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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 membraneous digestive enzymes.

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MATERIALS AND METHODS

1. Animals. Male Wistar rats weighing approximately 200g were injected
with streptozotocin dissolved in citrate buffer (pH 4.5) intraperitoneally
(70mg/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 con
centration using the glucose oxidase method (10). Only those rats with heavy
glucosuria (500mg/dl or more), which corresponds to a blood glucose level of 300
to 500mg/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-resticted rats). Water
was given ad libitum. On the twelfth day after the injection, rats were killed by
decapitation between 13.00 and 15.00hr. 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, 10cm 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•Ž 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 28mM in
sucrose and maltose, 2.8mM in isomaltose in 50mM sodium maleate buffer (pH
6.0). Alkaline phosphatase activity was determined by the method of Lowry (11).
Substrate concentration was 8mM in disodium p-nitrophenyl phosphate in 0.5M 2
- amino-2-methyl-l-propanol buffer (pH 10.0) with 0.2% of 1M MgCl2. Leucine
aminopeptidase activity was determined by the method of Goldbarg and
Rutenburg (12). Substrate concentration was 1.37mM in L-leucyl-ƒÀ-naphthylamide
hydrochloride mixed with an equal volume of 0.2M 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 (ƒÊmole-substrate hydrolyzed/mg protein/hour) and total activity
(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). 1.0ml of 20%
homogenate of jejunal mucosa was incubated with 0.48mg papain and 1.2mg

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cysteine-HCl for 90min at 37•Ž. After papain treatment , the incubation mixture
was centrifuged at 105,000•~g for 60min. Twelve ƒÊl 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)

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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-ƒÀ-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 6days; 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 bood 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., sucrose, isomaltase and maltase) in food non-restricted rats were not found to differ from those of control rats, but those in food restricted rats

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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 (70mg/kg). Each point represents means•}S. E. of 3 (control) or 5 (diabetic) rats.

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

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

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

Results are expressed as means•}S . E. (), number of animals; ALPase, alkaline
phosphatase; LAP, leucine aminopeptidase. a ƒÊmole-substrate hydrolyzed/mg
- protein/hr.; b ƒÊmole-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.

Results are expressed as means•}S. E. (), Number of animals . *,** Significantly

different from control rats at p<0.05, p<0.01, respectively. •›,•›•› Significantly different
from food non-restricted diabetic rats at p<0.05, p<0.01, respectively

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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 ƒÊg of S-I gave identical results in all groups, thus
indicating again that the diabetic and/or the nutritional status influenced the

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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. _??_, 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•}S. E. of five rats. *, **: significantly different from control rats at
p<0.05 or p<0.01, respectively. •¢,•¢•¢: 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.

Results are expressed as means•}S. E. ( ), Number of animals, a ƒÊmole-substrate
hydrolyzed/hr, b sucrase-isomaltase content. ** Significantly different from control rats
at p<0.01. •›•› Significantly different from food non-restricted diabetic rats at p<0.01.

quantity of enzyme, but not its catalytic properties.

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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 hyper
phagia (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 6days 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 ƒÊmole-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), spectific 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

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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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