarity in the magnitude of the increases in SGLT1 and GLUT5 mRNA and protein suggest that in NIDDM there is an increase in the abundance of SGLT1 and GLUT5 mRNAs and that these in turn give rise to a corresponding increase in levels of SGLT1 and GLUT5 proteins. To measure the abundance of GLUT2 protein by quantitative Western blotting requires the isolation of BLM; it is not possible to prepare BLM from the intestinal biopsy samples. Our data clearly demonstrate that the levels of GLUT2 mRNA are threefold higher in the intestine of patients with NIDDM compared with controls. In the intestine of the diabetic human, the increases in the abundance of the luminal monosaccharide transport proteins, SGLT1 and GLUT5, are twofold greater than those of the disaccharidases, the core microvillar structural protein villin, and β-actin. Although there is no significant difference in biopsy weight and total protein recovery between the control and diabetic groups, a 1.5-fold increase in the levels of the structural proteins could indicate diabetes-induced changes in intestinal structure, as observed in the villus hyperplasia reported in diabetic rats (14).

There are reports (6, 8, 13, 25) that mucosal hyperplasia occurs in rats with chronic diabetes, resulting in increased villus height. Hyperplasia alone could result in increased glucose absorption without any qualitative change in intestinal absorptive properties (25) due to increased absorptive surface. Fedorak and co-workers (13) measured increased induction of glucose transporters at the BBM, suggesting that diabetes may lead to more glucose transporters per cell. It has since been suggested (5, 6, 8) that in rats diabetes leads to an increased number of functionally mature enterocytes with a longer lifespan, coupled with a premature expression of hexose transporters in lower villus enterocytes. All of the above studies, however, are limited to streptozotocin-treated rats. Our results suggest that, although structural change may play a part in the diabetes-induced enhancement in intestinal monosaccharide absorption, there is also a further increase in the expression of both SGLT1 and GLUT5 protein abundance above this effect. There was no significant difference in the levels of β-actin mRNA between the control and diabetic samples, suggesting that regulatory mechanisms different from those responsible for the increases in SGLT1 and GLUT5 expression may be involved in the changes in β-actin protein abundance observed in the intestine of diabetic humans.

It has been proposed (20, 23) that GLUT2 is trafficked to the luminal membrane of rat jejunum, where it is responsible for the passive component of glucose absorption across the BBM, and in streptozotocin-induced diabetes the level of GLUT2 remaining in the BBM is increased, resulting in an enhanced capacity to absorb glucose. In our studies, BBMV isolated from the intestines of diabetic patients or healthy individuals could transport glucose in a Na⁺-dependent manner.

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**Fig. 6.** Northern blot analysis of total RNA isolated from duodenal biopsies of control subjects (C) and diabetic patients (D). Total RNA (5 μg/lane) was separated on a 1% agarose denaturing gel and transferred to nylon membrane (Hybond N, Amersham). Samples were Northern blotted with a 32P-labeled human SGLT1 cDNA probe, as described in METHODS. Membranes were sequentially stripped and reprobed for human GLUT5, β-actin, 18S rRNA, and human GLUT2. Autoradiographs were scanned, and signals from the control and diabetic samples were quantified densitometrically (Phoretix). Values are means ± SE given as densitometric units (n = 5).
There was, however, no cytochalasin B-sensitive 2-deoxy-b-glucopyranoside uptake in the same population of BBMV isolated from either control subjects or diabetic patients. Western analysis indicated the absence of GLUT2 protein in BBMV isolated from duodenal biopsies of both groups. It was notable that the antibody to human GLUT2 reacted with a specific protein of \( \sim 55 \text{ kDa} \) (36) in the BLMV but not BBMV isolated from human small intestine. This is not surprising, as it has been shown that in glucose-galactose malabsorption, resulting from mutations within the SGLT1 gene (24, 38), human infants cannot absorb glucose or galactose. Fructose, however, can be absorbed normally (38), indicating the presence of a transcellular route, via luminal GLUT5 and basolateral GLUT2, for the absorption of fructose. The presence of GLUT2 on the luminal membrane would forestall the chronic and potentially fatal diarrhea experienced by these children, suggesting that the expression of GLUT2 on the luminal membrane does not extend to humans. Work in our laboratory (29) using ovine intestine also indicates the absence of a functional facilitative glucose transporter on the luminal membrane of enterocytes.

Our results indicate that there is an increase in the expression of intestinal monosaccharide transporters in humans suffering from NIDDM. Although it is well established that in NIDDM one of the major defects is in the utilization of blood glucose by the peripheral tissues, the increased ability of the intestine to absorb monosaccharides further complicates the pathophysiology of this disease. It was noted that administration of hypoglycemic drugs, sulfonylureas and biguanides, did not affect the increased levels of monosaccharide transporters in the intestine of diabetic patients. Sulfonylurea drugs, gliclazide or glibenclamide, act mainly by augmenting insulin secretion (21). The biguanide drug metformin has a different mode of action; it exerts its effect mainly by decreasing gluconeogenesis and by increasing the peripheral utilization of glucose (21). This suggests that the enhanced expression of monosaccharide transporters is independent of any changes in blood glucose or insulin levels and may be due to alterations in the mechanisms and signaling pathways involved in regulating expression of the genes encoding for enterocyte sugar transporters. Although the apparent molecular mechanism involved is a coordinated increase in the levels of sugar transporter protein and mRNA, the molecular basis of this increase requires further investigation.

We thank Dr. D. Swallow (London, UK) for the lactate antibody, Dr. G. Holman (University of Bath) for the GLUT5 antibody, Dr. G. Gould (University of Glasgow) for the human GLUT2 cDNA, Dr. J. Hesketh (University of Newcastle-upon-Tyne) for the 18S rRNA cDNA, Dr. S. Maroux (Marseilles, France) for the sucrose antibody, and Dr. J. Ball (University of Cincinnati) for the Na\(^+\)/K\(^+\)-ATPase antibody.

This work was supported in part by a University of Liverpool Research Development Fund and by Biogenesis UK Limited.

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