EFFECT OF FOOD RESTRICTION ON INTESTINAL DISACCHARIDASES IN STREPTOZOTOCIN- INDUCED DIABETES OF RAT

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Summary The effect of food restriction on the intestinal weight and membrane digestive enzyme activities was observed in rats with diabetes induced by streptozotocin. The specific activities of disaccharidases of food non-restricted diabetic rats were not changed, but total activities were significantly increased due to the increase of intestinal mucosal weight. Restriction of food intake did not increase intestinal mucosal weight, but significantly increased the specific and total activities of disaccharidases in parallel with the increase of sucrase-isomaltase content.

On the other hand, experimental diabetes did not influence the activity of alkaline phosphatase and leucine aminopeptidase except for specific and total activities of alkaline phosphatase in food non-restricted rats.

Keywords streptozotocin, diabetic rat, intestinal disaccharidases, food restriction

It has been reported that experimental diabetes causes structural and functional changes in the alimentary tracts, *viz.*, intestinal hyperplasia or hypertrophy (1, 2), increase of intestinal glucose absorption (3, 4), and also alteration of the activities of membrane digestive enzymes, such as maltase, sucrase, lactase, trehalase, alkaline phosphatase, *etc.* (5-8).

The question exists whether these small intestinal (structural and functional) changes are related to the hyperphagia accompanying diabetes. Since Nakabou *et al.* (9) have shown that hyperplasia or hypertrophy in alloxan diabetic rats can be prevented in such rats with restricted food intake, it was considered pertinent to use this model to explore the effect of increased food intake in diabetic rats on intestinal membrane digestive enzymes.

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MATERIALS AND METHODS

1. *Animals.* Male Wistar rats weighing approximately 200 g were injected with streptozotocin dissolved in citrate buffer (pH 4.5) intraperitoneally (70 mg/kg). Control rats received citrate buffer alone. All rats were housed in metabolic cages and their body weight, food and water intake, and urinary volume were measured daily. Twenty-four hour urines were tested for glucose concentration using the glucose oxidase method (10). Only those rats with heavy glucosuria (500 mg/dl or more), which corresponds to a blood glucose level of 300 to 500 mg/dl (6) were used for study. Two days after the injection of streptozotocin, diabetic rats were divided into two groups. Rats in the first group had free access to the laboratory chow (Oriental Yeast Company). (These were food non-restricted rats). Rats of the other group were restricted as to the quantity of food which control non-diabetic rats consumed on the previous day (food-restricted rats). Water was given *ad libitum*. On the twelfth day after the injection, rats were killed by decapitation between 13.00 and 15.00 hr. Blood for glucose analysis was drawn by decapitation.

2. *Intestinal homogenate.* The intestine was removed, cut open, flushed with ice-cold saline, blotted with tissue paper and then weighed. Thereafter, the small intestine was divided into three segments, *viz*, 10 cm from pylorus as duodenum, and two equal lengths of the jejunum and ileum. For the study of disaccharidases, the middle segment (jejunum) was used. Intestinal mucosa, which was scraped with glass-slide, was homogenized with 4 volumes of distilled water to make 20% homogenate and was stored at -20°C until use. An aliquot of the homogenate was used for the assay of membrane digestive enzyme activities and sucrase-isomaltase complex (S-I complex) content.

3. *Enzyme assays and protein determination.* Disaccharidase activities were determined by the method of Dahlqvist (10). Substrate concentration was 28 mM in sucrose and maltose, 2.8 mM in isomaltose in 50 mM sodium maleate buffer (pH 6.0). Alkaline phosphatase activity was determined by the method of Lowry (11). Substrate concentration was 8 mM in disodium *p*-nitrophenyl phosphate in 0.5 M 2-amino-2-methyl-1-propanol buffer (pH 10.0) with 0.2% of 1 M MgCl_2 . Leucine aminopeptidase activity was determined by the method of Goldbarg and Rutenburg (12). Substrate concentration was 1.37 mM in L-leucyl- β -naphthylamide hydrochloride mixed with an equal volume of 0.2 M phosphate buffer (pH 7.0). Protein concentration was determined by the method of Lowry *et al.* (13) using bovine serum albumin as standard. Enzyme activities were expressed in two ways: specific activity ($\mu\text{mole-substrate hydrolyzed/mg protein/hour}$) and total activity ($\text{mmole-substrate hydrolyzed in total jejunum/hour}$).

4. *Immunoassay of sucrase-isomaltase content.* Content of S-I complex was determined with papain-solubilized supernatants from jejunal homogenate by immunoassay in an application of the method of Mancini *et al.* (14). 1.0 ml of 20% homogenate of jejunal mucosa was incubated with 0.48 mg papain and 1.2 mg

cysteine-HCl for 90 min at 37°C. After papain treatment, the incubation mixture was centrifuged at $105,000 \times g$ for 60 min. Twelve μ l of papain-solubilized supernatant was applied on the immunoplate containing S-I antiserum. The S-I content was determined from the diameter of the precipitin ring using a previously prepared S-I complex as standard. Antiserum to S-I complex was prepared (15) using S-I complex. This was purified as described before (16).

5. *Chemicals.* Streptozotocin was obtained from Sigma Chemical Company, maltose from Wako Pure Chemical Industries, Ltd., and sucrose from Kokusan Chemical Works, Ltd. L-Leucyl- β -naphthylamide hydrochloride was from Sigma Chemical Company and disodium *p*-nitrophenyl phosphate from Wako Pure Chemical Industries, Ltd. Papain and glucose oxidase were from Worthington Biochemicals Company. Isomaltose was kindly provided by Hayashibara Company, Ltd.

RESULTS

Our findings were compatible with those of other investigators; that the injection of streptozotocin induced a severe state of diabetes mellitus in rats. Water intake and urinary volume started to increase the next day after injection of streptozotocin and reached a plateau in 6 days; food intake was increased from the sixth day after injection. Despite hyperphagia, body weight did not increase in diabetic rats compared with control rats (Fig. 1).

The changes of intestinal mucosal weight and membrane digestive enzyme activities at the fifth day after injection of streptozotocin (just before the increase of food intake) are shown in Tables 1 and 2. Both specific and total activities of disaccharidases in the diabetic rats were increased significantly as compared with control rats; although the intestinal weight and jejunal mucosal weight did not change significantly. Alkaline phosphatase and leucine aminopeptidase activities were not increased. On the other hand, at the twelfth day after injection of streptozotocin (*i.e.*, after the increase of food intake) intestinal mucosal weight and total activity of disaccharidases were increased; specific activity of disaccharidases did not change (Table 3, Fig. 2).

Results of experiments studying the effect of food restriction on diabetic rats are shown in Table 3. Blood glucose, urinary glucose, whole intestinal weight and jejunal mucosal weight were significantly increased in food non-restricted rats compared with control rats. On the contrary, whole intestinal weight and jejunal mucosal weight did not increase in food-restricted rats. The increase in blood glucose levels in food-restricted rats was not so high as that in food non-restricted rats, but urinary glucose levels of food-restricted rats were significantly higher than those of food non-restricted rats.

On the twelfth day after the injection of streptozotocin, specific activities of disaccharidases (*i.e.*, sucrase, isomaltase and maltase) in food non-restricted rats were not found to differ from those of control rats, but those in food restricted rats

Fig. 1. Effects of diabetes on weight gain (A), food intake (B), water intake (C) and urinary volume (D). Diabetes was induced by the intraperitoneal injection of streptozotocin (70 mg/kg). Each point represents means $\pm$ S.E. of 3 (control) or 5 (diabetic) rats.

Table 1. Effects of 5 days of diabetes on rats.

	Control (4)	Diabetic (4)
Blood glucose (mg/dl)	133 $\pm$ 4	394 $\pm$ 32**
Urinary glucose (g/dl)	0.16 $\pm$ 0.04	11.38 $\pm$ 0.65**
Whole intestinal weight (g)	6.90 $\pm$ 0.64	7.65 $\pm$ 0.43
Jejunal mucosal weight (g)	1.98 $\pm$ 0.18	2.35 $\pm$ 0.14
Initial body weight (g)	199 $\pm$ 8	184 $\pm$ 10
Terminal body weight (g)	235 $\pm$ 14	199 $\pm$ 13

Results are expressed as means $\pm$ S.E. (), Number of animals. ** Significantly different from control rats at $p < 0.01$.

Table 2. Effect of 5 days of diabetes on rat jejunal membrane digestive enzyme activity.

		Maltase	Isomaltase	Sucrase	ALPase	LAP
Specific activity ^a						
Control	(3)	10.5 ± 0.6	1.43 ± 0.11	2.32 ± 0.19	29.4 ± 2.7	1.88 ± 0.19
Diabetic	(4)	14.9 ± 1.0*	2.03 ± 0.13*	3.52 ± 0.33*	28.2 ± 2.5	2.15 ± 0.22
Total activity ^b						
Control	(3)	2,554 ± 286	346 ± 41	562 ± 66	7,394 ± 1,581	460 ± 81
Diabetic	(4)	4,673 ± 319**	636 ± 37**	1,101 ± 82**	9,035 ± 1,264	678 ± 83

Results are expressed as means ± S.E. (), number of animals; ALPase, alkaline phosphatase; LAP, leucine aminopeptidase. ^a μ mole-substrate hydrolyzed/mg-protein/hr.; ^b μ mole-substrate hydrolyzed in total jejunum/hr. *** Significantly different from control rats at $p < 0.05$, $p < 0.01$, respectively.

Table 3. Effect of food restriction on diabetic rats.

		Control (5)	Diabetic	
			Food non-restricted (5)	Food-restricted (5)
Blood glucose (mg/dl)		113 ± 2	396 ± 27**	186 ± 52 ^{oo}
Urinary glucose (g/dl)		0.11 ± 0.01	9.62 ± 0.32**	13.30 ± 1.43***, ^o
Whole intestinal weight (g)	(g)	6.37 ± 0.34	8.77 ± 0.54**	6.15 ± 0.17 ^{oo}
Jejunal mucosal weight (g)	(g)	1.51 ± 0.07	2.33 ± 0.15**	1.22 ± 0.10 ^{oo}
Initial body weight (g)	(g)	197 ± 5	189 ± 7	188 ± 8
Terminal body weight (g)	(g)	270 ± 9	242 ± 5*	227 ± 8**

Results are expressed as means ± S.E. (), Number of animals. *** Significantly different from control rats at $p < 0.05$, $p < 0.01$, respectively. ^{oo} Significantly different from food non-restricted diabetic rats at $p < 0.05$, $p < 0.01$, respectively.

were markedly increased. Total activities were not changed in both groups of diabetic rats, but were markedly increased in comparison with control rats (Fig. 2). Activities of other membrane digestive enzymes, such as alkaline phosphatase and leucine aminopeptidase, were not influenced by food restriction.

To demonstrate that the changes in disaccharidase activities are due to the changes in enzyme content, S-I content was determined immunochemically. As shown in Table 4, S-I contents were markedly increased by restricted feeding. The increased S-I content paralleled an increase of specific activities of sucrase and isomaltase.

The expression of S or I per μ g of S-I gave identical results in all groups, thus indicating again that the diabetic and/or the nutritional status influenced the

Fig. 2. Effect of food restriction on jejunal membrane digestive enzyme activity of diabetic rats. a, maltase; b, isomaltase; c, sucrase; d, leucine aminopeptidase; e, alkaline phosphatase. $\square$, control; ▨ , food non-restricted diabetic; ▤ , food-restricted diabetic rats consuming diet equivalent to the amount consumed by control rats from second to twelfth day after injection of streptozotocin. Results are given as means $\pm$ S.E. of five rats. *, **: significantly different from control rats at $p < 0.05$ or $p < 0.01$, respectively. Δ , $\Delta\Delta$: significantly different from food non-restricted diabetic rats at $p < 0.05$ or $p < 0.01$, respectively.

Table 4. Effect of food restriction on sucrase-isomaltase contents and activities of diabetic rats on the twelfth day after injection of streptozotocin.

		Sucrase-isomaltase content ($\mu\text{g}/\text{mg-protein}$)	Sucrase activity ($\text{U}^a/\mu\text{g S-I}^b$)	Isomaltase activity ($\text{U}^a/\mu\text{g S-I}^b$)
Control	(5)	6.09 ± 0.27	0.592 ± 0.017	0.280 ± 0.006
Diabetic				
food non-restricted	(5)	6.58 ± 0.38	0.618 ± 0.042	0.298 ± 0.017
food restricted	(5)	$10.70 \pm 0.20^{**,\circ\circ}$	0.566 ± 0.022	0.266 ± 0.011

Results are expressed as means $\pm$ S.E. (), Number of animals. ^a $\mu\text{mole-substrate}$ hydrolyzed/hr, ^b sucrase-isomaltase content. ** Significantly different from control rats at $p < 0.01$. $\circ\circ$ Significantly different from food non-restricted diabetic rats at $p < 0.01$.

quantity of enzyme, but not its catalytic properties.

DISCUSSION

Rats with diabetes (induced by streptozotocin or alloxan) exhibited increased weight of intestines and activities of membrane digestive enzymes, especially disaccharidases, but the body weight gain was suppressed in spite of hyperphagia (1, 2). This present study confirmed these observations, however, it was shown that intestinal changes induced by streptozotocin depend on the stage of diabetes.

During the development of diabetes after the injection of streptozotocin, blood glucose levels reached a plateau within a day, and urinary volume and water intake reached a plateau on the sixth day after the injection. Food intake began to increase on the sixth day after the injection; thus, the period of 6 days after the injection seemed to be the period of adaptation to the diabetic state.

Before the increase of food intake, the specific activities of intestinal disaccharidases increased significantly compared with control rats, in good agreement with the results of Olsen and Rogers (5), but the weight of intestine did not change in comparison with control rats. On the other hand, after the increase of food intake, intestinal weight increased similarly, as shown by Nakabou *et al.* in alloxan-induced diabetic rats (9), but specific activities of disaccharidases were not increased.

Most reports have presented data on disaccharidase activities as specific activities (*i.e.*, expressed as μ mole-substrate hydrolyzed/unit of tissue protein/unit of time). Since specific activity is a value of a fraction (enzyme activity/tissue protein), a change in this value could be caused not only by a change in the numerator, but also by a change in the denominator. The denominator can change not only quantitatively but also qualitatively; *i.e.*, changes other than in the structure carrying the disaccharidases can occur in the tissue protein.

Specific activities of disaccharidases of food non-restricted rats were not increased, but total activities were significantly increased due to the increase of intestinal mucosal weight. Restriction of food intake significantly increased the specific activities of disaccharidases and in turn increased the total activities which were almost the same as those of food non-restricted rat.

These results strongly suggest that experimental diabetes causes an increase of total disaccharidase activities. Two mechanisms which increase total activities are operative, namely, 1) the increase of specific activity before the beginning of hyperphagia, and 2) the increase of the mass of intestinal mucosa after the beginning of hyperphagia (17). Furthermore, the increase of specific activities by restriction of food intake was shown to be due to the increase of enzyme content as measured by immunological assay.

According to some reports (6, 8), specific activity of alkaline phosphatase in diabetic rats increases. In our study, alkaline phosphatase activity was not changed in diabetic rats; only specific and total activities of alkaline phosphatase in food non-restricted rats were. Since alkaline phosphatase has been shown to be

influenced by food intake(8), it is likely that the increase of specific activity of alkaline phosphatase in diabetic rats could be related to the increase of food intake. This comparison thus indicates that the activity of disaccharidases seems to be controlled differently than those of other membrane digestive enzymes.

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