Originals

Ontogenic changes in proglucagon mRNA in BB diabetes prone and normal rats weaned onto a chow diet

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Summary Weaning onto chow diets causes the highest incidence of diabetes in the BB rat. Changes in gut development and absorption of nutrients in the diabetes prone rat and the subsequent effect on pancreatic function may play a role in the ultimate development of the disease. BB diabetes prone (dp) and BB normal (n) dams were fed chow diets. Pups were killed at various ages ranging from 7 to 30 days. BBdp rats had higher small intestine and colon weights expressed per body weight at all ages ($p < 0.0001$). RNA content (mg/g) in the jejunum, ileum and colon was higher in the BBdp rats beginning at the critical period at 21 days and maintained at 24 days and 30 days ($p < 0.0001$). Proglucagon message decreased with age in both BBdp and BBn animals ($p < 0.0001$). Levels of proglucagon mRNA were higher in BBdp compared to BBn animals only in the ileum at 10 days ($p < 0.01$). Adjusting for total

ileal and colonic RNA content resulted in BBdp animals having higher total colonic proglucagon mRNA at 21, 24 and 30 days ($p < 0.0001$). Plasma GLP-1(7–36) amide was more than doubled in BBdp compared to BBn animals ($p < 0.0005$) at 30 days. Expressing sodium-dependent D-glucose co-transporter (SGLT-1), GLUT2 and GLUT5 mRNA per total jejunal RNA shows increased transporter mRNA in BBdp compared to BBn rats at weaning (21 days) ($p < 0.05$). Radical differences exist between BBdp and BBn animals at ‘critical periods’ in both proglucagon and glucose transporter gene expression. These differences may help explain altered growth and diseases incidence between these two strains [Diabetologia (1997) 40: 871–878].

Keywords BB rat, proglucagon, ontogeny, intestine, glucose transporters, diabetes mellitus.

The bio-breeding (BB) rat is a useful animal model of human insulin-dependent diabetes mellitus (IDDM) [1, 2]. The BB rat displays many of the immune irregularities associated with the human disease. As in

human IDDM the syndrome is spontaneous and results in the autoimmune destruction of the insulin-producing beta cells of the pancreas [3, 4]. It is well established that both forms of diabetes, human and BB are autoimmune.

Investigations into the aetiology of IDDM suggest that both genetic and environmental factors are involved [5]. It is now well recognized that diet is an important factor influencing development of diabetes in the BB rat [6, 7]. Studies suggest that semi-purified diet may be protective whereas laboratory chow may be more diabetogenic in nature [8, 9].

Whether the protective effect of a semi-purified diet is due to the presence of a specific protective component or conversely the absence of a diabetogenic component is yet unknown. It is known, however, that the timing of initial exposure and duration of

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Abbreviations: BBdp, biobreeding diabetes prone; BBn, Bio-breeding normal; GLP-1, Glucagon-like peptide-1; SGLT-1, sodium dependent D-glucose co-transporter; GLUT2, sodium independent glucose transporter; GLUT5, sodium independent glucose transporter; MOPS, morpholinopropanesulphonic acid; DEPC, diethyl pyrocarbonate; SSC, standard saline citrate.

exposure influence the protective effect of semi-purified diets. There appears to be a 'critical window' in which exposure to protective diet must occur. In order to decrease the incidence of diabetes, the diet must be introduced prior to 30 days of age and continued for at least 100 days [10, 11].

Relatively little is known regarding the factors affecting the development of the intestine in the autoimmune BB rat, particularly around the time of weaning. The fact that the drug, cyclosporin A, often used to delay the onset of autoimmune diabetes in the rat causes a delay in the maturation of the small intestine during weaning in the rat [12] suggests that changes occurring in the intestine play a crucial role in disease incidence. It has also been shown that rats weaned onto chow diets demonstrate higher rates of growth than those weaned onto semi-purified diets [13, 14]. Pedersen et al. [15] showed that the risk of developing diabetes was greatest in the animals which displayed the highest body weight at 10–40 days of age. Changes in the absorption of nutrients from the intestine of diabetes prone animals and the subsequent effect on pancreatic function may play a role in the ultimate development of the disease. The role that proglucagon gene products, possibly trophic to the gut [16], and intestinal glucose transporters play in the pathogenesis of diabetes is unknown.

Using the diet which produces the highest incidence of diabetes in the BB rat, laboratory chow, we have examined the ontogenic development of the intestine in BBn (normal) and BBdp (diabetes prone) rats at 7, 10, 14, 21, 24 and 30 days of age. Gene expression of the gut hormone, proglucagon, and of intestinal glucose transporters was examined. In addition to characterization of intestinal mass and RNA content, plasma glucose and plasma GLP-1 concentrations were assessed.

Materials and methods

Animals. BBdp and BBn rat dams were obtained from the University of Alberta, Department of Agricultural, Food and Nutritional Science breeding colony. Original breeding pairs were obtained from Health Canada (Animal Resources Division, Health Protection Branch, Ottawa, Ontario, Canada). BBdp and BBn rat dams were given free access to standard laboratory chow (Rodent Laboratory Diet PMI no. 1500; PMI Feeds Inc., St. Louis, Mo., USA) and water ad libitum. Litters were culled to 8 pups. Male and female rat pups were removed from the dam and weaned onto standard laboratory chow at 21 days of age. Animals were housed in a temperature and humidity controlled room with a 12 h light/dark cycle. Animals killed at 24 and 30 days of age were group housed from weaning (21 days) until 30 days. Principles of laboratory animal care were followed (NIH publication No. 85–23, revised 1985). The protocol was approved by the University of Alberta Animal Welfare Committee.

Rat pups killed at 7 days and 10 days of age were removed from the dam and asphyxiated with CO₂ and then decapitated.

Animals killed between 14 and 30 days were anaesthetized and blood taken by cardiac puncture. For all animals the small intestine from the ligament of Treitz to the ileocecal valve and colon were excised, contents removed and tissues measured and weighed. The small intestine was divided by length into three equal segments. Tissues were immersed in liquid nitrogen and stored at –72 °C for later mRNA analysis.

Isolation of total RNA. Total RNA was isolated using Trizol (Gibco BRL, Burlington, Ontario, Canada). Isolation was according to the protocol provided with the reagent. RNA was dissolved in DEPC (diethyl pyrocarbonate) treated water and quantity and purity determined by ultraviolet spectrophotometry at 260 and 280 nm.

Northern blot analysis. Messenger RNA in all samples was measured using a Northern blot analysis procedure described by Fuller et al. [17] with modifications. Aliquots of 15 µg total RNA were dissolved in 10 µl gel loading buffer [50% deionized formamide (vol/vol), 2 mol/l formaldehyde, 1.3% glycerol (vol/vol), 0.02 mol/l morpholinopropanesulphonic acid (MOPS), 5 mmol/l sodium acetate, 1 mmol/l EDTA and 0.1% bromophenol blue (wt/vol)], boiled for 2 min to denature the RNA and then placed on ice for 5 min. Samples were centrifuged briefly and loaded onto 1% agarose (wt/vol) gels containing (0.66 mol/l) formaldehyde. RNA was then fractionated according to size by electrophoresis in the presence of a recirculating running buffer containing 0.02 mol/l MOPS, 5 mmol/l sodium acetate and 1 mmol/l EDTA (5 h at 100 V). After electrophoresis, the gels were soaked in two changes of 10 × standard saline citrate (SSC) (1.5 mol/l NaCl, 0.15 mol/l trisodium citrate, pH 7.0) and then blotted onto a MSI Nitropure nitrocellulose membrane (MSI Laboratories, Westboro, Mass., USA), employing the capillary method described by Southern [18]. The RNA was then fixed onto membranes by baking in vacuo at 80 °C for 2 h.

Prior to hybridization with the [³²P] ATP-labelled cDNA probe, membranes were prehybridized for 2 h at 65 °C in prehybridization buffer [6 × SSPE (0.18 mol/l NaCl, 0.01 mol/l sodium phosphate, 1 mmol/l EDTA, pH 7.4), 0.1% SDS (wt/vol), 5 × Denhart's solution (0.5 g Ficoll 400, 0.5 g poly(vinyl pyrrolidone) (PVP), 0.5 g bovine serum albumin (fraction V)]. Following prehybridization, membranes were incubated for 16 h at 65 °C in an identical volume of fresh hybridization buffer with the addition of labelled cDNA probe. The cDNA proglucagon probe (donated by Dr. P.J. Fuller, Prince Henry's Institute of Medical Research, Melbourne, Australia), GLUT2 cDNA probe (donated by Dr. G.I. Bell, Howard Hughes Medical Institute, University of Chicago, Ill., USA), GLUT5 cDNA probe (donated by Dr. G.I. Bell) and sodium-dependent D-glucose co-transporter (SGLT-1) cDNA probe (donated by Dr. N. Davidson, University of Chicago), were labelled by nick translation (Random Primers DNA Labelling System, Life Technologies, Burlington, Ontario, Canada) with [³²P] dATP (3000 Ci/mmol, Amersham Canada, Oakville, Ontario, Canada).

Following hybridization, membranes were washed 3 times for 20 min each at room temperature with 2 × SSPE, 0.1% (wt/vol) SDS. They were then washed once at 65 °C for 20 min with 0.1 × SSC, 0.1% (wt/vol) SDS. Membranes were heat sealed in plastic bags and then exposed at –70 °C to KODAK XAR5 film (Eastman Kodak, Rochester, N.Y., USA) using an intensifying screen (Dupont Canada, Mississauga, Ontario, Canada). For statistical analysis, the signals were quantified using laser densitometry (Model GS-670 Imaging Densitometer, BioRad Laboratories (Canada) Ltd., Mississauga, Ontario, Canada). The 28S and 18S ribosomal bands were quantified

Table 1. Effect of disease and age on body and intestinal weights

Parameter	Group	Days					
		7	10	14	21	24	30
Body weight (g)	BBdp	12.9 ± 1.8 ^a	24.2 ± 1.9 ^b	23.3 ± 1.6 ^b	37.6 ± 1.6 ^{c, x}	49.1 ± 1.9 ^{d, x}	92.5 ± 1.6 ^e
	BBn	16.1 ± 2.0 ^a	23.4 ± 1.7 ^b	22.5 ± 1.4 ^b	44.2 ± 1.7 ^{c, y}	65.2 ± 1.8 ^{d, y}	88.6 ± 1.8 ^e
Total small intestine weight (g)	BBdp	0.46 ± 0.1 ^a	0.79 ± 0.1 ^b	0.76 ± 0.1 ^b	1.83 ± 0.1 ^{c, x}	2.27 ± 0.1 ^{d, x}	4.40 ± 0.1 ^{e, x}
	BBn	0.57 ± 0.1 ^a	0.69 ± 0.1 ^a	0.70 ± 0.1 ^a	1.39 ± 0.1 ^{b, y}	3.40 ± 0.1 ^{c, y}	3.54 ± 0.1 ^{c, y}
Small intestine weight/body weight	BBdp	3.6 ± 0.2 ^a	3.4 ± 0.2 ^a	3.3 ± 0.2 ^a	4.8 ± 0.2 ^{b, x}	4.8 ± 0.2 ^b	4.8 ± 0.2 ^{b, x}
	BBn	3.7 ± 0.2 ^a	3.1 ± 0.2 ^b	3.1 ± 0.2 ^b	3.2 ± 0.2 ^{b, y}	5.2 ± 0.2 ^c	4.0 ± 0.2 ^{d, y}
Colon weight (g)	BBdp	0.05 ± 0.03 ^a	0.10 ± 0.03 ^a	0.11 ± 0.03 ^a	0.26 ± 0.03 ^b	0.37 ± 0.03 ^c	0.90 ± 0.03 ^{d, x}
	BBn	0.05 ± 0.03 ^a	0.08 ± 0.03 ^a	0.09 ± 0.02 ^a	0.22 ± 0.03 ^b	0.43 ± 0.03 ^c	0.60 ± 0.03 ^{d, y}
Colon weight/body weight	BBdp	0.39 ± 0.03 ^{a, x}	0.44 ± 0.03 ^{ab, x}	0.47 ± 0.03 ^{b, x}	0.68 ± 0.03 ^{c, x}	0.69 ± 0.03 ^c	0.98 ± 0.03 ^{d, x}
	BBn	0.30 ± 0.03 ^{a, y}	0.36 ± 0.03 ^{ab, y}	0.39 ± 0.02 ^{b, y}	0.50 ± 0.03 ^{c, y}	0.67 ± 0.03 ^d	0.67 ± 0.03 ^{d, y}

Values are mean ± SEM ($n = 8$ rats/treatment). Using p_{diff} values from SAS, superscripts 'a' to 'e' represent significant differences ($p < 0.05$) between ages within a row. Using p_{diff} values from SAS, superscripts 'x' and 'y' represent significant

($p < 0.05$) differences between disease groups within an age category. Values with different letters are significantly different from each other

from negatives of photographs of the membranes. These bands confirm the integrity of the RNA and were used as a denominator for densitometer values to compensate for any loading discrepancies.

Radioimmunoassay. Blood was collected from each rat into a chilled syringe. Aprotinin (Trasylol, 10000 KIU/ml, Bayer AG, Leverkusen, Germany) and EDTA were added to the blood at 500 KIU/ml and 1 mg/ml respectively. Blood was centrifuged at $1600 \times g$ for 15 min at 4°C and plasma stored at –70°C until analysis.

Glucagon-like peptide-I. GLP immunoreactive peptides were extracted from 2.2 ml of plasma using a SEP-COLUMN containing 200 mg of C_{18} (catalogue no. RIK-SEPCOL 1; Peninsula Laboratories, Belmont, Calif., USA) with Buffer A [0.1% trifluoroacetic acid (catalogue no. RIK-BA-1, Peninsula)] and Buffer B [60% acetonitrile (catalogue no. RIK-BB-1, Peninsula)] as elution solvents. The extraction was performed according to the protocol provided by Peninsula Laboratories. Radioimmunoassay was performed using a dextran coated charcoal method for separation of bound and free label. The GLP-1(7–36)amide antibody was a gift from Dr. C. MacIntosh of the University of British Columbia. GLP-1(7–36)amide standard and ^{125}I -GLP-1(7–36)amide were purchased from Peninsula Laboratories. The antiserum was c-terminally directed and detected amidated forms of GLP-1 with no significant cross-reactivity with non-amidated forms of GLP-1. The intra-assay coefficient of variation was 4.1%.

Plasma glucose determinations. Plasma glucose was determined using Sigma Diagnostics Glucose (Trinder) Reagent for the enzymatic determination of glucose at 505 nm (Sigma).

Statistical analysis. All data are expressed as mean ± SEM. Differences between BBdp and BBn rats at all time points (disease susceptibility versus time) were determined using the two-way ANOVA model in the general linear model procedure in SAS (Version 6.04, SAS Institute, Cary, N.C., USA). Statistical significance is defined as $p \leq 0.05$. P_{DIFF} values were used to determine significant differences between ages and BBn vs BBdp animals. For ease of presentation, use of the word 'disease' refers to the difference in 'susceptibility to disease' between the BBn and BBdp animals.

Results

Growth parameters. The effect of disease and age on growth and intestinal weight change are shown in Table 1. Body weight, total small intestine weight, small intestine weight as a proportion of body weight, colon weight, and colon weight as a proportion of body weight increased with increasing age ($p < 0.0001$). Only at 21 and 24 days did BBdp animals weigh less than BBn animals ($p < 0.0001$). Two critical time points (21 and 30 days) were associated with higher small intestine weight per body weight in BBdp than in BBn animals. At 30 days BBdp had greater colon weight than BBn ($p < 0.0001$). At all ages except 24 days BBdp had higher colon weight per body weight than BBn ($p < 0.05$).

Intestinal RNA content. Concentration (mg/g) of RNA in jejunum, ileum and colon increased significantly with age ($p < 0.0001$) but did not differ between BBdp and BBn animals (data not shown). Total RNA from the segments, jejunum, ileum and colon as well as total intestinal RNA increased significantly ($p < 0.0001$) with age (Table 2). The critical time point at which BBdp have higher total jejunal and ileal RNA content than BBn ($p < 0.0001$) appears to be 14 days. The critical time point at which BBdp have higher total colonic and intestinal RNA content than BBn appears to be 21 days (minimum $p < 0.05$).

Blood parameters. At 30 days of age BBdp had significantly ($p < 0.0005$) higher plasma GLP-1 than BBn animals (Fig. 1). The increase in GLP-1 was more than twofold.

No significant difference between BBn and BBdp animals was found for plasma glucose levels (Fig. 2). Within BBdp animals 30 day plasma glucose was higher than 14 day values ($p < 0.05$). Within BBn

Table 2. Effect of disease and age on intestinal RNA content

Parameter	Group	Days					
		7	10	14	21	24	30
Total jejunal RNA (mg)	BBdp	0.51 ± 0.3 ^a	1.04 ± 0.3 ^a	1.24 ± 0.3 ^a	3.02 ± 0.3 ^{b, x}	7.89 ± 0.3 ^{c, x}	9.41 ± 0.3 ^{d, x}
	BBn	0.68 ± 0.3 ^a	1.09 ± 0.3 ^a	1.01 ± 0.3 ^a	2.18 ± 0.3 ^{b, y}	4.14 ± 0.3 ^{c, y}	7.90 ± 0.3 ^{d, y}
Total ileal RNA (mg)	BBdp	0.60 ± 0.3 ^a	1.14 ± 0.3 ^a	0.90 ± 0.3 ^a	2.66 ± 0.3 ^b	6.67 ± 0.3 ^{c, x}	7.88 ± 0.3 ^{d, x}
	BBn	0.94 ± 0.3 ^a	1.12 ± 0.3 ^a	0.75 ± 0.3 ^a	2.05 ± 0.3 ^b	4.20 ± 0.3 ^{c, y}	6.93 ± 0.3 ^{d, y}
Total colonic RNA (mg)	BBdp	0.17 ± 0.1 ^a	0.27 ± 0.1 ^a	0.32 ± 0.1 ^a	1.17 ± 0.1 ^{b, x}	2.56 ± 0.1 ^{c, x}	3.81 ± 0.1 ^{d, x}
	BBn	0.15 ± 0.1 ^a	0.26 ± 0.1 ^a	0.34 ± 0.1 ^a	0.78 ± 0.1 ^{b, y}	1.31 ± 0.1 ^{c, y}	2.26 ± 0.1 ^{d, y}
Total intestinal RNA (mg)	BBdp	1.28 ± 0.6 ^a	2.42 ± 0.6 ^a	2.24 ± 0.6 ^a	6.86 ± 0.6 ^{b, x}	17.15 ± 0.6 ^{c, x}	21.10 ± 0.6 ^{d, x}
	BBn	1.75 ± 0.6 ^a	2.44 ± 0.6 ^a	2.10 ± 0.6 ^a	5.01 ± 0.6 ^{b, y}	9.62 ± 0.6 ^{c, y}	17.07 ± 0.6 ^{d, y}

Values are mean ± SEM ($n = 8$ rats/treatment). Using p_{diff} values from SAS, superscripts 'a' to 'e' represent significant differences ($p < 0.05$) between ages within a row. Using p_{diff} values from SAS, superscripts 'x' and 'y' represent significant differ-

ences ($p < 0.05$) between disease groups within an age category. Values with different letters are significantly different from each other

Fig. 1. Effect of age and disease on non-fasting plasma GLP-1 levels in BB rats. Values are mean ± SEM ($n = 10$ rats/treatment). One-way ANOVA was used to determine statistical significance. * Significantly different from BBn ($p < 0.0005$)

Fig. 2. Effect of age and disease on non-fasting plasma glucose levels in BB rats. Values are mean ± SEM ($n = 5-10$ animals/treatment). Significant effects are expressed on the figure

animals 30 day plasma glucose was higher than 21 day values ($p < 0.05$).

Proglucagon mRNA. At 14 days a significant decrease occurred in the abundance of proglucagon mRNA in the ileum of BBdp and BBn animals ($p < 0.0001$) (Fig. 3). BBdp had significantly higher ileal proglucagon mRNA ($p < 0.01$) than BBn at 10 days of age. By 14 days, with the initial introduction of food this pattern was reversed with BBn now demonstrating higher proglucagon mRNA than BBdp. Accounting for the total potential message in the ileum by multiplying proglucagon mRNA by total ileal RNA content shows that total potential proglucagon mRNA was elevated in 24 and 30 day BBdp compared to younger BBdp animals ($p < 0.0001$) (Fig. 4). In BBn animals, 21 days was the age at which higher total potential proglucagon mRNA was seen compared to younger animals ($p < 0.0001$). BBdp animals had lower total potential ileal proglucagon mRNA than BBn at 21, 24 and 30 days ($p < 0.05$).

In the colon 14 days again appears to be the critical period at which colonic proglucagon mRNA decreases from earlier ages ($p < 0.0001$) (Fig. 5). BBdp consistently had lower levels of colonic proglucagon mRNA than BBn with this difference reaching significance at 7 and 30 days ($p < 0.05$). Accounting for the total potential message in the colon by multiplying proglucagon mRNA by total colonic RNA content shows that total potential proglucagon mRNA was significantly increased at 21 days and remained higher through to 30 days in both BBdp and BBn animals ($p < 0.001$) (Fig. 6). Within BBdp animals, proglucagon mRNA is higher at 24 and 30 days ($p < 0.0001$) compared to BBn animals. This critical period appears to begin at 21 days ($p = 0.09$).

Glucose transporter mRNA. Effects of age and disease on glucose transporter mRNA are shown in Table 3. In BBdp rats SGLT-1 mRNA was lowest at 7 days and peaked at 21 days ($p < 0.05$). In the BBn rats the increase occurred later with significantly higher levels of mRNA seen at 24 and 30 days

Fig. 3. Effect of age and disease on ileal proglucagon mRNA expression in BB rats. Values are mean $\pm$ SEM ($n = 6$ rats/treatment). Significant effects are expressed in the figure. Each lane was loaded with 15 μ g of total RNA. Inset of autoradiograph

Fig. 5. Effect of age and disease on colonic proglucagon mRNA expression in BB rats. Values are mean $\pm$ SEM ($n = 6$ rats/treatment). Significant effects are expressed in the figure. Each lane was loaded with 15 μ g of total RNA. Inset of autoradiograph

Fig. 4. Effect of age and disease on ileal proglucagon mRNA expression adjusted for total ileal RNA content in BB rats. Values were obtained by multiplying total ileal RNA by ileal proglucagon mRNA levels. Values are mean $\pm$ SEM ($n = 6$ rats/treatment). Significant effects are expressed in the figure

Fig. 6. Effect of age and disease on colonic proglucagon mRNA expression adjusted for total colonic RNA content in BB rats. Values were obtained by multiplying total colonic RNA by colonic proglucagon mRNA levels. Values are mean $\pm$ SEM ($n = 6$ rats/treatment). Significant effects are expressed in the figure

($p < 0.05$). Disease was significantly different with BBdp animals having higher levels at 21 days ($p < 0.01$) and BBn showing highest levels at 30 days ($p < 0.0003$).

In BBdp rats levels of GLUT2 were higher at 10, 14 and 21 days compared to other ages ($p < 0.05$). In BBn only 10 days were higher than other ages ($p < 0.002$). For GLUT2 mRNA 14 and 21 days again appear to

be the critical period at which BBdp have significantly higher GLUT2 mRNA than BBn ($p < 0.02$).

In BBdp rats peak levels of GLUT5 were seen at 21 days ($p < 0.0002$). In BBn rats, levels of GLUT5 reached maximum values at 30 days ($p < 0.0001$) with 24 days also significantly ($p < 0.003$) higher than earlier time points. In BBdp animals significantly higher levels of GLUT5 mRNA were seen at 21 days

Table 3. Effect of disease and age on glucose transporter mRNA abundance in 15 µg total RNA

Parameter	Group	Days					
		7	10	14	21	24	30
SGLT-1 mRNA	BBdp	1.80 ± 0.12 ^a	1.71 ± 0.12 ^a	1.72 ± 0.13 ^a	2.19 ± 0.12 ^{b, x}	1.93 ± 0.12 ^b	1.95 ± 0.12 ^{b, x}
	BBn	1.68 ± 0.13 ^a	1.48 ± 0.12 ^a	1.74 ± 0.12 ^a	1.65 ± 0.13 ^{a, y}	2.13 ± 0.12 ^b	2.67 ± 0.12 ^{c, y}
GLUT2 mRNA	BBdp	0.24 ± 0.09 ^a	0.52 ± 0.10 ^b	0.82 ± 0.10 ^{b, x}	0.48 ± 0.08 ^{b, x}	0.32 ± 0.08 ^{ab}	0.27 ± 0.08 ^{ab}
	BBn	0.22 ± 0.19 ^a	0.65 ± 0.10 ^b	0.44 ± 0.10 ^{c, y}	0.26 ± 0.08 ^{ac, y}	0.30 ± 0.09 ^{ac}	0.39 ± 0.08 ^{ac}
GLUT5 mRNA	BBdp	1.52 ± 0.18 ^a	1.44 ± 0.20 ^a	1.69 ± 0.18 ^a	2.23 ± 0.16 ^{b, x}	1.24 ± 0.16 ^{a, x}	1.02 ± 0.16 ^{a, x}
	BBn	1.07 ± 0.17 ^{ab}	1.89 ± 0.18 ^{ac}	1.75 ± 0.18 ^{abc}	1.42 ± 0.18 ^{b, y}	1.97 ± 0.16 ^{c, y}	2.66 ± 0.16 ^{d, y}

Values are mean ± SEM ($n = 8$ rats/treatment). Using p_{diff} values from SAS, superscripts 'a' to 'e' represent significant differences ($p < 0.05$) between ages within a row. Using p_{diff} values from SAS, superscripts 'x' and 'y' represent significant differ-

ences ($p < 0.05$) between disease groups within an age category. Values with different letters are significantly different from each other

Table 4. Effect of disease and age on total potential glucose transporter mRNA abundance in total jejunal RNA

Parameter	Group	Days					
		7	10	14	21	24	30
SGLT-1 mRNA	BBdp	9.2 ± 7.6 ^a	18.5 ± 7.6 ^a	21.4 ± 8.5 ^a	67.4 ± 7.6 ^{b, x}	150.9 ± 7.6 ^{c, x}	183.0 ± 7.6 ^{d, x}
	BBn	11.7 ± 8.5 ^a	16.6 ± 7.6 ^{ab}	17.6 ± 7.6 ^{ab}	33.9 ± 8.5 ^{b, y}	98.2 ± 7.6 ^{c, y}	210.7 ± 7.6 ^{d, y}
GLUT2 mRNA	BBdp	1.3 ± 2.7 ^a	5.7 ± 2.5 ^a	11.5 ± 2.8 ^a	13.5 ± 2.5 ^{b, x}	24.7 ± 2.5 ^{c, x}	24.8 ± 2.5 ^c
	BBn	1.6 ± 2.7 ^a	7.3 ± 2.7 ^{ab}	4.8 ± 2.8 ^a	6.5 ± 2.5 ^{ab, y}	11.1 ± 2.7 ^{b, y}	31.6 ± 2.5 ^c
GLUT5 mRNA	BBdp	8.2 ± 12.3 ^a	14.8 ± 13.6 ^a	22.0 ± 12.3 ^a	67.6 ± 11.0 ^{bc, x}	98.6 ± 11.0 ^c	95.1 ± 11.0 ^{c, x}
	BBn	7.0 ± 12.0 ^a	21.3 ± 12.3 ^a	18.4 ± 12.3 ^a	32.0 ± 12.3 ^{a, y}	87.0 ± 11.0 ^b	208.4 ± 11.0 ^{c, y}

Values are mean ± SEM ($n = 8$ rats/treatment). Using p_{diff} values from SAS, superscripts 'a' to 'e' represent significant differences ($p < 0.05$) between ages within a row. Using p_{diff} values from SAS, superscripts 'x' and 'y' represent significant differ-

ences ($p < 0.05$) between disease groups within an age category. Values with different letters are significantly different from each other

($p < 0.0001$) while BBn animals only exhibited higher levels at 24 and 30 days ($p < 0.01$).

Values obtained for the purpose of accounting for the total potential message for glucose transporter mRNA by multiplying transporter mRNA by total jejunal RNA content are shown in Table 4. SGLT-1 in both BBdp and BBn was significantly elevated from 21 to 30 days compared to younger ages ($p < 0.0001$). At 21 days ($p < 0.007$) and 24 days ($p < 0.0001$) BBdp had higher levels of total SGLT-1 than BBn. By 30 days this pattern was reversed with BBn having higher levels of potential SGLT-1 mRNA than BBdp ($p < 0.009$).

For total potential GLUT2 mRNA BBdp animals had the highest abundance beginning at weaning (21 days) and remaining elevated to 30 days ($p < 0.001$). In BBn the increase was present later on at 24 days ($p < 0.02$) and again remained elevated at 30 days ($p < 0.0001$). The significantly greater abundance of potential GLUT2 message in BBdp at 21 days ($p < 0.03$) and 24 days ($p < 0.002$) than BBn again reinforces the changes occurring in the BBdp animals at the critical period of weaning.

Age patterns for total potential GLUT5 mRNA mimicked those seen with GLUT2. BBdp had higher levels of total GLUT5 at 21 days ($p < 0.003$) than BBn but as with other transporters, BBn had a greater abundance than BBdp at 30 days ($p < 0.0001$).

Discussion

Dramatic changes in intestinal morphology and enzymology occur from birth to the early post-weaning period. Diet as well as humoral growth factors influence the ontogeny of small intestinal morphology and enzymology [19, 20]. Changes occurring in the intestine of the autoimmune diabetes prone BB rat in the early postnatal period are of particular interest. This study examined the ontogenic development of the intestinal hormone, proglucagon and intestinal glucose transporters in chow-fed rats. For the first time we describe dramatic differences in intestinal growth and the expression of proglucagon, SGLT-1, GLUT2 and GLUT5 mRNA in BBn and BBdp rats from 7 to 30 days of age.

Weaning onto chow diets produces the highest incidence of diabetes in BB rats compared to feeding a semi-purified diet [8, 9]. BBdp rats at 21 and 30 days of age had significantly greater small intestine weight per body weight than BBn animals at these time points. At all ages examined except 24 days of age, colon weight per body weight was significantly higher in BBdp compared to BBn animals. These findings suggest that on a body weight basis, BBdp animals have a larger gut and greater capacity for nutrient absorption.

Chow diets may increase gut signalling which stimulates pancreatic beta-cell insulin secretion.

GLP-1(7–36)amide is a potent insulin secretagogue produced in the distal ileum and colon [21]. Previous work has demonstrated that feeding a high fiber diet upregulates proglucagon mRNA and increases GLP-1 and insulin secretion [22]. Colonic proglucagon mRNA adjusted for total colonic RNA content is significantly higher in BBdp rats compared to BBn rats at 24 and 30 days of age. The higher colonic proglucagon mRNA corresponds to the significantly elevated plasma GLP-1 seen in 30-day-old BBdp compared to BBn animals.

The higher levels of proglucagon mRNA seen at 24 and 30 days of age in the BBdp rats could potentially result in elevated levels of plasma GLP-1 and GLP-2. Recent studies have demonstrated that the prohormone convertase, PC1, is the sole enzyme responsible for the processing of proglucagon to the intestinal peptides, glicentin, oxyntomodulin, GLP-1(1–37/36NH₂), GLP-1(7–37/36NH₂) and GLP-2 [23, 24]. Therefore an increase in plasma GLP-1 as demonstrated in this study would suggest an increase in plasma GLP-2 as well.

GLP-1 is a potent insulin secretagogue and increased levels in plasma would signal increased insulin secretion from the pancreatic beta cells. GLP-2 has recently been shown to increase D-glucose maximal transport rate in the jejunum [25]. An increase of this nature along with the demonstrated upregulation of glucose transporter mRNA in BBdp animals at 21 days of age would further challenge the secretory function of the pancreatic beta cell. It is a well established fact that increasing insulin secretion by administration of cyclophosphamide accelerates the progression of beta-cell destruction [26, 27]. Conversely, by administering exogenous insulin in BBdp rats, the incidence of diabetes can be reduced [26]. Transplantation of normal histocompatible islets from Wistar-Furth rats still stimulates the immune attack in BB rats and suggests that an antigen normally expressed in pancreatic islets mediates the autoaggressive T-cell attack [28, 29]. The increase in proglucagon mRNA, therefore, resulting in increased GLP-1 and GLP-2 production, may play an important role in increasing the secretory potential of pancreatic islets and ultimate disease pathogenesis.

In streptozotocin induced diabetic models, studies have demonstrated that adaptation of intestinal glucose transport occurs in rats during experimental diabetes [30, 31]. Fedorak et al. [30] demonstrated that in the jejunum adaptation occurs in chronically diabetic rats via an increase in the density of glucose carriers in the upper villus region. The ileum is responsive in both acute and chronic states and results in an increase in carriers in the upper villus region as well as recruitment of carriers in the mid to lower villus region. The authors did not examine whether the increase in functional glucose uptake was associated

with an increase in the expression of intestinal glucose transporter mRNA. In the present study we show that as early as weaning, when there are no clinical symptoms of diabetes in the BBdp rat, the expression of SGLT-1, GLUT2 and GLUT5 mRNA is elevated above that observed in normal animals. The increased abundance of these genes increase the potential for the production of glucose transporter protein and increased nutrient uptake.

If increased levels of proglucagon mRNA signal an increase in pancreatic insulin secretion, the increased expression of intestinal glucose transporters may further exacerbate this phenomenon. Weaning appears to be a “critical period” for the protective or deleterious effects of diet on diabetes incidence in the BB rat. Adjusting for the total RNA content of the intestine further emphasizes the increase in glucose transporter expression seen at 21 days of age in BBdp animals. The higher abundance of transporter mRNA indicates a potential for increased nutrient uptake from the intestine at this “critical” time, suggested by this study to be from 44 days of age, at which time the pups may begin consuming the dams’ diet through to 21 days of age when weaning occurs. In human infants, many retrospective studies have shown a negative correlation between breast feeding as an infant and IDDM occurrence in adolescence [32, 33]. Breast feeding may confer protection by decreasing exposure to foreign antigens via solid food or formula. Indeed, Kostraba et al. [34] showed that individuals with IDDM were exposed to solid foods earlier than non-diabetic individuals.

Several animal studies have shown that higher growth rates are typically seen with chow diets [13, 14]. Pedersen et al. [15] recently demonstrated that the risk of developing diabetes later in life is strongly associated with higher body weight at 10–40 days of age. Lower plasma and pancreatic insulin levels were seen in animals that did not develop diabetes [15]. Again this observation has been recognized in human infants. Those infants fed formula grow more rapidly in the first year of life than those who are breast fed and this difference remains after the introduction of solid foods [35–37].

In summary, we have demonstrated that dramatic differences in intestinal development exist between BBdp and BBn animals when weaned onto chow diets. BBdp animals have significantly greater small intestinal and colon masses per body weight. These differences are especially evident in the early weaning period from 21 to 30 days of age. Expression of colonic proglucagon mRNA and the secretion of GLP-1, a potent insulin secretagogue is increased in BBdp animals. At weaning (21 days), glucose transporter mRNA is elevated in BBdp compared to BBn animals. Taken together, this evidence suggests that a potentially larger more active gut is associated with increased nutrient (glucose) uptake requiring greater

pancreatic involvement. These differences may ultimately contribute to disease pathogenesis.

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