Morphological Changes and Increased Sucrase and Isomaltase Activity in Small Intestines of Insulin-Deficient and Type 2 Diabetic Rats

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Abstract. The small intestine plays an important role in the digestion and absorption of many nutrients. To investigate the contribution of carbohydrate digestion to diabetes mellitus, we examined the morphological changes of the small intestine, and the expression of sucrose-isomaltase, which is one of the intestinal disaccharidas, in diabetic model rat, that is the streptozotocin-induced (STZ) diabetic rat (insulin-deficient model), and the Otsuka Long-Evans Tokushima Fatty (OLETF) rats and the Goto-Kakizaki (GK) rats (type 2 diabetic models). Intestinal hyperplasia was observed in STZ, OLETF, and GK rats. Moreover, in the small intestine of each diabetic strain, the proliferating cell nuclear antigen (PCNA)-labeling index, which is a marker of proliferation, was higher than in the respective control. Cdx1 and Cdx2, known to be transcriptional factors related to intestinal proliferation and differentiation, were more highly expressed in STZ, OLETF and GK rats than in the respective controls. These findings indicate that small intestinal hyperplasia, and thereby the resultant increase of total activity of disaccharidases such as sucrase and isomaltase in the entire small intestine, might be one of the reasons for postprandial hyperglycemia in diabetes mellitus.

Key words: Carbohydrate digestion, Small-intestinal disaccharidase, Diabetes mellitus, Hyperplasia, Rat


THE intestine plays an important role in the digestion and absorption of many nutrients [1]. Carbohydrates are necessary for energy and are ultimately digested by α-glucosidase and disaccharidase in the small intestine [1]. Carbohydrate digestion directly increases the postprandial blood glucose level. The degree of carbohydrate digestion depends on intestinal morphology, enzymes, and the types of carbohydrate involved.

Sucrase and isomaltase, which are well known small-intestinal disaccharidas and which play important roles in final carbohydrate digestion, are located on the brush border membranes, and include the sucrase-isomaltase (SI) complex [2, 3].

Diabetes mellitus is associated with increased intestinal digestion of carbohydrates, protein, and fat, as well as absorption of glucose, amino acid, and fatty acid. Moreover, hyperplasia and hypertrophy of the small intestine have been reported in alloxan- or streptozotocin-induced diabetic (STZ) rats and young Otsuka Long-Evans Tokushima Fatty (OLETF) rats (6-week-old), as compared to their respective controls [4–7]. However, in diabetes mellitus, the relationships between morphological changes in the small intestine,
and the activity and expression of α-glucosidase and disaccharidases, including sucrase and isomaltase, have yet to be clarified.

In addition, Cdx1 and Cdx2 are known transcriptional factors in the intestine [8, 9]. Cdx1 is related to the development and regeneration of intestinal epithelium [10], and Cdx2 is related to differentiation of the small intestine [11–13]. Cdx2 also plays a role in the regulation of the expression of various small-intestinal proteins, such as SI [12–14], lactase-phlorizin-hydrolase [14, 15], calbindin-D9K [16], and carbonic anhydrase I [17]. Therefore, the expression of these transcriptional factors in diabetes mellitus may affect small-intestinal differentiation and SI expression.

In this study, we investigated the contribution of carbohydrate digestion to diabetes mellitus, and we report herein on morphological changes in the small intestine, as well as on sucrase and isomaltase activities, and expressions of SI complex in various types of diabetic model rats, the insulin-deficient model, STZ rat; and two type 2 diabetic models, that is the OLETF rats [18] which displays obesity and hyperinsulinemia, and the GK rat [19], which displays dysfunctional insulin secretion against glucose. Moreover, we also investigated, in the small intestines of these diabetic model rats, the proliferating cell nuclear antigen (PCNA)-labeling index, which is a proliferation marker, and the mRNA expression levels of Cdx1 and Cdx2, which are transcriptional factors, related to proliferation and differentiation in the small intestine.

**Materials and Methods**

**Animals and study design**

For STZ-induced diabetic model rats (STZ rats), STZ (50 mg/kg body weight, in 100 μl of 50 mM sodium citrate, pH 4.5) was intraperitoneally injected into male Wistar rats (7 weeks old, Japan SLC, Shizuoka, Japan), and those rats with elevation of blood glucose (more than 25 mM) were used for experiments after 2 weeks. Control rats were injected with 100 μl of 50 mM sodium citrate (pH 4.5) only. Male OLETF rats and control male LETO rats (10 and 30 weeks old) were obtained from Otsuka Pharmaceutical Co., Tokushima, Japan. Male GK rats and control male Wistar rats (10 and 20 weeks old) were purchased from Clea Japan (Osaka, Japan). The animals were housed at 24 ± 2°C with light from 0800 to 2000 h; food (CE-2, Clea Japan) and water were available ad libitum. Each rat was anesthetized with 10 mg of pentobarbital and killed, the blood was collected, and a 5 cm length of the small intestine was obtained. Plasma glucose levels were determined with enzymatic assay kit (Glucose-B Test Wako, Wako Pure Chemical Industries, Osaka, Japan). Hemoglobin A1c (HbA1c) levels were determined by an aminophenyl-boronicagarose affinity chromatography method (Glyc-Affin, GHb, Seikagaku Kogyo, Tokyo, Japan). Plasma insulin levels were measured with radioimmuno assay using rat insulin as a standard (Novo Nordisk, Bausweir, Denmark). The small intestine was cut transversely into two segments of roughly equal length. The first segment was rinsed with ice-cold phosphate buffered saline, frozen in liquid nitrogen, and stored at −80°C for disaccharidase assay, immunoblotting, and RT-PCR analysis. The other segment was fixed in 4% formaldehyde solution and embedded in paraffin, and 3 μm thick sections were stained with hematoxylin and eosin (HE). The lengths of ten villi were measured in each part of the intestinal sections, using software (NIH Image), and villi length was shown as the average of the lengths of ten villi in each rat. Computed data were expressed as mean ± SEM.

**Mucosal protein content, sucrase and isomaltase activity**

The mucosa in 1 cm of the small intestine were collected by scraping with a glass slide, and homogenized in 1 ml of 100 mM potassium phosphate buffer, pH 6.8, with a Teflon homogenizer. After centrifugation at 3,000 x g for 10 min, the supernatant obtained was used as a crude enzyme solution. The activities of sucrase and isomaltase were assayed with 25 mM sucrose and isomaltose, respectively, as the substrate, according to the method described by Dahlqvist [20]. Mucosal protein content of the 1 cm of small intestine was estimated as described by Lowry et al. [21], using the crude enzyme solution, with bovine serum albumin as a standard.

**PCNA labeling index**

Anti-PCNA antibody was purchased from Medical & Biological Laboratories (Nagoya, Japan). The PCNA labeling index of the small intestine was determined
as the percentage of immunoreactive nuclei in 100 cells. In small intestine mucosa, the PCNA labeling index was expressed as the percentage of positive cells in longitudinally sectioned small intestine tubules. Computed data were expressed as mean ± SEM.

Immunoblotting of sucrase-isomaltase complex (SI) protein

The crude enzyme solution (10 μg of protein) was subjected to SDS-PAGE (7.5% gel), as described by Laemmli [22]. Proteins in the gel were transferred to a polyvinylidene difluoride membrane (Millipore Japan, Tokyo, Japan). Rat SI protein was purified from rat small intestine mucosa in a method modified from that described by Takesue et al. [23], and SI antibody was prepared in normal rabbits with purified SI protein (Sawady Technology, Tokyo, Japan). Absorbtional test of the SI antibody was performed with purified SI protein (data not shown). The membrane was treated with SI antibody, and then with alkaline phosphatase-conjugated goat anti-rabbit IgG antibodies. Immunoreactive proteins were detected by staining with 4-nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate 4-toluidine salt and the relative amount was estimated by scanning densitometry. The relative intensity of SI protein expression was calculated by normalizing the expression in each age-matched control rats as 1. Computed data were expressed as mean ± SEM.

RT-PCR analysis

Total RNA was extracted from the small intestine by the guanidinium thiocyanate-cesium chloride method [25, 26]. Complimentary DNA was synthesized from 5 μg of total RNA with a commercial kit (Ready-To-Go T-primed First-Strand Kit, Amersham-Pharmacia Biotech Japan, Tokyo, Japan). PCR primer pairs are shown as follows. Cdx1: sense primer 5’-AAGAC TCGGACCAAGGACAGTA-3’, antisense primer 5’-AUCTGCGGGAGGGCATGGGTCG-3’ (1–252) [27]; Cdx2: sense primer 5’-CGCAGCAGCTGG AATGGCTA-3’, antisense primer 5’-CCGGATGCT GAGGGTGATGA-3’ (139–295) [28]; beta-actin: sense primer 5’-ATCCGTAAAGA CCTCTATGC-3’, antisense primer 5’-AACCAGCTCAAGTAAACAGTC-3’ (862–1148) [29]. PCR reactions were catalyzed using Taq DNA polymerase (Toyobo, Osaka, Japan). The thermal cycling parameters consisted of an initial denaturation step for 3 min at 94°C, followed by 37 cycles consisting of 30 s at 94°C, 30 s at 62°C, 30 s at 72°C. The final extension step was for 7 min at 72°C. PCR products were resolved by 1.5% agarose gel electrophoresis. DNA was stained with ethidium bromide for fluorescence detection, and quantified by scanning densitometry.

Statistical analysis

Statistical evaluations were performed by Student’s

| Table 1. Body weight (BW), plasma glucose (PG), insulin and HbA1c, levels |
|----------------|----------------|----------------|----------------|
|               | BW (g)         | PG (mM)        | Insulin (pM)   | HbA1c (%)      |
| STZ           | 204 ± 8*       | 27.8 ± 0.3*    | 0.8 ± 0.4*     | 9.8 ± 1.4*     |
| Wistar        | 266 ± 7        | 5.0 ± 0.1      | 206 ± 88       | 5.6 ± 0.1      |
| GK 10 week    | 246 ± 4*       | 11.0 ± 1.2*    | 89 ± 11**      | 9.2 ± 0.3*     |
| Wistar 10 week| 270 ± 4        | 6.1 ± 0.5      | 192 ± 18       | 4.9 ± 0.2      |
| GK 20 week    | 336 ± 8*       | 14.4 ± 0.5*    | 49 ± 9*        | 9.5 ± 0.4*     |
| Wistar 20 week| 440 ± 9        | 5.4 ± 0.6      | 364 ± 154      | 5.9 ± 0.3      |
| OLET 10 week  | 361 ± 4*       | 12.2 ± 0.7*    | 1274 ± 183*    | 6.9 ± 0.6      |
| LETO 10 week  | 361 ± 4*       | 5.0 ± 0.2      | 401 ± 154      | 6.3 ± 0.2      |
| OLET 30 week  | 445 ± 5*       | 17.9 ± 4.2*    | 2209 ± 184*    | 13.2 ± 0.3*    |
| LETO 30 week  | 414 ± 7        | 6.7 ± 0.8      | 371 ± 292      | 6.6 ± 1.0      |

Data are expressed as means ± SEM. Each group of n = 5. *P<0.01, **P<0.05 vs age-matched control rats.
$t$ test. All data are shown as mean ± SEM, and statistical significance was defined as $P<0.05$.

**Result**

**Body weight, plasma glucose, plasma insulin and HbA$_{1c}$**

Body weight of insulin-deficient STZ rats was significantly lower than that of control Wistar rats; GK rats, a model of type 2 diabetes mellitus, weighed significantly less than control Wistar rats at 10 and 20 weeks old. However, the body weight of OLETF rats, also a model of type 2 diabetes mellitus, was significantly higher than that of control LETO rats at 10 and 30 weeks of age, when OLETF rats were obese. Plasma glucose levels of diabetic model rats were significantly higher than that of each control group of rats. Plasma insulin levels of STZ and GK rats were significantly lower than those of control rats at each age. However, since OLETF rats had significantly higher insulin than control LETO rats at each age, the OLETF rats showed hyperinsulinemia. The HbA$_{1c}$ levels of diabetic model rats were significantly higher than those of the respective control rats each week, except for the 10-week-old OLETF rats (Table 1).

**Morphological observation, mucosal protein content and PCNA labeling index in the small intestine**

HE-stained sections of the small intestine are shown in Fig. 1. Villi were significantly longer in diabetic model rats than in the respective age-matched control rats, and mucosal protein content in 1 cm of small intestine in diabetic model rats was also significantly higher than in the respective age-matched control rats (Table 2).

The PCNA labeling index is shown in Fig. 2. In diabetic STZ, GK and OLETF rats, the percentage PCNA positive area in each villus was wider than in the respective age-matched control rats.

**Immunoblotting of SI protein**

In the immunoblotting study, the expression levels of SI protein in STZ rats were significantly higher than in control Wistar rats (Fig. 3). However, the expressions in GK and OLETF rats were not significantly

![Fig. 1. Morphological observations of small intestine using HE-stained sections. STZ rat and control Wistar rat are shown in (A) and (B), respectively. OLETF rat and control LETO rat are in (C) and (D) at 10 weeks of age, (E) and (F) at 30 weeks, respectively. GK rat and control Wistar rat are in (G) and (H) at 10 weeks old, (I) and (J) at 20 weeks, respectively. Bar: 200 μm.](image)
Table 2. Villi length and mucosal protein content

<table>
<thead>
<tr>
<th></th>
<th>Villi length (μm)</th>
<th>Mucosal protein (mg/cm intestine)</th>
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<tbody>
<tr>
<td>STZ</td>
<td>329 ± 9*</td>
<td>4.7 ± 0.1*</td>
</tr>
<tr>
<td>Wistar</td>
<td>280 ± 7</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>GK</td>
<td>10 week 321 ± 7**</td>
<td>3.0 ± 0.1**</td>
</tr>
<tr>
<td></td>
<td>10 week 302 ± 8</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>GK</td>
<td>20 week 353 ± 7*</td>
<td>4.3 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td>20 week 324 ± 5</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>OLETF</td>
<td>10 week 381 ± 7*</td>
<td>3.6 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td>10 week 339 ± 5</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>LETO</td>
<td>10 week 424 ± 9**</td>
<td>6.1 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td>30 week 382 ± 7</td>
<td>4.6 ± 0.1</td>
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</table>

Villi length is shown as the average of the length of ten villi from each rat. Each group, n = 5. Data are expressed as means ± SEM. *p<0.01, **p<0.05 vs age-matched control rats.

Fig. 2. PCNA labeling indexes. The PCNA labeling index of the small intestine was determined as the percentage of immunoreactive nuclei in 100 cells. In small intestine mucosa, the PCNA labeling index was expressed as the percentage of positive cells in longitudinally sectioned small intestine tubules. Computed data were expressed as mean ± SEM. W: Wistar rats; STZ: STZ rats; GK: GK rats; L: LETO rats; O: OLETF rats. *p<0.01, **p<0.05 compared with age-matched control rats in each strain.

Fig. 3. SI immunoblotting. Immunoblotting with SI antibody in the small intestinal mucosa. The expression levels of the sucrase and isomaltase subunits of the SI complex were examined by immunoblotting, using an antibody against rat SI complex in STZ (S), GK (G), OLETF (O), and the respective controls, Wistar (W) and LETO (L) rats are different. Immunoblots of the sucrase and isomaltase subunits were quantified by scanning densitometry. Relative intensity of isomaltase subunit expression was calculated, and the expression in the respective age-matched control rats was normalized as 1. W: Wistar rats; S: STZ rats; G: GK rats; L: LETO rats; O: OLETF rats. *p<0.01, **p<0.05 compared with age-matched control rats in each strain.

Total activity of sucrase and isomaltase in small intestine

The total activity of sucrase and isomaltase in the mucosa of 1 cm of the small intestine in STZ, GK, and OLETF rats was significantly higher than in the respective control rats (Fig. 4).

Expression of Cdx1 and Cdx2 mRNA

The evaluation of mRNA expression of Cdx1 and Cdx2, which are transcription factors in the small intestine, in diabetic model rats, was performed by RT-PCR analysis (Fig. 5). The expression levels of Cdx1
and Cdx2 mRNA in STZ, GK, and OLETF rats were significantly higher than in age-matched control rats (Fig. 5).

Discussion

In this study, we found through morphological examination that intestinal epithelial hyperplasia occurs in STZ, OLETF, and GK rats. Indeed, it has been reported that intestinal hyperplasia is found in STZ rats [6] and 6-week-old OLETF rats [7], and that intestinal epithelial cells at the tips of villi are taller in alloxan-induced diabetic rats [4]. Moreover, we also found that the mucosal protein content of the small intestine was increased in STZ, OLETF, and GK rats. These results suggest that intestinal hyperplasia is induced not only in the insulin-deficient type, but also in type 2 diabetes mellitus, and that such hyperplasia induces an increase in the peripheral area of the intestinal lumen.

In our present study, immunoblotting analysis revealed that SI protein expression in STZ rats was higher than control but those in young OLETF and GK rats were not significantly different from that in age-matched control. Indeed, in the previous reports, increase of SI expression was only found in insulin-deficient STZ, alloxan and aged OLETF rats [30–34]. It has been reported that aged OLETF rats show low level of insulin secretion [33], and that insulin has a suppressive effect on the synthesis of the SI complex in small-intestinal epithelial cells [34]. It is plausible that young OLETF and GK rats might keep the insulin level for low expression of SI. On the other hand, the total activities of sucrase and isomaltase in 1 cm of intestine of STZ, OLETF and GK rats were significantly higher than those in age-matched control rats. Hence, our data is the first to reveal that total sucrase and isomaltase activities are increased in diabetes mellitus rats regardless of diabetic type. Moreover, our results suggest that increases of total sucrase and isomaltase activities in diabetes mellitus result from intestinal hyperplasia.

Proliferation and differentiation in the small intestine are related to Cdx1 and Cdx2, known transcriptional factors [8, 9]. We found that the mRNA expression levels of Cdx1 and Cdx2 were higher in diabetic model rats than in their respective controls. Moreover, it has been reported that transfection of the cdx2 gene to IEC-6, which is a cell line of rat small intestine, induces differentiation and SI expression [11], and that expression of CDX2 may trigger the initiation and development of intestinal metaplasia [34]. Since there are many transcriptional factors, and other transcriptional factors or interactions among two or more transcriptional factors in the small intestine may be related to intestinal differentiation, Cdx1 and Cdx2 can-
not be the only factors that induce differentiation in the small intestine in cases of diabetes mellitus. However, the present study suggests that the higher expression of Cdx1 and Cdx2 in diabetes mellitus is one of the mechanisms for increasing differentiation in the small intestine, and that hyperplasia may occur in the small intestine as a result. Increase of intestinal transcription factor in diabetes mellitus has been little studied. Alternatively, intestinal hyperplasia and increase of disaccharidase activities have been observed in starved state [35], and Cdx expression in small intestine has been induced in starvation in chicken [36]. We speculated that increase of transcriptional factors might occur in diabetes mellitus in the same way as in starved conditions.

In our present study, intestinal hyperplasia was observed, and the absolute activities of disaccharidases and α-glucosidases in the small intestine were increased in both insulin-deficient and type 2 diabetes mellitus. In other words, we found that carbohydrate digestion in the small intestine increased in both insulin-deficient and type 2 diabetes mellitus. α-Glucosidase inhibitors are usually used in the improvement of postprandial hyperglycemia. Indeed, administration of α-glucosidase inhibitors improves postprandial hyperglycemia [35–37], and the UKPDS reports that the administration of acarbose, which is one of the α-glucosidase inhibitors, has improved hyperglycemia and HbA1c for three years [38]. Therefore, it is suggested that increased digestion of carbohydrates may be one of the causes of postprandial hyperglycemia in diabetes mellitus, for which α-glucosidase inhibitors are available for its improvement.

Acknowledgments

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