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Invertase, Maltase, Lactase, and Peroxidase Activities in Duodenum of BB Rats

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The development of immune-mediated diabetes in BB rats may involve a defect of the gastrointestinal tract (GI), as suggested by increased gut permeability. This study aimed at measuring invertase, maltase, lactase, and peroxidase activities in the duodenum of diabetes-prone BioBreeding (BBdp) rats and control BioBreeding rats (BBc) given free access to NIH-07 diet up to the time of killing at 60–66 d of age. After washing the entire small intestine, the duodenal mucosa was scraped off in the first 5-cm segment from the pylorus and frozen in distilled water. Invertase, maltase, and lactase activities were measured by monitoring the conversion of [U-^{14}C]sucrose, [U-^{14}C]maltose, and [D-[1-^{14}C]glucose] lactose to radioactive hexoses, which were phosphorylated in the presence of adenosine triphosphatase and yeast hexokinase and then separated from their precursor by ion-exchange chromatography. Peroxidase activity was measured by a spectrophotometric procedure. In the BBdp rats, the activity of invertase, maltase, and lactase averaged, respectively, 70.2 ± 4.4, 81.2 ± 4.3, and 75.7 ± 4.1% (n = 16 and $p < 0.001$ in all cases) of the control values found in BBc rats of the same sex. Inversely, after exclusion of two female BBc rats with abnormally high plasma D-glucose concentration, the activity of peroxidase in the BBdp rats averaged 157.4 ± 20.0% (n = 16; $p < 0.02$) of the mean control value recorded in BBc rats of the same sex (100.0 ± 9.3%; n = 14). These findings are compatible with the view that a proinflammatory state of the GI associated with compromise function may precede the occurrence of pancreatic insulitis in BBdp rats and, possibly, human subjects with type 1 diabetes.

Key Words: BB rats; duodenum; invertase; maltase; lactase; peroxidase.

Introduction

Intestinal disaccharidases such as invertase, lactase, and maltase are enzymes located in the brush border of mature enterocytes *(1,2)*. Inflammatory mucosal damage observed in humans suffering from celiac disease results in a decrease in disaccharidase activities *(3–5)*. Diabetes-prone BioBreeding (BBdp) rats present some features similar to those of animals and humans suffering from celiac disease: an increased intestinal permeability occurring before the other clinical manifestations *(6–9)* and an influence of diet on the course of the disease *(3–5,10)*. Indeed, a gluten-free diet induces in humans a remission of celiac disease *(3–5)* while a hydrolyzed casein diet reduces the incidence of diabetes or delays its onset in BBdp rats *(10)*. Moreover antigliadin and endomysial antibodies associated with celiac disease are more prevalent in type 1 diabetes patients and their offspring than in the general population *(11,12)*.

The aim of the present study was to evaluate duodenal disaccharidases and peroxidase activities as markers of mucosal damage and inflammatory processes, respectively, in BBdp rats.

Results

Methodological Considerations

Under the present experimental conditions, the velocity of the reaction catalyzed by the three hydrolases under consideration was fairly stable up to at least min 20 (maltase and lactase) or min 60 (invertase) of incubation at 30ºC. The reaction velocity was proportional to the protein content of the samples up to 60 μg (maltase and invertase) or 300 μg (lactase) per assay. As judged from the coefficient of variation (CV) (i.e., the SD/mean ratio) of assays conducted in triplicate, the within-day precision averaged 3.5 ± 0.4 (n = 38) in the case of invertase, 2.9 ± 0.4% (n = 28) in the case of maltase, and 4.2 ± 0.4% (n = 38) in the case of lactase. The recovery of enzymatic activity after one cycle of freezing and thawing averaged 101.7 ± 4.3% (n = 15). As documented in Fig. 1, when the same samples with either low or high specific activity were examined on six successive days, the CV for the enzymatic determinations averaged 9.6 ± 1.5% (n = 6).

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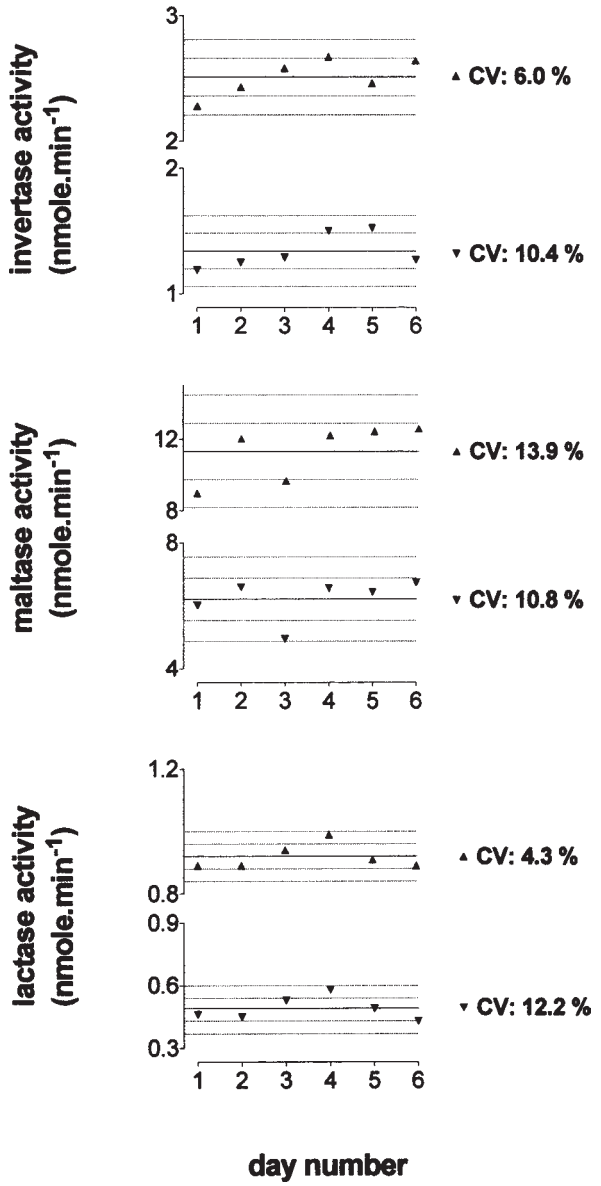

Fig. 1. Daily measurements of invertase, maltase, and lactase activity (nanomoles/minute) in samples with either high or low enzymatic activity. The CV, mean value (solid line), and mean ± 1 or 2 SDs (dotted lines) are indicated in each case.

As assessed with D-[U-^{14}C]glucose, D-[U-^{14}C]fructose, or a mixture of these two hexoses, respectively, the second step of the assay procedure—i.e., the phosphorylation of the monosaccharides—yielded a recovery of 95.3 ± 2.2% (n = 10), 95.0 ± 1.0% (n = 10), and 94.4 ± 2.1% (n = 20). The amount of phosphorylated monosaccharides formed in the presence of yeast hexokinase was also strictly proportional to the initial concentration of the hexose(s).

For all three hydrolases, there was a close correlation ($p < 0.001$) between the results of measurements made in either the scraped material or whole extract of successive segments of the small intestine (Fig. 2, left). In 18 such successive segments, the specific activity of the three hydrolases progressively increased to reach a peak value in the tenth segment and then was much lower in the last six segments (Fig. 2, right). The paired ratio in specific activity in the scraped/total material was not significantly different with each hydrolase, averaging 1.53 ± 0.09 in the case of invertase, 1.48 ± 0.05 in the case of maltase, and 1.34 ± 0.10 in the case of lactase (n = 14 in all cases).

The peroxidase activity of duodenal samples was assessed by the *O*-phenylenediamine method. The absorbance of the oxidized product peaked at 430 nm. With horseradish peroxidase (HRP), the initial reaction velocity, as judged by the change in optical density per minute, was proportional to the amount of HRP present in each sample (Fig. 3). The initial reaction velocity was also proportional to the protein content in each individual duodenal extract (data not shown). When the specific activity of peroxidase was measured in both a particulate and a soluble fraction obtained by centrifugating for 15 min at 40,000*g*, the recovery of peroxidase activity averaged 109.5 ± 4.2% (n = 11), and only 22.2 ± 3.5% (n = 12) of the enzymatic activity measured in the total extract was recovered in the supernatant fraction.

In some instances, the mean value for duodenal enzymatic activity was significantly different in male and female rats. For instance, in both control BioBreeding (BBc) and BBdp rats, the specific activity of peroxidase was significantly higher in female rats than in males. Two comparisons between BBc and BBdp rats were therefore performed. In the first analysis, results obtained in male and female rats were pooled. In the second analysis, the individual results recorded in both BBc and BBdp rats were all expressed relative to the mean control value found for the same enzyme in BBc rats of the same sex. The normalized results so obtained in male and female animals were then pooled.

Metabolic and Hormonal Status of BBc and BBdp Rats

All animals used in the present study were of comparable age (60–66 d). Close-to-equal numbers (seven to nine) of male and female rats were examined in the BBc and BBdp groups (Table 1).

The body weight was higher ($p < 0.001$) in male than female rats. The body weight of BBdp rats was not lower than that of BBc rats and the daily gain in body weight was comparable in BBc and BBdp rats of the same sex. Likewise, in the fed state, no significant difference in plasma D-glucose concentration was found between BBc and BBdp rats. The plasma insulin concentration was lower ($p < 0.001$), however, in BBdp rats (13.80 ± 2.76 μU/mL; n = 15) than in BBc rats (29.01 ± 2.76 μU/mL; n = 16). Likewise, the insulinogenic index (i.e., the paired ratio between plasma insulin and D-glucose concentrations) was significantly lower ($p < 0.05$ or less) in BBdp rats than in BBc rats, whether in male or female animals. This coincided with the fact that the insulin content of the pancreas (U/g wet wt) represented in the BBdp rats no more ($p < 0.02$) than 81.7 ± 6.4%

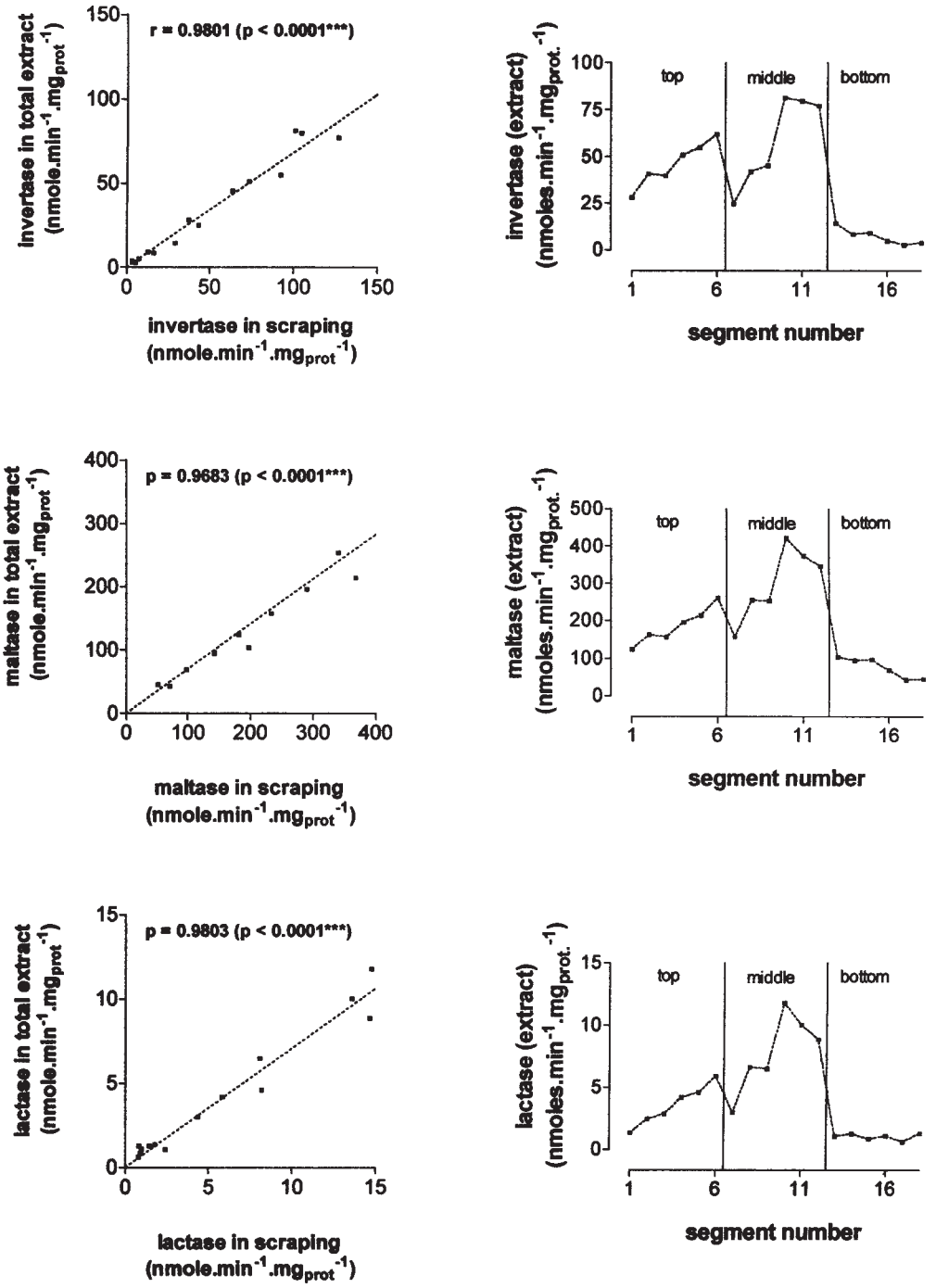

Fig. 2. (**Left**) Correlation among invertase, maltase, and lactase activity in paired intestinal samples tested in either total or scraped extracts; (**right**) pattern of invertase, maltase, and lactase activity in successive intestinal segments tested in total extracts.

(n = 16) of the mean control value found in BBc rats of the same sex (100.0 ± 3.7%; n = 16).

Histologic examination of the pancreas revealed incipient insulitis in only 2 of 16 BBdp rats.

Duodenal Enzymatic Activities in BBc and BBdp Rats

As documented in Table 2, the activity of invertase, maltase, and lactase was lower ($p < 0.01$ or less) in the duodenum of BBdp rats than in BBc rats. There was a significant correlation ($p < 0.015$ or less) between the individual values of all hydrolases in these animals (Fig. 4). There was no significant difference in the mean peroxidase specific activity of duodenal samples from BBc and BBdp rats (Table 2). Although there was no significant correlation (n = 31; $p >$ 0.5) between peroxidase activity and plasma D-glucose concentration, 2 female BBc rats with abnormally high plasma D-glucose concentrations (12.4 and 14.8 mM vs a mean value of 10.8 ± 0.2 mM for the other 14 BBc rats) displayed

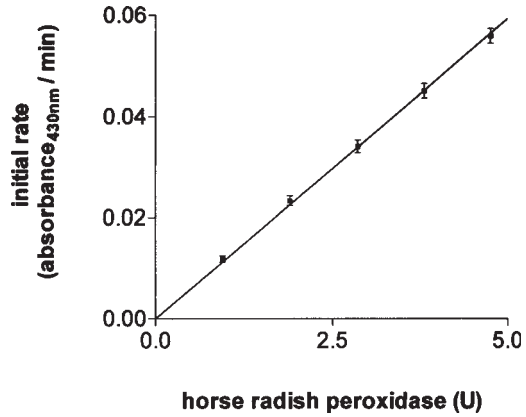

Fig. 3. Initial reaction velocity in assay of peroxidase in presence of increasing amounts of HRP. Mean values (±SEM) refer to six individual determinations.

Table 1
Metabolic and Hormonal Data in BBc and BBdp Rats

	BBc		Bdp	
	Male	Female	Male	Female
Number (*n*)	9	7	7	9
Age (d)	60–64	62–66	60–64	62–66
Body weight (g)	244 ± 4	179 ± 5	251 ± 6	192 ± 3
Gain in body weight (g/d)	4.90 ± 0.55	3.42 ± 0.24	5.10 ± 0.36	3.56 ± 0.36
Plasma D-glucose (m*M*)	10.96 ± 0.23	11.32 ± 0.75	10.82 ± 0.55[a]	10.45 ± 0.21
Plasma insulin (μU/mL)	28.72 ± 3.40	29.01 ± 2.76	19.02 ± 2.11[a]	10.32 ± 1.48
Paired plasma insulin/glucose ratio (U/mol)	2.62 ± 0.31	2.51 ± 0.33	1.75 ± 0.14[a]	0.99 ± 0.15
Pancreas insulin content (U/g wet wt)	3.23 ± 0.20	4.82 ± 0.18	2.83 ± 0.46	3.72 ± 0.17

[a] $n = 6$ (not 7).

Table 2
Enzymatic Activities in Duodenum of BBc and BBdp Rats

Rats	BBc	BBdp	*p*
Invertase (U/g)	32.0 ± 2.1 (16)	21.1 ± 1.3 (16)	<0.001
Maltase (U/g)	158.5 ± 7.4 (16)	128.5 ± 6.7 (16)	<0.0055
Lactase (U/g)	1.29 ± 0.06 (16)	1.00 ± 0.06 (16)	<0.0012
Peroxidase (U/g)	54.6 ± 7.7 (16)	82.2 ± 13.2 (16)	<0.09

a peroxidase activity (92.6 and 140.6 U/g) in excess of the upper limit of the 95% confidence interval (85.3 U/g) for the values recorded in the other 14 BBc rats (Fig. 5). When these two BBc rats were excluded from analysis of the results, the duodenal peroxidase activity (45.7 ± 4.9 U/g; $n = 14$) became significantly higher ($p < 0.021$) in BBdp rats than in BBc rats.

Identical conclusions were reached when comparison between BBc and BBdp rats was conducted on the normalized values calculated within each sex (as discussed earlier). Thus, in the BBdp rats, the activity of invertase, maltase, and lactase averaged, respectively, 70.2 ± 4.4, 81.2 ± 4.3, and 75.7 ± 4.1% ($n = 16$ in all cases) of the control values found in BBc rats of the same sex (100.0 ± 4.7, 100.0 ± 4.7, and 100.0 ± 3.4%; $n = 16$ in all cases). The mean values for the three hydrolases in BBdp rats were thus significantly lower ($p < 0.01$ or less) than those found in BBc rats. Inversely, after exclusion of the two female BBc rats with abnormally high plasma D-glucose concentration, the activity of peroxidase in the BBdp rats exceeded ($p < 0.02$) that found in BBc rats, averaging 157.4 ± 20.0% ($n = 16$) of the mean control value recorded in BBc rats of the same sex (100.0 ± 9.3%; $n = 14$).

Discussion

The brush-border membrane disaccharidases have already been shown to be increased in drug-induced (alloxan and streptozotocin) diabetic rats and in type 2 diabetes patients *(13–19)*. Moreover, experimental diabetes alters other intestinal functions such as active transport mechanisms for monosaccharides, amino acids, sodium, and bile acids (reviewed in refs. *16*). In rats with experimentally induced diabetes and in humans with non-insulin-dependent diabetes mellitus, there is an increase in the capacity of the intestine to absorb monosaccharides; humans overexpress different monosaccharide transporters *(20)*. Insulin therapy in humans showed a return of disaccharidase activities toward the nor-

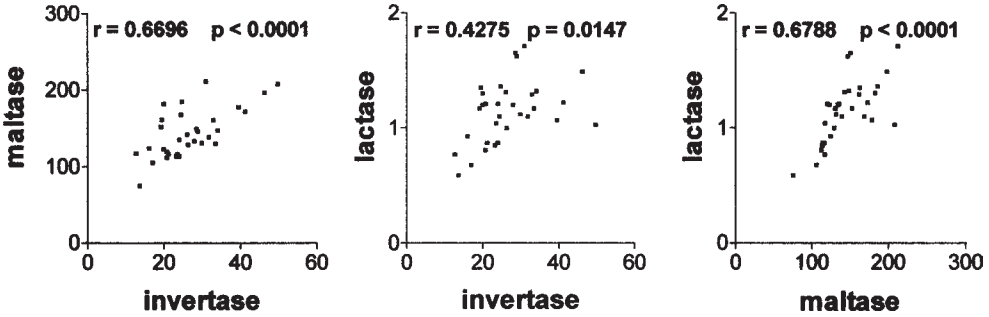

Fig. 4. Correlation among invertase, maltase, and lactase activity in 32 duodenal samples obtained from BBc and BBdp rats.

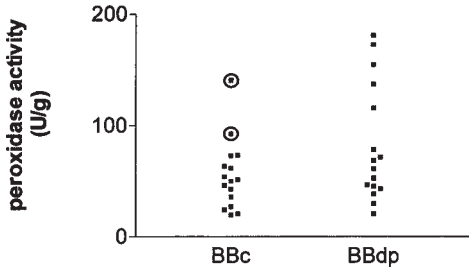

Fig. 5. Peroxidase activity in duodenal samples from BBc and BBdp rats. Abnormally high values recorded in two BBc rats are circled.

mal range *(13)*. Enhancement of sucrase activity is independent of gut intraluminal factors *(14,16)*.

Our study shows that an animal model of type 1 diabetes (BBdp rats) presents an opposite pattern: a decrease in disaccharidase activities in duodenum as found in human celiac disease. Different studies have shown a decrease in disaccharidases correlated with the severity of the mucosal damage *(3,4)* in celiac disease. One study *(19)* reported sucrase, maltase, and lactase activities lowered to 13.8–94.8% of the control values in duodenal biopsies from humans suffering from celiac disease at different stages of mucosal damage. Sucrase activity was considered a good indicator of mucosal response to gluten-free diet while lactase has persistently been shown to remain low *(3)*. Other studies have reported reduced disaccharidase activities associated with an increase in inflammatory cell infiltrate *(21–23)*. As far as we know, no studies reported data concerning disaccharidases in an animal model of celiac disease.

In the present experiments, like in prior studies conducted at approximately the same age *(24–26)*, the BBdp rats displayed an apparently normal plasma D-glucose concentration, despite a low plasma insulin concentration. It should be kept in mind, however, that in rats hyperglycemia only develops when 80–90% of the pancreatic gland is removed. Moreover, in the present animal model, the time course for the decrease in B-cell mass is quite variable in distinct BBdp rats. Although significant correlations were found between the plasma insulin concentration or insulinogenic index and either the invertase activity ($p < 0.005$ for the plasma insulin concentration and $p < 0.002$ for the insulinogenic index) or lactase activity ($p < 0.025$ for the plasma insulin concentration and $p < 0.02$ for the insulinogenic index), there are reasons to believe that the decrease in disaccharidase activity is not the consequence of insulin deficiency. First, no significant correlation was observed between plasma insulin concentration or insulinogenic index and maltase activity. Second, and more convincingly, a number of prior studies have documented that in alloxan or streptozotocin rats, the activity of intestinal invertase, maltase, and peroxidase is strikingly increased, independently of any dietary or intraluminal factors *(14–18)*. Hence, the present results would rather suggest that the lowering of plasma insulin concentration reflects the severity of the intestinal defect.

The altered activity of disaccharidases in the duodenum of BBdp rats coincided with an increase in peroxidase activity. The latter finding is compatible with a possible inflammation of the duodenum.

In conclusion, our study demonstrates that a duodenal proinflammatory state may precede the occurrence of pancreatic insulitis of BBdp rats. Further investigations are necessary to evaluate the extent along the gut, the time course, and the diet dependency of this duodenum anomaly.

Materials and Methods

The experimental protocol was approved by the Comité d'Ethique et du Bien-Etre Animal of Brussels Free University.

Animals

Male and female BBdp and nondiabetes-prone BBc rats were obtained from the colonies maintained at the Animal Resources Division of Health Canada (Ottawa, Canada) and transferred 45–48 d after birth to Brussels, Belgium. Their body weight was monitored from the d 9 to 14–16 after arrival in Brussels, during which period they were housed in groups of three to five in separate cages. The animals were given free access to tap water and NIH-07 diet *(27)* up to the time of killing by decapitation at 60–66 d of age. The BBdp rats fed the NIH-07 diet become diabetic at 60–120 d of age.

Blood Collection and Preparation of Pancreas and Intestine

Blood was collected in heparinized tubes for the measurement of plasma D-glucose *(28)* and insulin *(29)* concentrations. The pancreas and intestine were rapidly removed. The pancreas was divided into two parts. The first part was weighed and immediately homogenized in acid-alcohol *(30)* for measurement of its insulin content *(31)*, while the second part was stored at 4ºC in phosphate-buffered saline (pH 7.0) containing 10% formol for later histologic examination.

Determination of Invertase, Maltase, and Lactase Activities

The entire small intestine from the pylorus up to the ileocecal opening and the large intestine (cecum excluded) were washed with cold 0.9% saline and trimmed free of all mesenteric tissues. The first 5-cm segment from the pylorus was sampled. The duodenal mucosa was scraped off with a glass slide; cells were transferred in 3 mL of cold distilled water and immediately frozen. After thawing and ultrasonication (three times for 10 s each on ice), invertase, maltase, and lactase activities were measured in a two-step method, as suggested by Dahlqvist *(32)*, consisting of an incubation in the presence of the appropriate substrate followed by determination of D-glucose. Our procedure was based on the use of appropriate labeled substrates: [U-^{14}C] sucrose (NEN, Boston, MA) for invertase activity that liberates D-[U-^{14}C]glucose and D-[U-^{14}C]fructose, [U-^{14}C] maltose (Amersham, Little Chalfont, UK) for maltase activity that liberates 2 mol of D-[U-^{14}C]glucose, and [D-[1-^{14}C] glucose]lactose (Amersham) for lactase that produces 1 mol of labeled D-glucose and 1 mol of unlabeled D-galactose. The first step was conducted in a reaction mixture (100 µL) consisting of HEPES-NaOH buffer (50 m*M*, pH 6.5) containing 6 m*M* $MgCl_2$, 60 m*M* KCl, and 10 m*M* KH_2PO_4. After an incubation of 15 min at 30ºC in the presence of the appropriate substrate (28 m*M*), the enzymatic reaction was stopped by heating at 85ºC for 10 min. In the second step, the labeled products were phosphorylated (assay volume: 500 µL) at 37ºC during 2 h in the presence of 3.6 U/mL of yeast hexokinase (Roche, Mannheim, Germany) and 3.2 m*M* adenosine triphosphate dissolved in the same buffer as just mentioned but adjusted to pH 7.5.

After separation of the phosphorylated products (D-glucose and D-fructose) from their precursor by ion-exchange chromatography, the former products were counted by liquid scintillation. All measurements were made in triplicate. Under the present experimental conditions, the radioactivity of the blank (sample omitted) corresponded to 1.16 ± 0.02% (n = 6), 0.76 ± 0.02% (n = 6), and 0.18 ± 0.03% (n = 6) of the radioactivity present in the assay mixture for [U-^{14}C]sucrose, [U-^{14}C]maltose, and [D-[1-^{14}C]glucose]lactose, respectively. At the concentration of the substrates used in these experiments (28 m*M*), the disaccharidases are >50% saturated, with higher concentrations of the substrates resulting in substrate inhibition *(32)*.

Determination of Peroxidase Activity

To determine peroxidase activity *(33,34)*, scraping homogenates were diluted (1:1) in hexadecyltrimethyl-ammonium bromide (HTAB) buffer (1% HTAB in 100 m*M*, pH 6.0 phosphate buffer). These diluted samples were freeze-thawed three times in a dry ice/acetone bath and sonicated three times for 10 s each on ice. Peroxidase activity was then measured spectrophotometrically at 30ºC. For this purpose, 0.1 mL of sample was mixed with 2.9 mL of a 50 m*M*, pH 6.0, phosphate buffer containing 0.167 mg/mL of *O*-phenylenediamine-dihydrochloride (Sigma, St. Louis, MO) and 158 µ*M* hydrogen peroxide (Merck, Darmstadt, Germany). The change in absorbance at 430 nm was recorded on a Beckman DU®-65 spectrophotometer (Beckman, Fullerton, CA). The rate of reaction was linear at least for 2 min. One unit of peroxidase activity was defined as a one absorbance unit increase per min under the present experimental conditions.

Statistical Analyses

All results are presented as mean values (±SEM) together with the number of individual determinations (n). The statistical significance of differences between mean values was assessed by use of student's t-test, or analysis of variance whenever appropriate.

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