

## Disaccharidase activity in the intestinal tract of Wistar–Furth, diabetes-resistant and diabetes-prone BioBreeding rats

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Diabetes-prone BioBreeding (BBdp) rats often present an enteropathy that may precede the onset of autoimmune insulinitis. The aim of the present study was to assess the influence of sex, the time course, the strain specificity, the distribution along the intestinal tract and the effect of diet for the changes in the activity of gut invertase, maltase and lactase found in BBdp rats, as compared with both Wistar–Furth (WF) and diabetes-resistant BioBreeding (BBc) rats. These hydrolases were measured, therefore, at day 10, 30, 45, 70, 95 and 120 in three intestinal segments of WF, BBc and BBdp rats fed, after weaning, either a protective high-casein diet, which decreases the incidence of diabetes in the BBdp rats, or one of two diabetogenic diets (National Toxicology Program; NTP or wheat-gluten-based; WG). Except for a somewhat lower lactase activity in the BBdp rats, no obvious difference in hydrolyase activity between the three strains of rats was observed at day 10. Between days 30 and 120, however, the activity of the hydrolases, especially that of invertase and lactase, was lower in the BBdp rats than in either the WF or BBc rats, at least when considering the animals fed either the NTP or WG diet. These findings support the view that BBdp rats exposed to a diabetogenic diet develop an enteropathy well before the onset of autoimmune insulinitis, in a manner somehow comparable with the situation found in some type 1 diabetic patients, in whom coeliac disease may be diagnosed before diabetes onset.

### BioBreeding rats: Enteropathy: Disaccharidases

It was recently documented that diabetes-prone BioBreeding (BBdp) rats often present an enteropathy that may precede the onset of autoimmune insulinitis. It is characterised by increased crypt length and mitotic activity, increased numbers of intra-epithelial lymphocytes, increase in peroxidase activity, decreased glucagon-like peptide-1 content, decreased disaccharidase activities and increased gut permeability (Meddings *et al.* 1999; Courtois *et al.* 2002; Graham *et al.* 2002; Hardin *et al.* 2002; Malaisse *et al.* 2002). In the present study, the activity of invertase, maltase and lactase was measured at day 10, 30, 45, 70, 95 and 120 in three segments of the intestinal tract in Wistar–Furth (WF) rats, diabetes-resistant BioBreeding (BBc) rats and BBdp rats. The rats were fed, after weaning, either a protective hydrolysed casein-based (HC) diet, which inhibits the development of diabetes in BBdp rats (MacFarlane *et al.* 2003), or one of two diabetes-promoting diets, namely the National Toxicology Program-2000 (NTP) or a wheat-gluten-based (WG) semi-purified AIN-Q36 diet. Based on these 2578 individual measurements, the present study aimed mainly to assess the influence of sex, time course, strain specificity, distribution along the intestinal tract and the effect of diet on the activity of the three hydrolases.

### Materials and methods

#### Animals

Male and female BBdp rats and control BBc rats were obtained from the colonies maintained at the Animal Resources Division of Health Canada (Ottawa, Canada). WF rats were purchased from Charles River (St Constant, Quebec, Canada). All animals were raised under specific-pathogen-free conditions and were weaned at 23 d of age and given free access to food and water. Rats were killed at 10, 30, 45, 70, 95 or 120 d of age. Experiments were approved by the local Animal Care Committee in Ottawa and by the Comité d’Ethique et du Bien-Etre Animal of Brussels Free University (Belgium).

#### Diets

After weaning, from the 23rd day up to the time of killing, the groups of rats were fed one of three diets (MacFarlane *et al.* 2003). The NTP-2000 (NTP) diet (Zeigler Bros., Gardners, PA, USA), mainly plant-based (milk-free), had wheat as the major component (37%) and contained approximately 14.6% protein, 8.2% fat, 9.9% crude

**Abbreviations:** BBc, diabetes-resistant BioBreeding; BBdp, diabetes-prone BioBreeding; HC, hydrolysed casein-based; NTP, National Toxicology Program; WF, Wistar–Furth; WG, wheat-gluten-based.

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fibre, 52% carbohydrate, and 10.7% moisture. The WG diet consisted of 22.5% wheat gluten (ICN Biochemicals, Cleveland, OH, USA) and was based on the AIN-Q36 formulation. The hydrolysed casein (HC) diet contained 20.0% casein hydrolysate (pancreas S enzymic hydrolysate; Redstar Bioproducts, Mississauga, Ontario, Canada) as a source of amino acids. The two semi-purified diets were isoenergetic and isonitrogenous. The NTP diet was irradiated before use and contained low levels of chemical and microbial contaminants.

### Gut sample collection

After the rats were killed, the entire small intestine from the pylorus until the ileocaecal opening was quickly removed and washed with cold 0.9% (w/v) saline. Gut pieces were trimmed free of all mesenteric tissues and divided into three equal parts designated as top, middle and bottom. Each part was divided into six different segments of equal length which were immediately frozen. After ultrasonication ( $3 \times 10$  s on ice) in 3 ml cold distilled water, disaccharidase activities were measured in the 6th segment of the proximal, median or distal part of the gut.

### Measurement of disaccharidase activity and protein

The activity of invertase, maltase and lactase was assessed by methods described elsewhere (Courtois *et al.* 2002). Briefly, after 15 min incubation at 30°C in the presence of [ $U$ - $^{14}C$ ]sucrose, [ $U$ - $^{14}C$ ]maltose or [ $D$ -[ $1$ - $^{14}C$ ]glucose]-lactose and heating for 10 min at 85°C, the  $^{14}C$ -labelled hexoses were phosphorylated in the presence of yeast hexokinase and their phosphorylated products separated by ion-exchange chromatography. The assay of protein was made according to the method of Lowry *et al.* (1951).

### Management of data and statistics

All results are presented as mean values and standard errors of the mean together with the number of individual determinations ( $n$ ). The statistical significance of the differences between mean values was assessed by the use of the Student's  $t$  test or by ANOVA.

## Results

### Invertase

At the age of 10 d (i.e. before weaning), in the animals fed dam's milk, the activity of invertase was often below the limit of detection, especially in the top segments (Table 1). No obvious influence of sex was detected at this young age. Pooling all available data, the activity of invertase averaged no more than 4.8 (SEM 2.2) nmol/min per mg ( $n$  30) in male rats and 4.0 (SEM 1.1) nmol/min per mg ( $n$  35) in female animals. The activity of invertase was comparably low ( $P > 0.2$ ) in the top segment (3.1 (SEM 1.8) nmol/min per mg;  $n$  36) and bottom segment (5.9 (SEM 1.2) nmol/min per mg;  $n$  32). No significant difference in invertase activity ( $P > 0.3$ ) was found between the BBc rats (1.6 (SEM 0.7) nmol/min per mg;  $n$  21) and the BBdp rats (2.6 (SEM 0.7) nmol/min per mg;  $n$  23).

**Table 1.** Invertase activity (nmol/min per mg) in the intestinal tract of rats\* (Mean values and standard errors of the mean)

Day	Diet	WF rats						BBc rats						BBdp rats						
		Top		Mid		Bottom		Top		Mid		Bottom		Top		Mid		Bottom		
		Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	
10	Milk	7.1	5.4	12																
30	HC	41.7	4.2	5	50.0	2.3	5	13.0	9.9	2.5	12	3.4	1.4	9	1.8	0.7	12	40.0	3.5	11
	NTP	56.1	3.2	5	54.4	8.9	5	2.9	13.0	3.1	5	28.3	12.2	5	29.7	1.1	10	32.5	8.5	10
	WG	43.0	3.9	5	56.3	11.6	5	31.2	11.8	0.7	5	20.6	9.5	5	28.0	2.5	10	49.7	12.9	10
45	HC	34.4	5.9	5	54.9	3.7	5	4.3	4.3	1.1	5	4.2	0.9	5	29.3	2.6	9	69.9	6.8	10
	NTP	32.3	3.4	5	51.7	1.1	5	2.6	2.6	0.3	5	3.3	0.4	5	34.9	3.2	10	56.6	4.4	10
	WG	35.1	2.2	5	53.6	11.5	5	15.0	4.5	4.5	5	4.8	1.2	5	32.0	2.6	10	77.7	2.5	10
70	HC	41.4	9.5	3	41.5	4.4	4	3.7	3.7	0.6	4	4.4	0.5	4	38.0	2.9	9	40.0	4.0	9
	NTP	45.7	9.4	3	31.5	4.8	4	2.9	4.6	0.5	4	5.2	0.4	5	33.0	3.1	6	41.2	3.3	7
	WG	41.6	8.1	3	49.8	7.6	4	4.6	4.6	0.8	4	6.4	1.0	4	36.0	3.9	7	51.2	4.4	6
95	HC	42.7	2.8	5	52.9	1.7	5	8.0	8.0	3.7	5	5.4	2.0	4	37.6	7.8	9	47.4	4.3	9
	NTP	61.3	10.4	5	39.6	2.0	5	5.7	5.7	2.4	5	11.8	4.2	5	60.5	7.8	7	45.0	5.6	7
	WG	47.2	3.9	5	54.4	3.6	5	6.7	6.7	3.5	5	16.9	7.9	5	45.6	11.3	5	61.9	7.2	5
120	HC	19.5	3.9	5	30.8	4.8	5	6.1	6.1	1.8	5	7.3	3.0	4	17.8	2.6	6	27.4	1.1	6
	NTP	28.7	1.7	5	22.4	4.2	5	9.8	9.8	2.9	4	8.1	2.1	5	25.4	5.1	6	24.0	4.4	6
	WG	24.4	2.0	4	29.2	5.6	4	9.2	9.2	3.6	4	6.8	1.3	6	19.8	2.9	7	39.2	8.6	7

WF, Wistar-Furth; BBc, diabetes-resistant BioBreeding; BBdp, diabetes-prone BioBreeding; HC, hydrolysed casein-based; NTP, National Toxicology Program; WG, wheat-gluten-based.

\* For details of animals, diets and procedures, see pp. 201–202.

The activity of invertase appeared somewhat higher in the WF rats (8.5 (SEM 2.9) nmol/min per mg;  $n$  24). Such a difference only achieved statistical significance ( $P < 0.05$ ), however, when comparing the WF rats with either the BBc rats or both the BBc and BBdp rats (2.1 (SEM 0.5) nmol/min per mg;  $n$  44).

At 30 d of age, sizeable invertase activity was found in the top, mid and bottom segments, with overall mean values of 36.5 (SEM 1.5) nmol/min per mg ( $n$  59), 44.9 (SEM 2.4) nmol/min per mg ( $n$  59) and 13.8 (SEM 2.1) nmol/min per mg ( $n$  60), respectively. A comparable situation prevailed at older ages (45, 70, 95 and 120 d). Except in the bottom segment, in which the invertase activity was always much lower than in the top or mid segment, there was almost always a trend towards lower invertase activity in female rats than in male animals of the same age (30 to 120 d). Thus, in the former animals, the invertase activity of the top and mid segments averaged 89.1 (SEM 2.0) % ( $n$  280;  $P < 0.001$ ) of the mean values found at the same intestinal level in male rats of the same age (100.0 (SEM 1.9) %;  $n$  245).

The distribution pattern of invertase activity along the intestinal tract was grossly comparable in the rats examined between days 30 and 120. For instance, the value found in the bottom segment averaged at 30 and 120 d, respectively, 37.8 (SEM 5.9) % ( $n$  60) and 33.0 (SEM 3.6) % ( $n$  47) of the mean corresponding value found at the same age in the top segment (100.0 (SEM 4.0) %;  $n$  59 and 100.0 (SEM 5.8) %;  $n$  48). The invertase activity in the mid segment was equal to or higher than that found in the top segment. Thus, relative to the latter value, it averaged 122.9 (SEM 6.6) % ( $n$  59) at 30 d, 178.8 (SEM 6.4) % ( $n$  59) at 45 d, 104.3 (SEM 4.4) % ( $n$  48) at 70 d, 97.1 (SEM 4.1) % ( $n$  50) at 95 d, and 128.2 (SEM 8.6) % ( $n$  48) at 120 d.

The activity of invertase remained fairly stable between days 30 and 95. Overall mean invertase activity (top and mid segments) was 40.7 (SEM 1.5) nmol/min per mg ( $n$  118) at day 30. At day 45, overall invertase activity was 49.0 (SEM 1.8) nmol/min per mg ( $n$  120), at day 70 it was 45.1 (SEM 1.5) nmol/min per mg ( $n$  93) and at day 95 it was 50.4 (SEM 1.7) nmol/min per mg ( $n$  100). At day 120, however, it was decreased ( $P < 0.001$ ) to 27.2 (SEM 1.3) nmol/min per mg ( $n$  96).

The mean value for invertase activity averaged in the WF rats 96.6 (SEM 2.8) % ( $n$  139;  $P > 0.35$ ) of the corresponding mean measurements made at the same level (top and mid segments) and same age (30 to 120 d) in the BBc rats fed the same diet (100.0 (SEM 2.4) %;  $n$  146).

At day 30, the activity of invertase in the three segments (top, mid and bottom) in the BBdp rats averaged 77.1 (SEM 5.2) % ( $n$  88;  $P < 0.01$ ) of the mean corresponding values (100.0 (SEM 8.1) %;  $n$  45) found at the same age and at the same level of the intestinal tract in the BBc rats fed the same diet. A decrease of invertase activity in the BBdp rats was also observed in most cases in older animals (days 45 to 120), but it failed on occasion to achieve statistical significance. All available data collected between days 30 and 120 in the top and mid segments were then pooled. The invertase activity in the BBdp rats averaged 91.0 (SEM 2.5) % ( $n$  242;  $P < 0.02$ ) of the mean

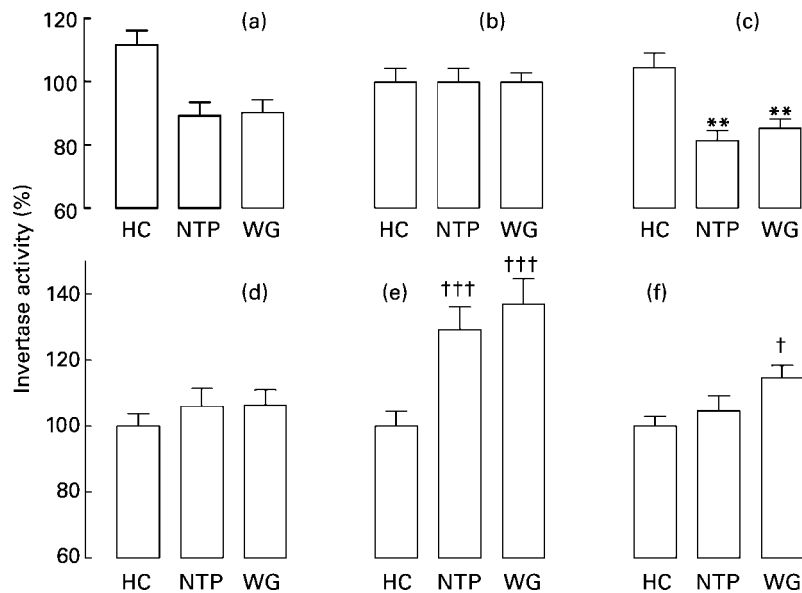
corresponding values (100.0 (SEM 2.4) %;  $n$  146) found at the same age and at the same level in the BBc rats fed the same diet.

Three factors must be taken into account when considering the latter percentages. First, the decrease in invertase activity found in the BBdp rats was not identical at all ages. In the top and mid segments, the invertase activity was decreased to 84.3 (SEM 4.7) % at day 30 ( $n$  58;  $P < 0.06$  v. the mean corresponding values found at the same level of the intestinal tract and at the same age in the BBc rats fed the same diet, in this and following cases). The invertase activity was decreased to 72.8 (SEM 3.6) % at day 70 ( $n$  44;  $P < 0.001$ ) and 86.3 (SEM 5.7) % at day 120 ( $n$  38;  $P < 0.12$ ). The overall mean value was 81.2 (SEM 2.7) % ( $n$  140;  $P < 0.001$ ). On days 45 and 95, however, the invertase activity in the BBdp rats appeared little affected, averaging 111.0 (SEM 5.8) % at day 45 ( $n$  60;  $P > 0.2$ ) and 94.8 (SEM 5.7) % at day 95 ( $n$  42;  $P > 0.5$ ), with an overall mean value of 104.3 (SEM 4.2) % ( $n$  102;  $P > 0.45$ ).

Second, the results obtained in different segments of the intestinal tract were not identical. All available data recorded between days 30 and 120 were pooled and the results expressed relative to the mean corresponding values found at the same level and at the same age in the BBc rats fed the same diet. The activity of invertase was decreased to 79.2 (SEM 2.6) % ( $n$  121;  $P < 0.001$ ) in the top segment, whilst averaging 102.7 (SEM 4.0) % ( $n$  121;  $P > 0.6$ ) in the mid segment of the BBdp rats.

Last, the results obtained with different diets in the BBdp rats were also different from one another (Fig. 1). At days 30, 70 and 120, the activity of invertase was obviously decreased in the BBdp rats, when expressed relative to those found at the same level of the intestinal tract in the BBc rats of the same age fed the same diet. On these days the mean values for the measurements made in the top and mid segments were almost always (i.e. in five out of six cases) lower in the animals fed the NTP diet than in those fed either the HC or WG diet. All measurements recorded between the ages of 30 and 120 d (including days 45 and 95) in the BBdp rats fed the NTP diet were next taken into account. Even then, the activity of invertase in the top segment averaged 78.3 (SEM 4.7) % ( $n$  39;  $P < 0.005$ ) of the corresponding values found at the same level of the intestinal tract and at the same age in the BBc rats also fed the NTP diet. Likewise, the relative activity of invertase averaged, in the mid segment, 85.0 (SEM 5.3) % ( $n$  40;  $P = 0.09$ ). The overall mean value (top and mid segments) was 81.7 (SEM 3.5) % ( $n$  79;  $P < 0.005$ ). For the purpose of comparison, the same overall mean value (top and mid segments) averaged in the BBdp rats fed the HC and WG diet, respectively, 103.6 (SEM 5.2) % ( $n$  88;  $P > 0.6$ ) and 85.9 (SEM 3.1) % ( $n$  75;  $P < 0.005$ ). Thus, the HC diet indeed protected ( $P < 0.001$ ) against the decrease in invertase activity otherwise observed in the BBdp rats fed the NTP diet. In the BBdp rats fed the WG diet, however, a significant decrease in invertase activity was still observed, such a decrease being comparable ( $P > 0.35$ ) with that found in the same type of animals when fed the NTP diet.

In the 30- to 120-d-old WF rats, invertase activity in the top and mid segments averaged, in the animals fed the NTP



**Fig. 1.** The activity of invertase in the top and mid segments of Wistar–Furth (WF; (a) and (d)), diabetes-resistant BioBreeding (BBc; (b) and (e)) and diabetes-prone BioBreeding (BBdp; (c) and (f)) rats examined between day 30 and 120 and fed either the hydrolysed casein-based (HC), National Toxicology Program (NTP) or wheat-gluten-based (WG) diet is expressed relative to the mean corresponding values found at the same level of the intestinal tract and at the same age either in BBc rats fed the same diet ((a), (b) and (c)) or in rats of the same strain fed the HC diet ((d), (e) and (f)). Mean values are derived from forty-five to fifty individual measurements in WF and BBc rats, and seventy-five to eighty measurements in the BBdp rats. Standard errors of the mean are represented by vertical lines. Mean values are significantly different from those for the BBc rats: \*\*  $P < 0.005$ . Mean values are significantly different from those for the rats of the same strain fed the HC diet: †  $P < 0.01$ , †††  $P < 0.001$ .

diet, 106.1 (SEM 5.6) % ( $n$  47;  $P > 0.3$ ) of the mean corresponding values found at the same level of the intestinal tract and at the same age in rats fed the HC diet (100.0 (SEM 3.6) %;  $n$  47). Similarly, invertase activity in the top and mid segments averaged, in the animals fed the WG diet, 106.8 (SEM 4.4) % ( $n$  45;  $P > 0.2$ ). Surprisingly, however, in the rats fed the NTP diet, the invertase activity in the top segments was higher (127.2 (SEM 7.7) % *v.* 100.0 (SEM 6.3) %;  $n$  23 in both cases;  $P < 0.01$ ) than in the rats fed the HC diet. However the activity of invertase was lower in the mid segments (85.8 (SEM 5.6) % *v.* 100.0 (SEM 3.8) %;  $n$  24 in both cases;  $P < 0.05$ ).

In the BBc rats, an increase in invertase activity in the animals fed the NTP diet, as already observed in the top segments of the WF rats, was also often observed in the mid segments. Pooling all results recorded between day 30 and 120 in both the top and mid segments, the invertase activity of the BBc rats fed the NTP diet averaged 130.6 (SEM 6.9) % ( $n$  50;  $P < 0.001$ ) of the mean corresponding values found in the BBc rats fed the HC diet (100.0 (SEM 4.7) %;  $n$  46). In the BBc rats, even the WG diet increased such a percentage to 138.3 (SEM 7.7) % ( $n$  50;  $P < 0.001$ ).

In the BBdp rats, the NTP diet failed to increase significantly the activity of invertase. Thus, the measurements made between day 30 and 120 in the top and mid segments in the BBdp rats fed the NTP diet averaged 105.0 (SEM 4.7) % ( $n$  79;  $P > 0.35$ ) of the mean corresponding values found at the same level of the intestinal tract and at the same age in the BBdp rats fed the HC diet (100.0 (SEM 3.3) %;  $n$  88). In the BBdp rats, however, the WG diet increased invertase activity to 114.7 (SEM 4.6) % ( $n$  75;  $P < 0.01$ ) of the corresponding values found in the BBdp rats fed the HC diet.

Nevertheless, the latter percentage was lower ( $P < 0.01$ ) than that found in the BBc rats, as was also the case when comparing, according to the same criteria, the BBdp and BBc rats both fed the NTP diet ( $P < 0.005$ ).

#### Maltase

At day 10, no significant sex-related difference in maltase activity was observed whether in the WF, BBc or BBdp rats and whether in the top or bottom segments of the intestinal tract. Likewise, no significant difference in maltase activity was found between the top and bottom segments, whether in the WF, BBc or BBdp rats. Pooling all available data, such an activity averaged 43.0 (SEM 4.0) ( $n$  24), 31.7 (SEM 3.8) ( $n$  21) and 38.7 (SEM 4.3) ( $n$  23) nmol/min per mg, respectively in the WF, BBc and BBdp rats (Table 2).

The maltase activity was much higher at older ages, with overall mean values of 207.5 (SEM 6.5) ( $n$  178), 193.1 (SEM 8.0) ( $n$  180), 203.8 (SEM 10.5) ( $n$  142) and 223.8 (SEM 8.9) ( $n$  149) nmol/min per mg at days 30, 45, 70 and 95 respectively. At day 120, however, the activity of maltase was decreased to 61.2 (SEM 2.6) % ( $n$  143) of the mean values found at the same level of the intestinal tract at day 95 (100.0 (SEM 3.7) %;  $n$  149).

From day 30 to 120 inclusive, there was a trend towards lower values for maltase activity in female rats than in male animals. For instance, in the top and mid segments, the values found in female rats averaged 95.0 (SEM 1.9) % ( $n$  280;  $P < 0.05$ ) of the mean corresponding values found at the same age and at the same level of the intestinal tract in male rats (100.0 (SEM 1.8) %;  $n$  245). Such a difference failed to achieve statistical significance, however,

**Table 2.** Maltase activity (nmol/min per mg) in the intestinal tract of rats\* (Mean values and standard errors of the mean)

Day	Diet	WF rats						BBc rats						BBdp rats								
		Top		Mid		Bottom		Top		Mid		Bottom		Top		Mid		Bottom				
		Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n			
10	Milk	35.5	5.3	12	50.5	5.3	12	33.6	4.8	12	29.1	6.2	9	39.5	7.5	12	216.9	19.4	10	37.9	4.4	11
30	HC	238.4	31.9	5	217.8	36.5	5	163.4	23.4	5	231.2	67.4	5	187.1	9.9	10	170.8	18.9	10	175.5	32.9	10
	NTP	249.8	22.8	5	76.2	8.0	5	171.3	27.3	5	168.1	44.7	5	156.0	14.9	10	170.8	18.9	10	160.8	26.6	10
	WG	211.7	25.9	5	351.8	56.0	5	140.2	15.3	5	209.8	12.5	5	194.1	22.5	9	234.8	11.9	9	239.6	18.5	10
45	HC	227.2	8.4	5	370.8	39.5	5	179.7	7.4	5	99.6	12.2	5	176.6	11.1	10	393.7	30.4	10	81.9	13.5	10
	NTP	187.2	16.5	5	244.4	20.4	5	159.1	9.8	5	196.8	24.3	5	161.4	8.8	10	249.4	24.1	10	90.2	9.9	10
	WG	220.4	15.4	5	274.4	21.5	5	193.3	13.3	5	327.1	50.7	5	160.1	7.6	10	335.0	21.9	10	132.8	25.2	10
70	HC	210.4	17.3	3	359.3	68.7	4	188.5	24.5	5	93.0	10.8	5	198.1	15.0	9	419.7	39.2	9	68.2	9.8	9
	NTP	204.3	28.5	3	237.1	41.0	4	228.4	30.8	5	269.3	40.4	5	171.9	15.0	6	222.2	13.9	7	73.2	10.9	7
	WG	217.8	33.0	3	354.7	68.8	4	230.0	11.0	4	380.4	79.4	4	225.1	27.2	7	298.4	17.3	6	131.5	24.5	7
95	HC	247.1	29.0	5	108.2	33.2	5	206.0	30.2	4	131.0	46.9	4	195.3	26.1	9	320.4	14.8	9	114.9	25.8	8
	NTP	343.1	75.1	5	104.3	26.5	5	245.1	36.7	5	138.1	33.7	5	246.5	31.8	7	183.4	24.1	7	115.5	26.7	7
	WG	262.9	34.9	5	115.2	43.1	5	259.6	30.9	5	338.0	25.1	5	247.5	22.8	5	290.7	27.9	5	189.7	51.7	5
120	HC	91.3	10.7	5	67.2	12.4	5	95.2	2.9	4	88.6	10.4	4	85.6	8.9	6	247.2	27.8	6	92.4	17.5	6
	NTP	134.0	13.5	5	100.7	51.3	4	113.0	18.0	5	161.5	22.2	5	97.0	18.9	6	161.7	20.8	6	56.0	7.6	6
	WG	131.4	11.6	4	74.7	14.1	4	102.8	4.5	6	116.4	23.7	6	76.7	14.4	7	209.1	42.1	7	101.4	12.7	5

WF, Wistar-Furth; BBc, diabetes-resistant BioBreeding; BBdp, diabetes-prone BioBreeding; HC, hydrolysed casein-based; NTP, National Toxicology Program; WG, wheat-gluten-based. \*For details of animals, diets and procedures, see pp. 201–202.

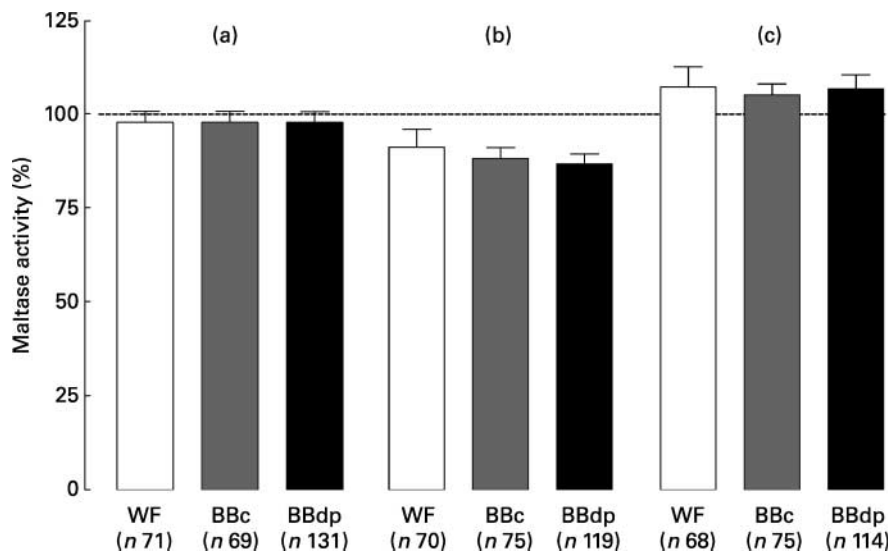
when the measurements made in the bottom segments were also included, because of one abnormal percentage in the rats aged 95 d. If this value is ignored, the maltase activity in the bottom segment in the female rats averaged 85.3 (SEM 4.2) % (*n* 110; *P* < 0.025) of the mean corresponding value found at the same age in male rats (100.0 (SEM 4.9) %; *n* 105).

At all ages between day 30 and 120, the highest maltase activity was found in the mid segments. The values recorded in the top and bottom segments averaged respectively 67.0 (SEM 1.5) % (*n* 262) and 46.6 (SEM 2.2) % (*n* 265) of the mean values found at the same age in the mid segments (100.0 (SEM 2.1) %; *n* 265).

In the WF rats (aged 30 to 120 d), the overall mean value for maltase activity was not significantly different in the animals fed either the NTP or WG diet and those fed the HC diet. Thus, relative to the mean value found at the same age and at the same level of the intestinal tract (top, mid or bottom segment) in the rats fed the HC diet (100.0 (SEM 3.7) %; *n* 71), maltase activity averaged 92.3 (SEM 6.2) % (*n* 70) in the rats fed the NTP diet. Relative maltase activity was 115.4 (SEM 7.4) % (*n* 68) in the animals fed the WG diet. There was a trend, however, for the NTP diet to decrease maltase activity, with lower mean values than in the animals fed the HC diet in eleven out of fifteen group comparisons. Such a decrease only achieved statistical significance (*P* < 0.001) in the mid segments, where the activity of maltase averaged, in the rats fed the NTP diet, 75.8 (SEM 4.3) % (*n* 24) of the mean corresponding value found at the same age in the rats fed the HC diet (100.0 (SEM 4.5) %; *n* 24). The overall mean value for maltase activity in the WF rats fed the NTP diet was also lower (*P* < 0.02) than that found in the WF rats fed the WG diet (see earlier for the relevant percentages).

A comparable, albeit not identical, situation was found in the BBc and BBdp rats. Thus, in the BBc rats, the NTP diet significantly decreased the activity of maltase between day 30 and 120. This was as judged from either the measurements made in the mid segments (74.0 (SEM 5.2) % *v.* 100.0 (SEM 5.9) %; *n* 23–25; *P* < 0.005) or from the overall mean values derived for all available measurements (88.1 (SEM 4.3) % *v.* 100.0 (SEM 3.8) %; *n* 69–75; *P* < 0.05). The WG diet slightly increased (*P* < 0.05) maltase activity to 112.5 (SEM 4.1) % (*n* 75) of the mean value found at the same age and at the same level of the intestinal tract in the BBc rats fed the HC diet. Likewise, in the BBdp rats, the maltase activity was lower in the animals fed the NTP diet rather than the HC diet. This was as judged from either the measurements made in the mid segments (64.6 (SEM 3.4) % *v.* 100.0 (SEM 3.6) %; *n* 40–44; *P* < 0.001) or from the overall mean value calculated from all available data (86.1 (SEM 3.5) % *v.* 100.0 (SEM 3.1) %; *n* 119–131; *P* < 0.005). The WG diet again increased (*P* < 0.02) maltase activity to 115.1 (SEM 5.4) % (*n* 114) of the values found at the same age and at the same level of the intestinal tract in the BBdp rats fed the HC diet. Fig. 2 illustrates the close analogy of the diet-induced changes in maltase activity in the WF, BBc and BBdp rats.

When fed the HC diet, the maltase activity in the WF rats examined between day 30 and 120 was higher



**Fig. 2.** The activity of maltase at day 30 to 120 in Wistar–Furth (WF), diabetes-resistant BioBreeding (BBc) and diabetes-prone BioBreeding (BBdp) rats fed the National Toxicology Program (NTP; (b)) or wheat-gluten-based (WG; (c)) diet is expressed relative to the mean corresponding values found at the same age and at the same level of the intestinal tract (top, mid and bottom segments) in the rats of the same strain fed the hydrolysed casein-based (HC; (a)) diet. Values are means, with standard errors of the mean represented by vertical lines. The number of individual measurements is indicated.

( $P < 0.02$ ) in the top and mid segments than in the BBc rats of the same age also fed the HC diet. Such was not the case, however, in the bottom segments. Pooling all available data, the maltase activity in the WF rats fed the HC diet averaged 107.4 (SEM 4.7) % ( $n = 71$ ;  $P > 0.2$ ) of the corresponding mean value found at the same age and at the same level of the intestinal tract in the BBc rats also fed the HC diet (100.0 (SEM 3.8) %;  $n = 69$ ). Likewise, the overall mean value for maltase activity in the BBdp rats fed the HC diet (99.9 (SEM 3.4) %;  $n = 131$ ) was not significantly different from that found in the BBc rats fed the same diet.

The mean maltase activity in the WF rats fed the NTP diet was higher than that found at the same age and at the same level of the intestinal tract in the BBc rats also fed the NTP diet (113.1 (SEM 6.3) %;  $n = 70$  v. 100.0 (SEM 4.0) %;  $n = 75$ ). This difference was not significant ( $P > 0.07$ ). In the BBdp rats fed the NTP diet, the activity of maltase averaged 87.6 (SEM 6.6) % ( $n = 30$ ) at day 30 of the corresponding mean values found at the same age and at the same level of the intestinal tract (top, mid and bottom segments) in the BBc rats also fed the NTP diet. Relative maltase activity was 83.3 (SEM 5.3) % ( $n = 20$ ) at day 70 and 88.0 (SEM 7.9) % ( $n = 19$ ) at day 120, with an overall mean value of 86.5 (SEM 3.9) % ( $n = 69$ ), significantly lower ( $P < 0.05$ ) than that found in the BBc rats (100.0 (SEM 5.4) %;  $n = 45$ ). Nevertheless, between day 30 and 120, the overall mean value for maltase activity in the BBdp rats fed the NTP diet (99.2 (SEM 3.8) %;  $n = 120$ ) was not different from that found at the same age and at the same level of the intestinal tract in the BBc rats fed the same diet.

Relative to the mean value found at the same age (day 30 to 120) and at the same level of the intestinal tract (top, mid and bottom segments) in the BBc rats fed the WG diet (100.0 (SEM 3.1) %;  $n = 75$ ), maltase activity averaged 108.4 (SEM 5.9) % ( $n = 68$ ) in the WF rats fed the WG

diet. Relative maltase activity averaged 100.9 (SEM 4.0) % ( $n = 114$ ) in the BBdp rats fed the WG diet. In this respect, no significant difference ( $P > 0.19$  or more) was thus found between the three types of rats.

#### Lactase

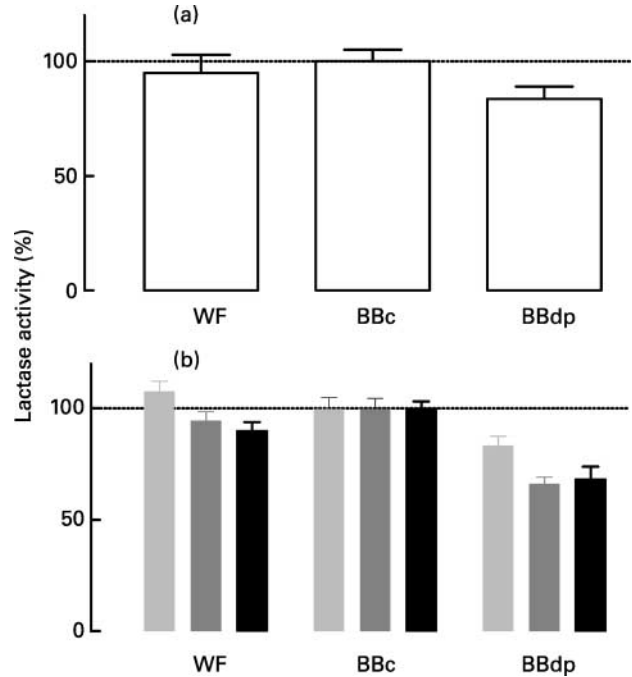
At day 10, lactase activity averaged 39.0 (SEM 2.9) nmol/min per mg ( $n = 24$ ) in the WF rats, 44.2 (SEM 3.7) nmol/min per mg ( $n = 21$ ) in the BBc rats and 35.1 (SEM 2.2) nmol/min per mg ( $n = 23$ ) in the BBdp rats (Table 3). The measurements made in female rats averaged 100.1 (SEM 5.2) % ( $n = 35$ ;  $P > 0.99$ ) of those made in male animals at the same level of the intestinal tract (top or bottom segments) and in the same type of rats (100.0 (SEM 5.8) %;  $n = 30$ ). The activity of lactase in the WF and BBdp rats averaged, respectively, 95.0 (SEM 7.9) % ( $n = 24$ ) and 83.6 (SEM 5.3) % ( $n = 23$ ) of the mean corresponding values found at the same level of the intestinal tract in BBc rats of the same sex (100.0 (SEM 5.1) %;  $n = 18$ ). The difference between the BBc and BBdp rats achieved statistical significance ( $P < 0.05$ ) as judged from either such percentages or the absolute values mentioned earlier (Fig. 3).

At later ages (day 30 to 120), the lactase activity was much lower whether in the top, mid or bottom segments of the intestinal tract and whether in the WF, BBc or BBdp rats. The overall mean values, in male and female rats respectively, were 6.1 (SEM 0.2) and 6.0 (SEM 0.2) nmol/min per mg ( $n = 122$  and 139) in the top segments, and 7.7 (SEM 0.3) and 6.7 (SEM 0.3) nmol/min per mg ( $n = 123$  and 141) in the mid segments. The overall mean values were only 0.9 (SEM 0.1) and 1.2 (SEM 0.1) nmol/min per mg ( $n = 125$  and 139) in the bottom segments. These data clearly document the variation in lactase activity along the intestinal tract, and indicate the lack of any marked sex difference. Likewise, between day 30

**Table 3.** Lactase activity (nmol/min per mg) in the intestinal tract of rats\* (Mean values and standard errors of the mean)

Day	Diet	WF rats						BBc rats						BBdp rats								
		Top		Mid		Bottom		Top		Mid		Bottom		Top		Mid		Bottom				
		Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n			
10	Milk	45.4	2.6	12	32.6	4.5	12	42.2	5.8	12	46.7	4.2	9	31.3	3.2	12	4.3	0.4	10	39.2	2.4	11
30	HC	7.9	1.1	5	1.1	0.2	5	6.7	1.3	5	2.6	0.9	5	5.5	0.5	10	2.9	0.4	10	1.4	0.4	10
	NTP	5.8	0.6	5	0.4	0.0	5	5.6	0.6	5	2.0	0.8	5	2.8	0.4	10	2.9	0.4	10	1.2	0.4	10
	WG	3.8	0.4	5	1.8	0.5	5	4.8	0.7	5	1.5	0.2	5	4.2	0.6	9	3.9	0.3	9	1.2	0.2	10
45	HC	9.1	0.7	5	0.8	0.1	5	6.7	1.3	5	1.4	0.3	5	6.2	0.3	10	9.5	0.7	10	0.8	0.1	10
	NTP	4.5	0.6	5	0.5	0.0	5	6.1	0.3	5	0.6	0.0	5	4.7	0.5	10	5.6	0.3	10	0.6	0.2	10
	WG	5.9	0.6	5	2.5	1.6	5	6.1	0.4	5	0.7	0.1	5	3.9	0.4	10	5.1	0.4	10	1.1	0.4	10
70	HC	9.5	1.0	3	0.7	0.4	4	9.7	0.6	5	0.8	0.1	5	7.7	0.8	9	8.6	1.1	9	0.4	0.1	9
	NTP	6.0	0.7	3	0.4	0.1	4	7.1	1.1	5	0.5	0.1	5	4.2	0.4	6	3.5	0.2	7	0.3	0.0	7
	WG	9.3	0.7	4	0.7	0.2	4	9.2	0.5	4	0.7	0.1	4	6.4	0.5	7	4.4	0.5	6	0.7	0.2	7
95	HC	7.3	1.0	5	1.6	1.3	5	7.8	1.6	4	2.4	1.9	4	7.3	0.9	9	9.0	1.3	9	1.4	0.9	8
	NTP	8.0	1.0	5	0.6	0.2	5	7.6	1.3	5	2.3	1.6	5	5.0	0.8	7	3.2	0.7	7	0.8	0.2	7
	WG	10.0	0.8	5	1.0	0.3	5	10.0	0.2	5	3.2	1.7	5	8.6	1.5	5	8.4	1.2	5	2.2	1.2	5
120	HC	4.9	0.6	5	0.6	0.1	5	6.1	0.6	4	0.6	0.1	4	4.5	0.8	6	9.7	1.9	6	1.3	0.7	6
	NTP	4.6	0.4	5	0.6	0.2	4	5.5	0.7	5	0.6	0.1	5	3.7	0.8	6	4.7	0.8	6	0.4	0.0	6
	WG	6.5	0.6	4	0.5	0.1	4	6.1	0.4	6	1.2	0.6	6	3.2	0.9	7	6.2	1.8	7	0.8	0.2	7

WF, Wistar-Furth; BBc, diabetes-resistant BioBreeding; BBdp, diabetes-prone BioBreeding; HC, hydrolysed casein-based; NTP, National Toxicology Program; WG, wheat-gluten-based. \* For details of animals, diets and procedures, see pp. 201–202.

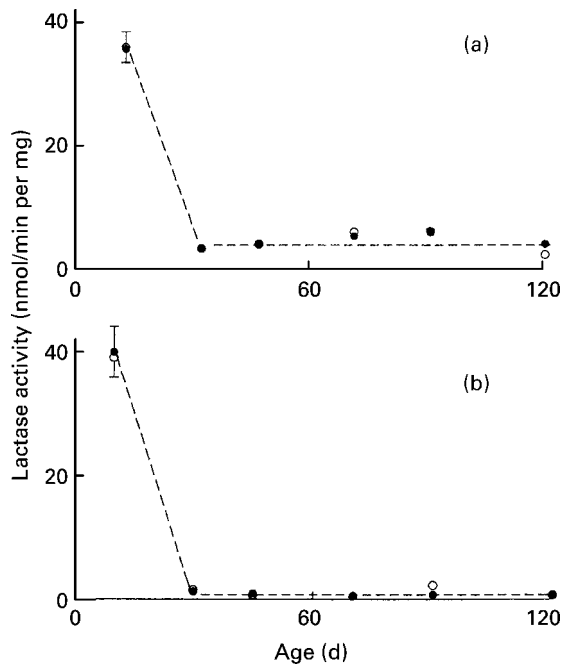


**Fig. 3.** The activity of lactase in Wistar-Furth (WF), diabetes-resistant BioBreeding (BBc) and diabetes-prone BioBreeding (BBdp) rats on day 10 fed dam's milk (a) and on days 30 to 120 (b) is expressed relative to the mean corresponding values found at the same level of the intestinal tract (top and bottom segments, at day 10; top and mid segments at day 30 to 120) and at the same age in BBc rats fed the same diet. At days 30 to 120, the results refer to animals fed either the hydrolysed casein-based (HC; ■) diet, the National Toxicology Program (NTP; ■) diet or the wheat-gluten-based (WG; ■) diet. Mean values are shown for twenty-three to thirty individual measurements in (a) and, in (b), for forty-five to forty-seven measurements made in the WF rats, 146 measurements made in the BBc rats and seventy-five to eighty-eight measurements in the BBdp rats. Standard errors of the mean are represented by vertical lines.

and 120, there was little difference in lactase activity as a function of age (Fig. 4).

Between day 30 and 120, the lactase activity in the top and mid segments of the WF rats was not significantly different from that recorded at the same level of the intestinal tract and at the same age in the BBc rats fed the same diet. Thus, in the WF rats, it averaged 97.6 (SEM 2.4) % (*n* 139; *P* > 0.45) of the mean corresponding values found in the BBc rats (100.0 (SEM 2.5) %; *n* 146). In the BBdp rats, however, the activity of lactase was decreased to 83.2 (SEM 4.3) % (*n* 88; *P* < 0.02) of that found in the BBc rats when fed the HC diet. Lactase activity was decreased to 66.2 (SEM 2.9) % (*n* 79; *P* < 0.001) and 68.3 (SEM 5.7) % (*n* 75; *P* < 0.001) when fed the NTP and WG diets, respectively. The latter two percentages, which were not significantly different from one another (*P* > 0.7), were both lower (*P* < 0.05 or less) than that recorded in the BBdp rats fed the HC diet (Fig. 3). The bottom segments were not included in this analysis because of the low values for lactase activity found at this level of the intestinal tract.

Relative to the mean values found at the same level of the intestinal tract (top or mid segments) and at the same



**Fig. 4.** Lactase activity in the top (a) or bottom (b) segment of male (●) and female (○) rats of increasing ages. Mean values are shown for fourteen to eighteen individual measurements at day 10 and for twenty to thirty-five measurements at days 30 to 120.

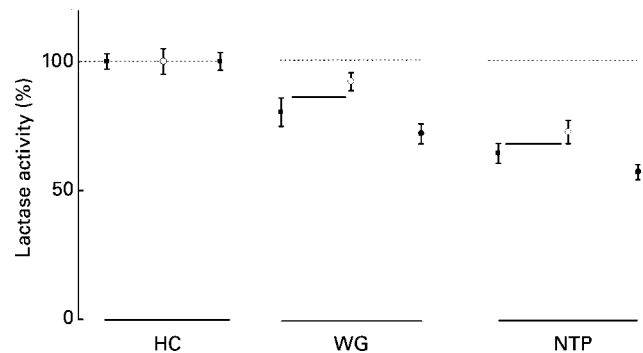
age (30 to 120 d) in the rats fed the HC diet, lactase activity in the animals fed the NTP diet was decreased to 65.9 (SEM 3.9) % ( $n$  47;  $P < 0.001$ ) in the WF rats. Relative lactase activity in the animals fed the NTP diet was decreased to 74.3 (SEM 4.6) % ( $n$  50;  $P < 0.001$ ) in the BBc rats. The measurements made in the animals fed the NTP diet were decreased to 58.4 (SEM 3.0) % ( $n$  79;  $P < 0.001$ ) in the BBdp rats. The former two percentages were not significantly different from one another ( $P > 0.15$ ). That found in the BBdp rats was lower ( $P < 0.01$ ), however, than the overall mean value recorded in both the WF and BBc rats (70.2 (SEM 3.0) %;  $n$  97). When fed the WG diet, the mean activity of lactase was also decreased to 82.0 (SEM 5.6) % ( $n$  45;  $P < 0.005$ ) in the WF rats, 93.9 (SEM 3.6) % ( $n$  50;  $P > 0.3$ ) in the BBc rats, and 73.4 (SEM 3.9) % ( $n$  75;  $P < 0.001$ ) in the BBdp rats. Once again, the percentages found in the WF and BBc rats were not significantly different from one another ( $P > 0.05$ ), with an overall mean value of 88.3 (SEM 3.3) % ( $n$  95;  $P < 0.01$ ), significantly higher ( $P < 0.005$ ) than that found in the BBdp rats. In all three strains, the decrease in lactase activity was more pronounced ( $P < 0.03$  or less) in the animals fed the NTP rather than the WG diet (Fig. 5).

## Discussion

In the prolongation of a recent report (Courtois *et al.* 2002), the present work deals with the activity of three hydrolases in the intestinal tract of WF, BBc and BBdp rats fed three different diets (HC, NTP and WG) and examined at various ages.

In the case of invertase, the salient findings were:

- (i) a marked increase of activity between day 10 and 30,



**Fig. 5.** The activity of lactase at days 30 to 120 in the top and mid segments of the intestinal tract in Wistar-Furth (WF; ■), diabetes-resistant BioBreeding (BBc; ○) and diabetes-prone BioBreeding (BBdp; ●) rats fed either the wheat-gluten-based (WG) or the National Toxicology Program (NTP) diet is expressed relative to the mean corresponding values found at the same level of the intestinal tract and at the same age in rats of the same strain fed the hydrolysed casein-based (HC) diet. Mean values are shown for forty-five to fifty individual measurements in the WF and BBc rats and for seventy-five to eighty-eight measurements in the BBdp rats, with standard errors of the mean represented by vertical lines. (—), Overall mean value found in WF and BBc rats fed either the WG or NTP diet.

followed by a close-to-steady value up to day 95 and a later decrease at day 120;

- (ii) a somewhat higher activity in male rats than in female animals from day 30 to 120;
- (iii) a lower activity in the bottom segment than in either the top and mid segments of the intestinal tract in the animals examined between day 30 and 120;
- (iv) a comparable activity in the WF and BBc rats but lower activity in the BBdp rats, such a decrease being most pronounced at days 30, 70 and 120, affecting preferentially the top segments and achieving statistical significance only in the BBdp rats fed either the NTP or WG diet;
- (v) an increased activity in the BBc rats fed the NTP or WG diet rather than the HC diet, such an increase being either absent or less pronounced in the BBdp rats.

It could be argued, therefore, that the decreased activity of invertase in the BBdp rats fed the NTP or WG diet reflects a lesser capacity of these animals to increase the activity of this enzyme in the intestinal tract in response to the administration of these diabetogenic diets, as distinct from the protective HC diet.

The measurements of maltase activity revealed, as essential features, a similar time course to that found for invertase (day 10 < day 30 to 95 > day 120), a minor sex difference (female < male), and a distribution pattern also comparable with that found for invertase (mid segments > top segments > bottom segments). The diet exerted relatively little effect on maltase activity, the trend being towards a lower value in the animals fed the NTP diet and a higher value in the animals fed the WG diet, when compared with the measurements made in rats fed the HC diet. There was also little difference in maltase activity between the three types of rats with somewhat higher mean values in the WF rats than in the BBc rats. The only difference between the BBdp and BBc rats



consisted of an occasional decrease of maltase activity in the BBdp rats fed the NTP diet. Taken as a whole and when compared with the information collected in the case of invertase and lactase activity, these findings suggest that all hydrolases examined in the present study do not display identical changes as a function of either the strain of rats or their dietary intake.

In the case of lactase activity, the time course represented a mirror image of that found with either invertase or maltase, the values recorded at day 10 being much higher than those recorded at older ages. At day 10, the activity of lactase appeared somewhat lower in the BBdp rats than in the BBc rats. Although the activity was not significantly different in the top and bottom segments at day 10, with mean respective values of 39.6 (SEM 1.9) and 38.8 (SEM 2.4) nmol/min per mg ( $n$  32–36), it was much lower in the bottom segments than in the mid and top segments at older ages. Most importantly, the activity of lactase, which was comparable in the WF and BBc rats, was lower in the BBdp rats than in the BBc rats, such a decrease being most marked in the animals fed the NTP or WG diet, rather than the HC diet. When compared with the situation found in rats fed the HC diet, the NTP diet and, to a lesser extent, the WG diet decreased lactase activity in all three strains of rats, such a decrease being indeed more pronounced in the BBdp rats than in the WF or BBc rats.

Taken as a whole, the present data indicate that the activity of hydrolases is, as a rule, decreased in BBdp rats fed a diabetogenic diet (NTP or WG) whilst such is not the case either in WF rats or in BBdp rats fed the HC diet. Such impairment was most obvious in the case of invertase and lactase. To the extent that such results reflect an alteration of the functional state of intestinal cells, they support the concept that a still ill-defined enteropathy prevails in BBdp rats fed a diabetogenic diet well before the onset of autoimmune insulinitis. In this respect, the situation found in BBdp rats is reminiscent of that recently identified in some type 1 diabetic patients, in whom coeliac disease was diagnosed before diabetes onset with a high prevalence of diabetic ketoacidosis at

diabetes onset and also a high prevalence of other autoimmune diseases (Valerio *et al.* 2002).

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