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Dietary lipid modulation of Na^+/glucose co-transporter (SGLT1), Na^+/K^+ ATPase, and ornithine decarboxylase gene expression in the rat small intestine in diabetes mellitus

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Nutrient transport is increased in streptozotocin-induced diabetes mellitus (DM) in rats. Variations in dietary lipid composition have been shown to modulate nutrient transport during intestinal adaptation in DM. Here, we examined Na^+/glucose cotransporter (SGLT1), Na^+,K^+-ATPase and ornithine decarboxylase (ODC) gene expression in the small intestine in DM. One week after the animals had been given streptozotocin or injected with vehicle, they were randomly allocated to receive either standard chow or semisynthetic isocaloric diets with triglycerides enriched with polyunsaturated fatty acid (PUFA) or saturated fatty acid (SFA). Two weeks later they were killed and brush border membrane (BBM) and basolateral membrane (BLM) fractions and RNA were isolated from the mucosa of the proximal and distal small intestine. Western and Northern blotting were performed with antibodies and cDNA probes specific to SGLT1, the Na^+,K^+-ATPase α_1 and β_1 subunit isoforms, and ODC. Increased levels of immunodetectable SGLT1, Na^+,K^+-ATPase α_1 and β_1 subunit, and ODC expression and their corresponding mRNAs were observed in DM versus control rats fed chow. All of these parameters were increased in diabetic and in nondiabetic rats fed SFA as compared with PUFA. These findings suggest that the increased glucose uptake in diabetes, and in response to feeding a saturated as compared with a polyunsaturated diet, is achieved by transcriptional events that modulate transport function and cell proliferation. (J. Nutr. Biochem. 8:673–680, 1997) © *Elsevier Science Inc. 1997*

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Introduction

The intestinal uptake of glucose is mediated by the Na^+/glucose transporter (SGLT1) in the intestinal brush border membrane (BBM) (reviewed in Ref. 1) SGLT1 activity is up-regulated in animals with streptozotocin-induced diabetes mellitus (reviewed in Ref. 2). The associated diabetic changes, including intestinal mucosal hyperplasia, weight loss, hyperglycemia, and glucosuria return toward baseline as the enhanced absorption of glucose is normalized in diabetic rats fed an isocaloric semisynthetic polyunsaturated diet as compared with saturated diet.[3,4]

Adaptive changes in SGLT1 activity occur in chow-fed diabetic rats,[5–8] and the increased maximal transport rate

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(Vmax) for glucose uptake is associated with increased abundance of SGLT1 protein and mRNA,[9] as well as increased activity and abundance of Na^+/K^+ ATPase protein and its corresponding mRNA.[10] The Na^+/K^+ ATPase is responsible for establishing and maintaining the Na^+ gradient required for the activity of the Na^+/glucose cotransporter, SGLT1.[11] The intestinal mucosal hyperplasia seen in experimental diabetes mellitus is associated with increased levels of ornithine decarboxylase activity,[12] the rate-limiting enzyme in the biosynthesis of polyamines (reviewed in Ref. 13).

The present study was undertaken to determine the effects of semisynthetic isocaloric saturated fatty acid (SFA) and polyunsaturated fatty acid (PUFA) lipid-containing diets on the expression of SGLT1 and Na^+/K^+ ATPase in this model system. The results suggest that the increased glucose uptake in diabetes and in response to feeding a saturated as compared with a polyunsaturated diet, is achieved by both transcriptional and posttranscriptional events that modulate transport function and cell proliferation.

Methods and materials

Animals and diets

Adult male Sprague Dawley rats weighing 200–250 g were assigned randomly to receive either streptozotocin (75 mg/kg) or normal saline intraperitoneally. The animals were fed standard Purina rat chow for 1 week, at which time the blood glucose was taken to ensure that the animals were diabetic (i.e., fasting blood glucose >250 mg/dL). Then on day 8, the diabetic or nondiabetic control animals were randomized to be fed standard rat chow, or an isocaloric semisynthetic SFA or PUFA diet.[3] These diets were continued for 2 weeks. The animals were housed in pairs and kept in a room at temperature 21°C, with 12 hr of light and 12 hr of darkness. Water and food were supplied ad libitum. The principles for the care and use of laboratory animals approved by the Canadian Federation of Biological Societies were observed.

Tissue preparation

One week after the animals had been given streptozotocin or vehicle, they were randomly assigned to be fed chow, SFA, or PUFA for 2 weeks. They were killed by the injection of sodium thiopental (250 mg/kg body weight). Forty centimeters of jejunum and ileum beginning 2 cm distal to the ligament of Treitz and proximal ileocecal valve, respectively, were flushed with ice-cold 0.9% saline and opened along the mesenteric border. Jejunal and ileal mucosal epithelial cells were harvested by gently scraping the intestinal mucosa with a spatula. The mucosal scrapings were snap frozen in liquid nitrogen and stored at −70°C until the time of assay.

Preparation of membrane fractions

BBM were isolated from the jejunal and ileal mucosa as described elsewhere.[14] All steps were performed at 4°C. Mucosal scrapings were homogenized using a Polytron homogenizer in an ice-cold buffer containing 500 mM mannitol, 10 mM HEPES buffer (pH 7.4) in the presence of a protease inhibitor cocktail (aprotinin, 5 μg/mL; leupeptin, 2.5 μg/mL; phenylmethylsulfonyl fluoride, 0.5 μM/mL). The homogenate was diluted 6-fold with deionized water, to which 1 M $MgCl_2$ was added to a final concentration of 10 mM. The suspension was agitated for 20 min, and then centrifuged at 3,000 × g for 15 min. The supernatant was then centrifuged at 20,000 × g for 30 min, and the resulting pellet was resuspended in a 100 mM mannitol, 10 mM HEPES (pH 7.4). The final pellet obtained after centrifugation at 20,000 × g for 30 min was resuspended in 10 mM Tris (pH 7.4) and aliquots were stored at −70°C. The final pellet was enriched 15- to 18-fold in sucrase activity with respect to the whole homogenate.

A crude basolateral membrane (BLM) fraction was obtained by a series of differential centrifugation steps.[15] All steps were performed at 4°C. Mucosal scrapings were homogenized using a Polytron homogenizer in a buffer containing 0.25 M sucrose, 150 mM NaCl, 30 mM Tris (pH 7.5) in the presence of the same protease inhibitor cocktail used for the preparation of BBM. The homogenate was centrifuged for 15 min at 6,000 × g, the pellet was rehomogenized in the same buffer and centrifuged for 15 min at 6,000 × g. The supernatant was then centrifuged for 45 min at 45,000 × g to yield a final BLM fraction. The final pellet was resuspended in homogenization buffer, and aliquots were stored at −70°C. The BLM fraction was enriched 10- to 12-fold in Na^+,K^+-ATPase activity over the initial homogenate, and contained negligible amounts of sucrase activity.

The activities of sucrase, Na^+/K^+ ATPase and ODC were measured as described previously,[16] and protein was measured using the Bio-Rad protein assay reagent.

Immunoblotting

Aliquots of BBM (for SGLT1) and BLM (Na^+/K^+ ATPase) fractions, and of the final supernatant (ODC) after BLM preparation (80 μg protein), were separated by SDS-PAGE (7.5% gradient resolving gel) and were transferred to nitrocellulose according to the method of Towbin et al.[17] Alternatively, aliquots (15 μg) of protein were immobilized on nitrocellulose membrane using a slot blot manifold (GIBCO BRL). In each instance, the efficiency of protein transfer was verified by Ponceau S staining of the membranes. The nitrocellulose membranes were incubated overnight in Tris-buffered saline with 0.5% Tween 20 (TTBS, pH 7.5) containing 5% (w/v) skim milk to block nonspecific protein binding sites. The membranes were then probed for 2 hr at room temperature with the following antibodies: anti-SGLT1 (raised against the C terminal amino acids 564–575 of the rabbit SGLT1 sequence, and generously provided by Dr. K. Takata, Tokyo), affinity purified rabbit anti-rat α_1 and β_1 Na^+/K^+ ATPase isoform specific antisera (Upstate Biotechnology, Lake Placid, NY USA), and anti-rat specific ODC antisera (generously provided by Dr. A.E. Pegg, Hershey, Pennsylvania).

After incubation in primary antibody, the membranes were washed briefly with TTBS (pH 7.5) to remove any unbound primary antibody. The reaction product was visualized after incubation for 1 hr at room temperature with [^{125}I] goat anti rabbit IgG (New England Nuclear, 1:1500 dilution) and X-ray exposure (Kodak XAR 5 film) for 24 hr with an intensifying screen at −70°C. The molecular weights of the identified proteins were determined by comparison with prestained molecular weight markers that were run on the same gels. The fluorograms were quantitated by densitometry, and the signals were normalized to those present in controls fed chow which were assigned a value of 1.0.

RNA isolation, northern, and slot blot analysis

RNA was isolated according to the method of Chomczynski and Sacchi.[18] Equal amounts (20 μg) of total RNA were denatured with formamide, fractionated on agarose-formaldehyde gels, and transferred to positively-charged nylon membranes (Boehringer Mannheim) using conventional capillary blotting techniques.[19] UV inspection of the gel after ethidium bromide (10 μg/mL)

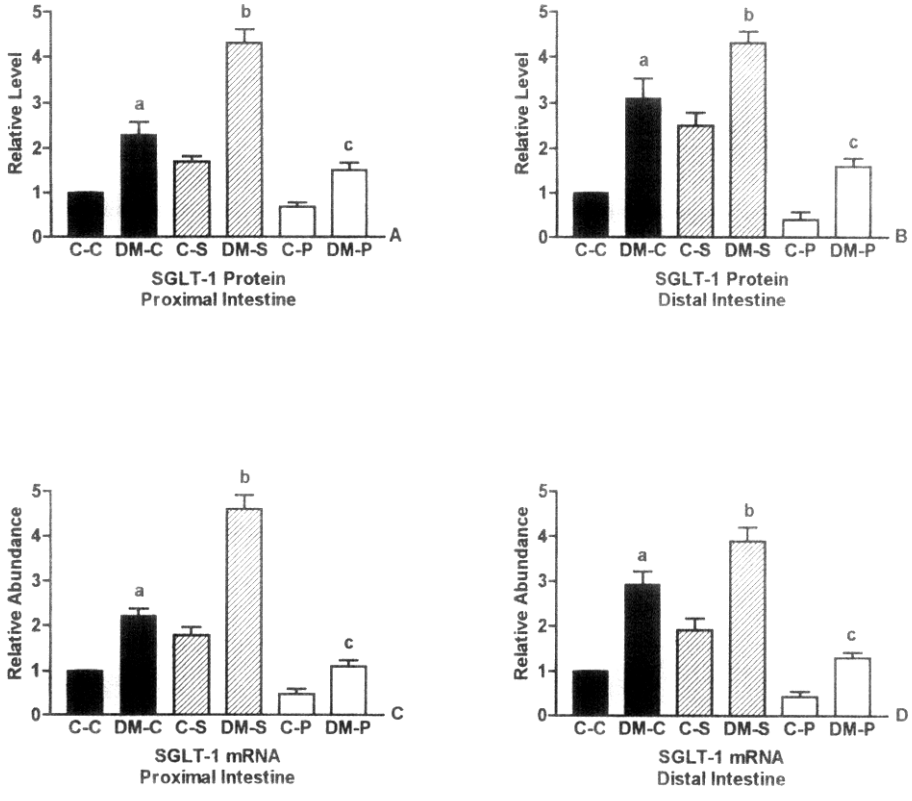

Figure 1 Levels of SGLT1 protein and SGLT1 mRNA abundance in the proximal (panels a and c) and distal (panels b and d) small intestine in control (C) and diabetic (DM) rats fed standard chow (C), saturated fatty acid (S), or polyunsaturated (P) fatty acid containing isocaloric diets. a,b,c = $P < 0.05$ compared with levels measured in corresponding controls.

staining confirmed both the presence of equivalent amounts of 18S and 28S ribosomal RNA per lane, and as well confirmed the integrity of the RNA.

The following cDNA probes were used: a 2.1 kb *Eco* RI SGLT1 fragment (Dr. N.O. Davidson, University of Chicago), 300 bp *Eco* RI − *Pst* I Na+/K+ ATPase $\alpha 1$ and *Nco* I − *Stu* I β_1 fragments (Dr. J. Lingrel, University of Cincinnati), and a 1.9kb *Eco* RI ODC fragment (Dr. Perry J. Blackshear, Howard Hughes Institute, North Carolina). The cDNA fragments were labeled with digoxigenin labeled dUTP (Boehringer Mannheim) by the random primer procedure described by Feinberg and Vogelstein.[20] The membranes were prehybridized (7% SDS, 50% formamide, 5X SSC, 2% blocking reagent (Boehringer Mannheim), 50 mM sodium phosphate (pH 7.0), 0.1% *N*-laurylsarcosine) at 42°C for 4 hr. Hybridization was performed for 12 to 16 hr at 42°C in the same solution containing 50 ng/mL of digoxigenin-labeled probes. The posthybridization washes and detection steps were performed as described elsewhere.[21] The RNA for slot-blot analysis was treated in a similar manner. Samples containing 6 μg RNA were blotted on nylon membrane (Boehringer Mannheim) using a slot-blot manifold (GIBCO BRL). Quantitation of the relative levels of abundance of the specific mRNAs were quantitated by densitometry, using a Hoefer Scientific Instruments scanning densitometer as described elsewhere.[22] Each blot was additionally probed with a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA (American Type Culture Collection, Rockville, MD USA), a constitutively expressed transcript, to normalize for minor variations in the amount of RNA loaded per sample. The signal intensity of each specific mRNA was expressed relative to the level of mRNA abundance determined in controls fed chow which was assigned a value of 1.0. For slot-blot analysis, a minimum of four fluorograms was evaluated for each data point. Finally, RNA preparations of control and diabetic rats were fractionated on the same agarose gel or slot-blot to control for intra-assay variability.

Data analysis

The results were expressed as mean ± SEM. The statistical significance of the differences between groups was determined by analysis of variance (ANOVA) with the minimum level of significance set at $P < 0.05$.

Results

SGLT1 protein expression and mRNA abundance

The levels of immunodetectable SGLT1 proteins expressed in the jejunal and ileal mucosa of control and diabetic rats were determined by Western blot analysis of BBM fractions (*Figure 1*). Immunoblotting with polyclonal SGLT1 antisera revealed a 74-kDa protein species as described elsewhere.[1]

Quantitation of immunoblots demonstrated increased levels of SGLT1 in the jejunum and ileum in diabetic (DM), as compared with control animals (C) fed chow (*Figure 1* a,b). Feeding nondiabetic control rats SFA or PUFA increased and decreased the levels of SGLT-1, respectively, in both regions of the small intestine. The difference reached statistical significance only in the distal small intestine. The levels of SGLT-1 in both regions in the DM groups were significantly greater than those measured in control rats on similar dietary regimens. In both control and DM rats, SGLT-1 levels were greater in the proximal as compared to the distal small intestine (data not shown).

Northern blotting with the SGLT1 cDNA probe revealed a 4.5 kb transcript.[1] In all diet groups, SGLT-1 levels were similar in the jejunum and ileum in control and DM rats (data not shown). Densitometric quantitation of slot blots

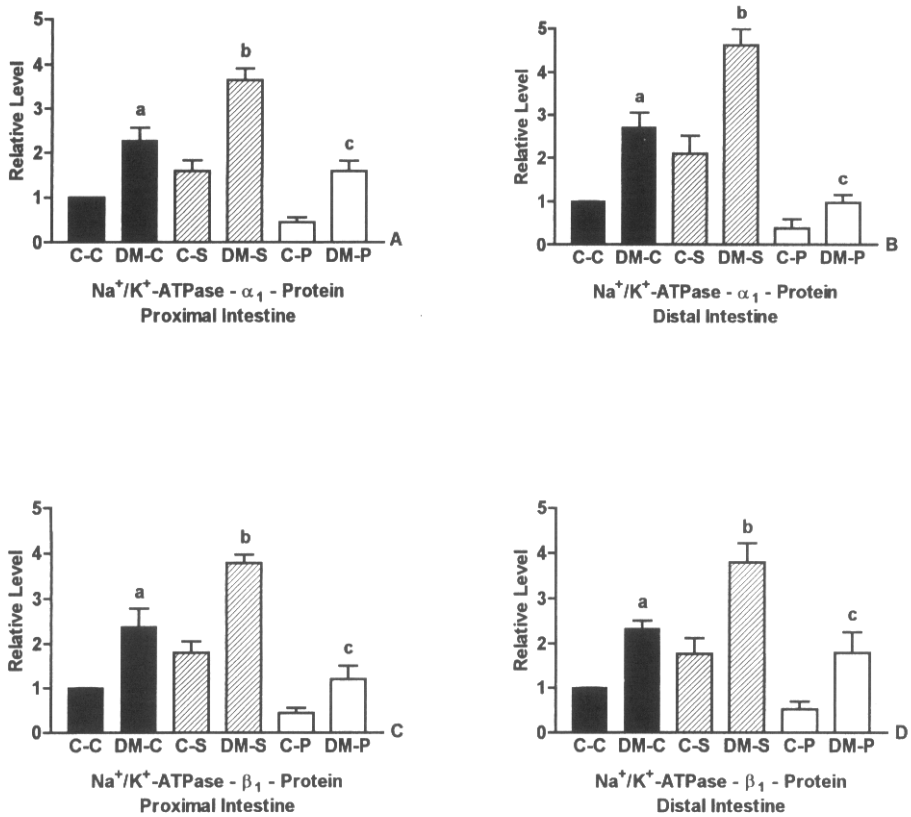

Figure 2 Levels of Na^+,K^+-ATPase α_1 and β_1 isoform protein in the proximal (panels a and c) and distal (panels b and d) small intestine in control (C) and diabetic (DM) rats fed standard chow (C), saturated fatty acid (S), or polyunsaturated (P) fatty acid containing isocaloric diets. a,b,c = $P < 0.05$ compared with levels measured in corresponding controls.

revealed important differences between control and DM groups (*Figure 1* c,d). In all dietary groups, SGLT-1 mRNA abundance was greater in the proximal and distal small intestine of DM rats as compared with the levels of SGLT-1 mRNA abundance measured in controls. In both regions of the small intestine, levels of SGLT-1 mRNA abundance in both control and DM rats were higher in rats fed SFA than in those fed PUFA.

Na^+/K^+-ATPase α_1 and β_1 subunit isoform protein expression and mRNA abundance

Immunoblotting with Na^+/K^+-ATPase α_1 isoform antisera revealed a 110-kDa protein species, whereas the β_1 antisera revealed a 55 kDa protein.[23] The levels of immunodetectable Na^+/K^+-ATPase α_1 and β_1 isoforms were greater in the proximal as compared with the distal small intestine in both control and DM rats in all of the diets examined (data not shown). Feeding control rats SFA resulted in increased levels of Na^+/K^+-ATPase α_1 and β_1 isoforms in both regions of the small intestine (*Figure 2* a–d). By contrast, feeding PUFA produced decreases in the levels of these Na^+/K^+-ATPase isoforms in the small intestine.

Compared with controls, the levels of immunodetectable Na^+/K^+-ATPase α_1 and β_1 isoforms protein was increased in all dietary groups in both the proximal and distal small intestine in DM rats (*Figure 2* a–d). The pattern of response to dietary lipids in DM rats was similar to that measured in controls for both the α_1 and β_1 Na^+/K^+-ATPase isoforms.

Northern blotting with the Na^+/K^+-ATPase α_1 and β_1 cDNA probes revealed 3.7 and 2.7 kb transcripts, respectively.[21] The levels of mRNA abundance of Na^+/K^+-ATPase α_1 and β_1 isoforms were similar in the proximal and distal small intestine in control and DM animals (data not shown). Feeding SFA to controls increased, whereas feeding PUFA decreased the levels of mRNA abundance of Na^+/K^+-ATPase α_1 and β_1 isoforms in both regions of the small intestine (*Figure 3*). A similar pattern of responses to SFA and PUFA were observed in DM rats. However, in the DM rats the levels of mRNA abundance of Na^+/K^+-ATPase α_1 and β_1 isoforms in both regions of the small intestine were greater than those measured in controls.

Finally, the levels of Na^+/K^+-ATPase enzyme activity were measured in the proximal and distal regions of the small intestine in control and DM animals (*Figure 3* e,f). The alterations in Na^+/K^+-ATPase α_1 and β_1 isoform protein levels and mRNA abundance, noted above, were paralleled by corresponding changes in Na^+/K^+-ATPase activity.

ODC protein expression and mRNA abundance

Western blots performed with the ODC monoclonal antibody disclosed a 50-kDa protein species.[13] In both groups of rats the levels of immunodetectable ODC protein were greater in the proximal as compared with the distal small intestine (data not shown).

In controls, feeding SFA increased, whereas feeding PUFA decreased the levels of immunodetectable ODC protein in the small intestine (*Figure 4* a and b). The levels of ODC protein were increased in the proximal and distal small intestine in DM rats fed chow, SFA, or PUFA compared with those levels measured in controls. Dietary

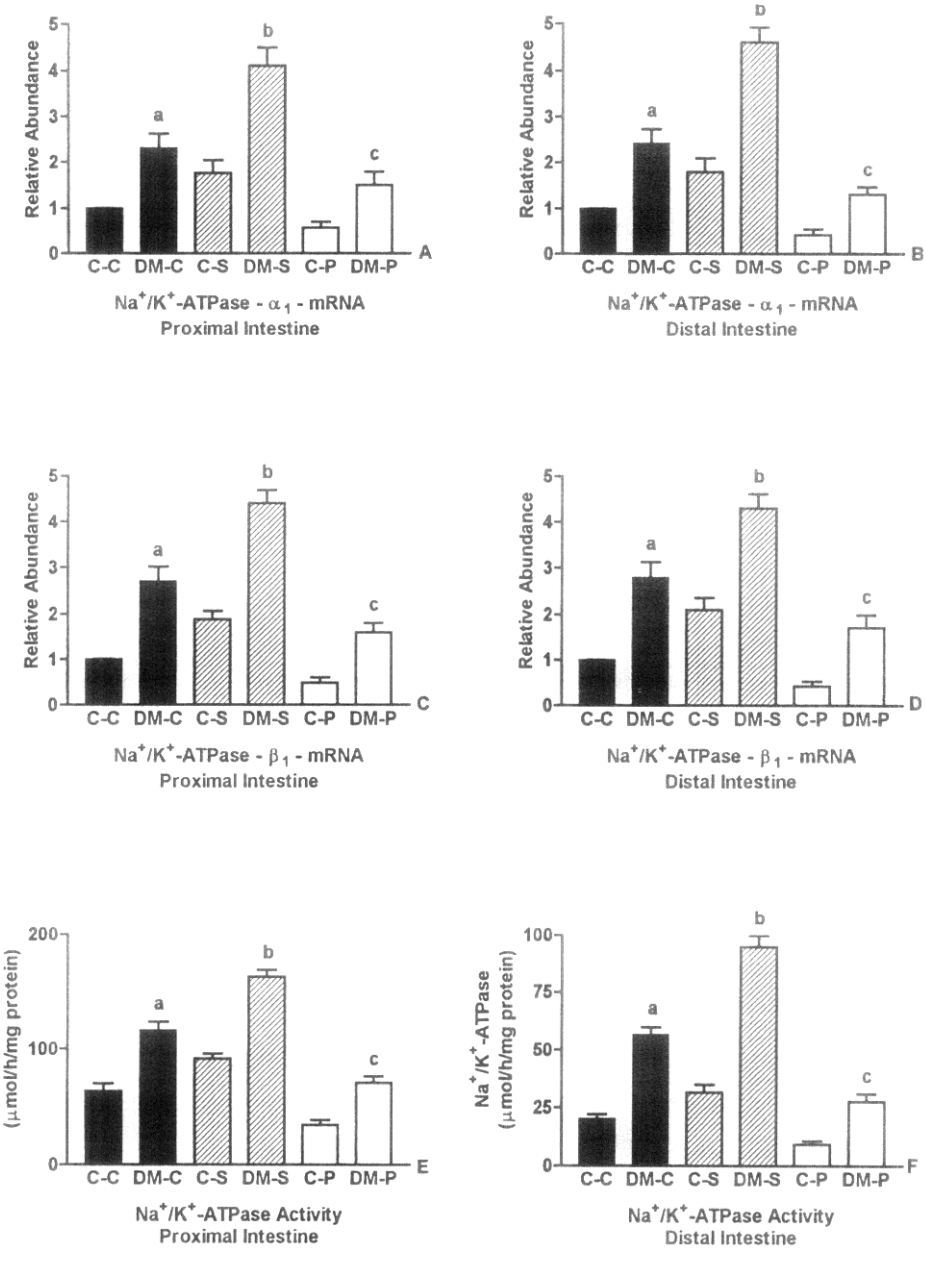

Figure 3 Levels of Na^+,K^+-ATPase α_1 and β_1 isoform mRNA abundance and Na^+,K^+-ATPase activity in the proximal (panels a,c and e) and distal (panels b,d, and f) small intestine in control (C) and diabetic (DM) rats fed standard chow (C), saturated fatty acid (S), or polyunsaturated (P) fatty acid containing isocaloric diets. a,b,c = $P < 0.05$ compared to levels measured in corresponding controls.

manipulation in DM rats produced a similar pattern of changes in ODC protein levels compared with controls.

Northern blotting with the ODC cDNA probes revealed a 2.6-kb transcript.[13] In control rats fed SFA the levels of ODC mRNA abundance were increased in the jejunum and ileum, whereas the levels of ODC mRNA abundance were decreased in control rats fed PUFA (*Figure 4* c, d). Whereas a similar pattern of responses to SFA and PUFA were observed in DM rats, the levels of ODC mRNA abundance in the jejunum and ileum were greater in the DM rats irrespective of diet. The levels of ODC mRNA abundance were greater in the proximal compared to the distal small intestine in control and DM animals (data not shown).

The ODC enzyme activities were measured in the proximal and distal regions of the small intestine in control and DM animals (*Figure 4* e,f). The alterations in immunodetectable ODC protein as well as the changes in the levels of ODC mRNA abundance were accompanied by corresponding changes in ODC activity.

Discussion

The enterocyte transporters for the major dietary sugars have been cloned, and there is a growing body of experimental evidence pertaining to their expression in the basal state and in response to dietary manipulation [reviewed in Refs. 1,22,23]. Glucose and galactose are transported across the BBM domain of the enterocyte by SGLT1.[1] By contrast, the absorption of fructose across the enterocyte BBM is mediated by a specific Na^+-independent facilitative transporter, termed GLUT5.[22] The transport of all three sugars across the basolateral membrane domain of the enterocyte is mediated by a single facilitative transport protein, termed GLUT2.[22] The uptake of glucose across the BBM, mediated

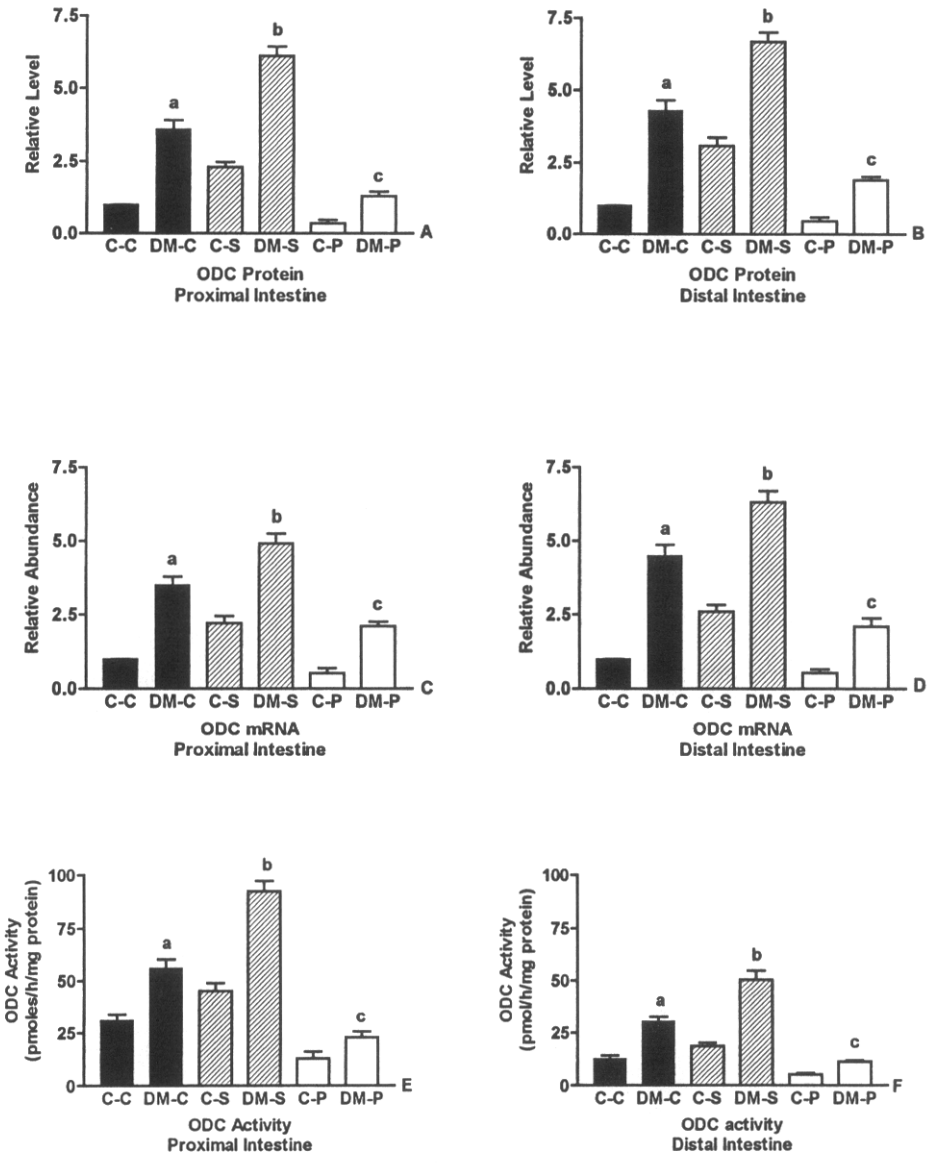

Figure 4 Levels of ODC protein, mRNA abundance and ODC activity in the proximal (panels a,c, and e) and distal (panels b,d, and f) small intestine in control (C) and diabetic (DM) rats fed standard chow (C), saturated fatty acid (S), or polyunsaturated (P) fatty acid containing isocaloric diets. a,b,c = $P < 0.05$ compared to levels measured in corresponding controls.

by SGLT1, requires a Na^+ gradient across the BBM, which is established and maintained by the Na^+/K^+ ATPase.[1,11]

The absorption of a variety of nutrients is increased in experimental diabetes mellitus [reviewed in Ref. 2]. The BBM uptake of glucose is increased in DM rats as a result of the up-regulation of the maximal transport rate (Vmax).[5–8] The increased SGLT1 activity has been recently shown to be accompanied by increases in SGLT1 protein and mRNA abundance.[9] In earlier studies from our laboratory, we have shown that in diabetic as well as in nondiabetic control rats, feeding a semisynthetic isocaloric diet enriched with SFA up-regulates the value of the Vmax, whereas feeding a PUFA reduces glucose uptake and improves the clinical control of the diabetes.[3,4] Thus, these initial observations form the framework for the present study.

In nondiabetic control rats fed chow, the previously reported increases and decreases in SGLT1 activity in response to feeding SFA and PUFA, respectively,[3,4] are associated with corresponding immunodetectable SGLT1 protein and changes in SGLT1 mRNA abundance. Together, these observations suggest that dietary lipid composition is capable of altering SGLT1 gene expression in the intact mammalian small intestine. In the present study, we observed a proximal-to-distal decline in SGLT1 protein levels, whereas SGLT1 mRNA abundance was similar in both regions of the small intestine. These findings add further support to the contention that the expression of SGLT1 is controlled primarily at the posttranscriptional level.[1]

The findings of increased SGLT1 protein and mRNA observed here in diabetic rats fed chow are in agreement with the findings of Burant et al.[9] The current model, based on glucose transport studies [reviewed in Ref. 2], analysis of phlorizin (i.e., specific SGLT1 inhibitor) binding to the BBM,[24] and measurement of SGLT1 gene expression,[9] together suggest that the increased glucose transport in experimental DM is explained by a premature expression of hexose transporters as enterocytes migrate along the crypt-villus axis. This would give rise to a cumulative increase in

SGLT1 protein as cells differentiate along the crypt-villus axis.

In the present study, feeding control rats SFA increased the levels of SGLT1 protein and RNA in the jejunum and ileum (*Figure 1*), whereas PUFA decreased both of these parameters. Irrespective of diet, the levels of SGLT1 protein and RNA in the proximal and distal small intestine were greater in the diabetic rats compared with controls (*Figure 1*). Taken together, the findings reported in the present study suggest that the previously reported dietary induced alterations in SGLT1 activity[3,4,25,26] reflect changes in the cellular events that determine SGLT1 gene expression along the crypt-villus axis.

We observed increased levels of immunodetectable α_1 and β_1 Na^+/K^+-ATPase isoforms in the jejunum as compared with the ileum in control rats fed chow; in contrast, levels of mRNA abundance encoding the α_1 and β_1 Na^+/K^+-ATPase isoforms were similar in both regions of the small intestine. These observations are in agreement with the findings of Gianella and coworkers[27] and Wild and Thomson.[28] As in the case of SGLT1, it would seem that the expression of Na^+/K^+-ATPase is controlled primarily by cellular events acting at the posttranscriptional level.

Feeding SFA increased the levels of Na^+/K^+ ATPase α_1 and β_1 isoform protein and RNA in the jejunum and ileum (*Figures 2* and *3*), whereas feeding PUFA decreased the levels of expression of both of these Na^+/K^+ ATPase isoforms. In each diet group, the levels of Na^+/K^+ ATPase α_1 and β_1 isoform protein and mRNA in the proximal and distal small intestine were greater in the diabetic rats (*Figures 2* and *3*). Considering the pivotal role that Na^+/K^+ ATPase plays in glucose transport across the BBM, it is not surprising that the alterations in SGLT1 expression observed in the present work are paralleled by corresponding changes in Na^+/K^+ ATPase gene expression and activity.

In diabetic rats there is an increased mucosal mass as compared with nondiabetic control rats fed the same diet [reviewed in Ref. 2]. The enzyme ODC catalyzes the biosynthesis of polyamines, which in turn play a central role in cell proliferation and differentiation [reviewed in Ref. 13]. Increased levels of ODC activity and enhanced concentrations of the polyamines putrescine and spermidine have been observed in the rat jejunum in a model of DM.[12] The increases in jejunal ODC activity in DM are related to enhancement in tyrosine kinase activity.[29] The findings reported here confirm and extend these previous observations. The levels of ODC activity, protein, and mRNA abundance were increased in the proximal and distal small intestine in DM (*Figures 4* and *5*). Dietary modification does not result in alterations in either the mucosal mass or crypt cell production rate.[26,30] In controls, ODC expression is increased by feeding SFA and decreased by feeding PFA. These observations support the previously reported findings concerning the effects of dietary fat content on rat colonic ODC activity.[31] In the present work, SFA increased and PUFA decreased ODC expression in the small intestine in DM (*Figures 4* and *5*). Thus, these alterations in ODC expression seem to be caused by the effects of DM, and are modulated further by the dietary lipid content. Changes in the level of expression of ODC, and consequently the levels of intracellular polyamines, may represent an important mechanism in the cellular signalling pathways that lead to increased enterocyte turnover and enhanced SGLT1 expression and activity. Evidence for this suggestion also comes from the observation that polyamines appear to play a role in regulating SGLT1 activity in LLC-PK1 cells, which share some properties with enterocytes.[32] There is a growing body of experimental evidence which suggests that the intracellular pathways regulated by protein kinase C control, at least in part, alters the expression of both ODC and SGLT1 in the normal enterocyte[33–35] and Na^+,K^+-ATPase activity in rat kidney cortical tubules.[36] However, the importance of intracellular signalling through protein kinase C in modulating nutrient transporter gene expression in the small intestine in experimental DM and response to changes in dietary lipids remains to be defined.

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