Dietary Effects on Insulin and Nutrient Metabolism in Mesenteric Lymph Node Cells, Splenocytes, and Pancreatic Islets of BB Rats

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The present studies were performed to determine if a protective diet has different effects on the metabolic activity or function of islet cells, as well as the metabolic activity of mesenteric lymph node (MLN) cells and spleen cells, from BioBreeding (BB) rats. Diabetes-prone BB (BBdp) rats and control non–diabetes-prone BB (BBc) rats were fed for about 20 days either a mainly plant-based diabetogenic diet, NIH-07 (NIH), or a protective semipurified diet with hydrolyzed casein (HC) as the amino acid source. At 6 to 8 weeks of age, BBdp rats had high plasma D-glucose and low insulin concentrations, low insulin content, and low metabolic and secretory responses to D-glucose in isolated pancreatic islets. Islet metabolism, as measured by accumulation of ^{14}C-acidic metabolites, amino acids, and the ratio of D-[U-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization was increased in control rats fed HC ($P < .05$); a similar trend in BBdp rats was not significant. Feeding the HC diet increased islet insulin content ($P < .01$) by 13% in BBdp and 23% in BBc rats; other metabolic and hormonal variables were unaffected. Compared with BBc rats, BBdp rats displayed higher rates of L-[U-^{14}C]glutamine oxidation, D-[5-^{3}H]glucose utilization, and D-[U-^{14}C]glucose oxidation in MLN cells, but not in splenocytes. There was a dramatic decrease of L-[U-^{14}C]glutamine oxidation in MLN cells from BBc and BBdp rats fed HC. Glycolysis was decreased in control rats. We conclude that the protection afforded by feeding BBdp rats a HC diet is associated with increased insulin in target β cells and downregulation of metabolic activity in gut-associated MLN cells. Metabolic activity in splenocytes, cells representative of the systemic immune system, was less affected. These data suggest that diet-induced metabolic changes occur in the islets and nearby cells of the gut immune system in the period before classic insulitis. Changes in the islets were smaller in comparison to the dramatic remodeling of nutrient catabolism in MLN cells. MLN downregulation may reflect baseline metabolic activity in the absence of diabetogenic (or other) food antigens and further highlights an important interaction between diabetogenic food antigens and the gut immune tissues.

THE DEVELOPMENT OF spontaneous autoimmune diabetes in diabetes-prone BioBreeding (BBdp) rats depends to a large extent on components in the diet.[1] The highest incidence is found in animals fed mainly plant-based diets such as Purina 5001 or NIH-07 (NIH), whereas those fed a protective, hydrolyzed casein (HC)-based diet, or other diets based on partially hydrolyzed or intact nondiabetogenic protein sources, show a decreased diabetes frequency and severity of insulitis.[1,2] During the period between 30 and 50 days of age, a time when insulitis is absent or minimal, BBdp rats weaned onto a diabetes-promoting, mainly plant-based diet show decreased islet cell area[3] and enhanced β-cell major histocompatibility complex (MHC) class I expression[4] compared with those weaned onto a HC diet. By 70 days of age, the more prevalent and fulminant inflammation in the pancreas of NIH-fed BBdp rats is characterized by increased expression of mRNA for the Th1 cytokine, interferon gamma (IFN-γ).[3] By contrast, the sparse mononuclear cell infiltration in animals fed HC is associated with decreased expression of IFN-γ mRNA and increased expression of the Th2 cytokine, interleukin-10 (IL-10), and transforming growth factor beta (TGF-β), a Th3 cytokine. These results show that diets which induce widely different pancreatic inflammation and numbers of diabetes cases are associated with early changes in the pancreas followed by changes in the distribution of immune cells at a time when classic insulitis is found. Thus, certain diet components affect both the target islet cells and the infiltrating immunocytes.

Although the mechanisms by which selected diets control diabetes expression remain unclear, there are data to support the proposal that diet-related changes in islet antigenicity and homeostasis precede effects on the inflammatory infiltrate that can be observed with the light microscope.[1,3,4] Others have reported that immune activation of BB rat lymphocytes is associated with enhanced glutamine metabolism,[5] and there is evidence that islet activity is important in the autoimmune process.[6,7] There are no detailed studies of metabolism in islets from animals fed protective or diabetogenic diets. Therefore, we believe it is important to determine if diets capable of modifying the diabetes outcome have different, temporally related effects on the metabolism of isolated islets or cells of the systemic and gut-associated lymphoid tissue.

We tested the hypothesis that these diets have different effects on the metabolic activity or function of islet cells and affect the metabolic activity of mesenteric lymph node (MLN) cells and spleen cells. For this, we determined the influence of two diets with markedly different diabetogenicity, the protective HC diet and the diabetes-inducing NIH diet, on (1) the metabolism, insulinotropic action of D-glucose, and insulin content in isolated pancreatic islets and (2) the metabolism of L-glutamine and D-glucose in MLN cells and splenocytes from diabetes-prone or control (non–diabetes-prone) BB rats aged about 50 days (BBdp and BBc).

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MATERIALS AND METHODS

The NIH-07 (NIH) diet is a standard, mainly cereal-based mixture composed of 82.5% plant materials. It includes dried skim milk (5%), fish meal (10%), soybean meal (12%), alfalfa meal (4%), corn gluten meal (3%), ground corn (24.5%), ground hard winter wheat (23%), wheat middlings (10%), brewer's yeast (2%), molasses (1.5%), and soybean oil (2.5%), plus minerals and vitamins. The HC-based diet contained 20% HC (Redstar Bioproducts, Tara, Ontario, Canada), 51% cornstarch, 12% sucrose, 7% soya oil, and 5% cellulose-type fiber (Solka-Floc), supplemented with 3.5% AIN-93G mineral mix, 1.0% AIN-93G vitamin mix, 0.2% choline bitartrate, and 0.3% L-cystine (ICN Biochemicals, Cleveland, OH).

Male and female BBdp rats and BBc rats were obtained from the colonies maintained at the Animal Resources Division of Health Canada (Ottawa, Ontario, Canada) and were transferred around weaning to Brussels (Belgium). During transport, the animals had access to the NIH diet. They were then weighed and housed in groups of 3 to 5 rats of the same sex in separate cages with free access to tap water and either powdered NIH or HC diet for approximately 20 days. There were 4 groups of animals: HC-fed BBc, NIH-fed BBc, HC-fed BBdp, and NIH-fed BBdp. At the end of the study, nonfasted animals were weighed and killed by decapitation and the tissues were removed.

Blood was collected in heparinized tubes for the measurement of plasma D-glucose[8] and insulin[9] concentrations. A single batch of about 400 to 500 islets was prepared by the collagenase procedure[10] from 3 to 5 rats from each group. From each batch, 2 groups of 15 islets each were sonicated in 250 μL H_2O for measurement of protein content.[11] Ten to 16 further groups of 8 islets each were incubated for 120 minutes at 37°C in a HEPES- and bicarbonate-buffered medium (1.0 mL) equilibrated against a mixture of O_2/CO_2 (95:5 vol/vol) and containing bovine serum albumin (5 mg/mL) and, as required, 2.8 or 16.7 mmol/L D-glucose. After removal of the incubation medium for measurement of insulin release,[10] each group of islets was sonicated in 1.0 mL 0.1-m/L phosphate buffer (pH 7.4) containing bovine serum albumin (10 mg/mL) for determination of insulin content.[10] Last, 6 to 8 groups of 20 islets each were incubated for 120 minutes at 37°C in the same HEPES- and bicarbonate-buffered medium (40 μL) at each of the 2 concentrations of D-glucose (2.8 or 16.7 mmol/L) in the presence of D-[5-^{3}H]glucose and D-[U-^{14}C]glucose (NEN, Boston, MA) for measurement of ^{3}HOH, $^{14}CO_2$, and ^{14}C-labeled acidic metabolites and amino acid production by methods described elsewhere.[12-14] Thus, after recovery of $^{14}CO_2$ and ^{3}HOH, radioactive acidic metabolites and amino acids were separated by ion-exchange chromatography. All results are expressed as picomoles of substrate equivalent, taking into account the specific radioactivity of the exogenous nutrient present in the incubation medium. The metabolic behavior of islets isolated by the collagenase procedure is similar to that of microdissected islets.[15]

Splenocytes from the same 3 to 5 rats were isolated[16] by disruption of the spleen, filtration, hemolysis of erythrocytes (incubation in H_2O for 1 minute at 20°C), and centrifugation, and were eventually resuspended (5×10^6 cells/mL) in the same HEPES- and bicarbonate-buffered medium as described before. After trypan blue staining, viable cells were counted with a hemocytometer. A similar procedure was used for the isolation of lymphocytes from MLNs. Groups of 2×10^5 splenocytes or mesenteric lymphocytes were incubated for 120 minutes at 37°C in the same medium (80 μL) containing either 1.0 mmol/L L-glutamine mixed with a trace amount of L-[U-^{14}C]glutamine (NEN) or 7.0 mmol/L D-glucose mixed with trace amounts of both D-[5-^{3}H]glucose and D-[U-^{14}C]glucose. L-[U-^{14}C]glutamine oxidation and several variables of D-glucose catabolism were measured.

All results are presented as the mean ± SE, together with the number of individual determinations (n) or degrees of freedom. The statistical significance of differences between mean values was assessed by Student's 2-tailed *t* test and ANOVA. No litter effects were detected in the present study.

RESULTS

Metabolic and Hormonal Status

Animals were in the fed state when euthanized. Plasma glucose was slightly higher in BBdp animals (9.35 ± 0.36 mmol/L, n = 33) versus control rats (8.01 ± 0.41 mmol/L, n = 32, $P < .02$). The plasma insulin concentration and insulinogenic index (ratio of plasma insulin to glucose) were much lower ($P < .001$) in BBdp rats than in control animals. No significant effect of diet on these variables was detected in control or BBdp rats (Table 1).

Insulin Release and Content

The protein content of the islets was lower ($P < .01$) in BBdp rats (1.17 ± 0.08 μg/islet, n = 16) than in control animals (1.61 ± 0.13 μg/islet, n = 16). The insulin content of the islets was also lower ($P < .001$) in BBdp rats (750 ± 18 μgU/islet, n = 112) than in control animals (1,243 ± 38 μU/islet, n = 112).

Basal insulin output, measured in islets incubated for 120 minutes at 2.8 mmol/L D-glucose, was slightly but not significantly lower in BBdp rats (6.7 ± 1.4 μgU/islet, n = 48) versus control animals (11.4 ± 1.9 μU/islet, n = 48). However, in the presence of 16.7 mmol/L D-glucose, the release of insulin was significantly lower ($P < .001$) in BBdp rats (184.0 ± 9.5 μU/islet, n = 64) versus BBc rats (266.3 ± 10.5 μU/islet, n = 56). When the output of insulin was expressed relative to the paired insulin content, no significant difference was detected between control and BBdp animals (Table 2).

The mean total amount of insulin released during incubation and recovered in the islets after incubation was slightly higher at high (16.7 mmol/L) compared with low (2.8 mmol/L) D-glucose, indicating that proinsulin biosynthesis was stimulated. This effect was significant ($P < .001$) in control rats, with the 16.7/2.8 mmol/L ratio averaging 113.9% ± 3.8% ($df = 102$), but was not statistically significant in BBdp animals, where the 16.7/2.8 mmol/L ratio did not exceed 105.1% ± 4.2% ($df = 110$).

Table 1. Metabolic and Hormonal Status in BBc and BBdp Rats by Diet

	BBc		BBdp	
Parameter	NIH	HC	NIH	HC
No. of rats	15	17	16	17
Age (d)	51.8 ± 0.3	51.2 ± 0.9	47.1 ± 0.3	47.8 ± 0.3
Body weight (g)	172 ± 7	169 ± 6	173 ± 5	159 ± 5
Duration of treatment (d)	19.8 ± 1.2	20.2 ± 1.1	17.3 ± 0.1	18.4 ± 0.2
Weight gain during treatment (g/d)	5.6 ± 0.3	5.5 ± 0.3	6.1 ± 0.3	5.2 ± 0.2
Plasma glucose (mmol/L)	8.18 ± 0.67	7.86 ± 0.52	8.67 ± 0.58	9.99 ± 0.39
Plasma insulin (μU/mL)	34.2 ± 3.3	45.1 ± 6.3*	20.6 ± 1.4	22.5 ± 2.8
Plasma insulin/glucose ratio (U/mol)	4.76 ± 0.74	6.46 ± 1.09*	2.46 ± 0.16	2.25 ± 0.29

*n = 16.

Table 2. Islet Insulin Content and Release in BBc and BBdp Rats by Diet

Parameter	BBc		BBdp	
	NIH	HC	NIH	HC
Insulin content (μU/islet)	1,117 ± 41 (56)	1,369 ± 59 (56)*	703 ± 26 (56)	797 ± 23 (56)*
Insulin output (μU/islet per 120 min)				
2.8 mmol/L D-glucose	9.5 ± 1.2 (24)	13.2 ± 3.6 (24)	8.2 ± 2.5 (24)	5.1 ± 1.1 (24)
16.7 mmol/L D-glucose	272.6 ± 10.0 (32)	257.9 ± 20.8 (24)	182.7 ± 10.0 (32)	185.3 ± 16.4 (32)
Insulin output/content (%)				
2.8 mmol/L D-glucose	0.83 ± 0.12 (24)	0.86 ± 0.13 (24)	1.15 ± 0.38 (24)	0.65 ± 0.13 (24)
16.7 mmol/L D-glucose	27.22 ± 1.82 (32)	22.99 ± 2.67 (24)	29.82 ± 1.45 (32)	25.90 ± 2.34 (32)
Insulin output + content (% of paired mean basal value)				
2.8 mmol/L D-glucose	100.0 ± 2.8 (24)	100.0 ± 2.6 (24)	100.0 ± 3.1 (24)	100.0 ± 3.0 (24)
16.7 mmol/L D-glucose	114.1 ± 4.5 (32)	113.7 ± 3.7 (24)	106.8 ± 4.6 (32)	103.3 ± 4.7 (32)

NOTE. The number of islet cultures is shown in parentheses.
*$P < .01$ *v* NIH.

There was no statistically significant diet-related difference in insulin output, insulin output to content, or insulin output plus content. However, there was a significant diet-related increase in insulin content in the islets of animals fed the HC diet in both BBc (23%, $P < .01$) and BBdp (13%, $P < .01$) rats (Table 2).

Islet Metabolism

At both 2.8 and 16.7 mmol/L D-glucose, the conversion of D-[5-^{3}H]glucose to ^{3}HOH and D-[U-^{14}C]glucose to $^{14}CO_2$ and ^{14}C-labeled acidic metabolites and amino acids was lower ($P < .005$) in islets from BBdp rats compared with control animals. In both types of rats, the increase in hexose concentration invariably augmented ($P < .001$ in all cases) the production of these radioactive metabolites.

The paired ratio between D-[U-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization, which was also increased ($P < .001$) as a result of the increase in hexose from 2.8 to 16.7 mmol/L in both control and diabetes-prone rats, was not lower in BBdp compared with BBc animals. At the high concentration of D-glucose (16.7 mmol/L), it was slightly higher ($P < .02$) in diabetes-prone rats (49.6% ± 1.2%, n = 60) than in control rats (46.1% ± 0.9%, n = 63).

No major diet-related difference in metabolic variables was found in either type of rat. If anything, the trend was for higher values for the variables listed in Table 3 in the case of animals fed the HC diet rather than the mixed NIH diet. However, this difference was statistically significant only in control rats, in which the production of ^{14}C-labeled acidic metabolites (both at 2.8 and 16.7 mmol/L D-glucose) and amino acids (only at 16.7 mmol/L D-glucose), as well as the ratio between D-[U-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization (again only at 16.7 mmol/L D-glucose), was significantly higher ($P < .05$) in animals fed the HC diet compared with the NIH diet.

Splenocyte Metabolism

The oxidation of L-[U-^{14}C]glutamine (1.0 mmol/L) in splenocytes was not significantly affected by the rat type or diet (Table 4). Similarly, the utilization of D-[5-^{3}H]glucose (7.0 mmol/L) was not significantly different in control and BBdp rats fed the

Table 3. Islet Metabolism in BBc and BBdp Rats by Diet

Parameter	BBc		BBdp	
	NIH	HC	NIH	HC
D-[5-^{3}H]glucose utilization*				
2.8 mmol/L D-glucose	45.3 ± 2.2 (31)	41.3 ± 3.3 (30)	28.4 ± 2.2 (31)	34.5 ± 3.5 (32)
16.7 mmol/L D-glucose	168.1 ± 7.3 (32)	171.2 ± 10.7 (31)	111.0 ± 5.8 (30)	125.3 ± 7.8 (30)
D-[U-^{14}C]glucose oxidation*				
2.8 mmol/L D-glucose	14.2 ± 0.6 (32)	12.6 ± 0.7 (32)	9.1 ± 0.4 (32)	10.0 ± 0.9 (32)
16.7 mmol/L D-glucose	71.0 ± 3.4 (32)	79.6 ± 4.1 (31)	55.6 ± 2.6 (32)	60.8 ± 2.7 (31)
D-[U-^{14}C]glucose conversion to acidic metabolites*				
2.8 mmol/L D-glucose	11.3 ± 0.7 (30)	16.1 ± 0.9 (31)†	10.0 ± 1.1 (31)	10.5 ± 1.4 (29)
16.7 mmol/L D-glucose	31.2 ± 2.7 (27)	41.6 ± 3.4 (29)†	23.3 ± 2.8 (32)	23.0 ± 2.5 (32)
D-[U-^{14}C]glucose conversion to amino acids*				
2.8 mmol/L D-glucose	8.8 ± 0.4 (32)	8.6 ± 0.5 (31)	5.7 ± 0.4 (31)	6.0 ± 0.3 (29)
16.7 mmol/L D-glucose	27.3 ± 1.1 (30)	32.5 ± 1.7 (32)†	19.6 ± 1.2 (32)	20.5 ± 1.3 (32)
D-[U-^{14}C]glucose oxidation/D-[5-^{3}H]glucose utilization (paired ratio, %)				
2.8 mmol/L D-glucose	32.3 ± 0.7 (31)	32.2 ± 0.9 (30)	33.6 ± 1.3 (31)	34.5 ± 3.5 (32)
16.7 mmol/L D-glucose	44.3 ± 1.3 (32)	47.9 ± 1.1 (31)†	49.8 ± 2.0 (30)	49.4 ± 1.4 (30)

NOTE. The number of islet cultures is shown in parentheses.
*Results expressed as pmol D-glucose equivalents/islet per 120 min.
†$P < .05$ *v* BBc rats fed NIH.

Table 4. Splenocyte Metabolism in BBc and BBdp Rats by Diet

Parameter	BBc		BBdp	
	NIH	HC	NIH	HC
L-[U-^{14}C]glutamine oxidation*	2.91 ± 0.20 (27)	2.71 ± 0.17 (27)	2.79 ± 0.07 (27)	2.45 ± 0.20 (27)
D-[5-^{3}H]glucose utilization*	14.77 ± 1.22 (25)	15.68 ± 0.34 (26)	17.87 ± 1.56 (25)	23.03 ± 2.20 (27)
D-[U-^{14}C]glucose oxidation*	0.76 ± 0.06 (28)	1.10 ± 0.07 (27)‡	0.82 ± 0.04 (26)	1.22 ± 0.13 (26)§
D-[U-^{14}C]glucose conversion to acidic metabolites*	7.66 ± 0.58 (28)	9.97 ± 0.50 (28)‡	10.79 ± 0.86 (27)	11.17 ± 0.98 (28)
D-[U-^{14}C]glucose conversion to amino acids*	0.36 ± 0.04 (28)	0.53 ± 0.04 (28)‡	0.39 ± 0.04 (27)	0.52 ± 0.06 (28)
D-[U-^{14}C]glucose oxidation/D-[5-^{3}H]glucose utilization†	5.48 ± 0.33 (24)	7.31 ± 0.54 (26)‡	5.96 ± 0.68 (25)	5.36 ± 0.19 (27)

NOTE. The number of spleen cell cultures is shown in parentheses.

*Results expressed as pmol L-glutamine or D-glucose equivalents/10^3 cells per 120 min.

†Paired ratio expressed as percent.

‡$P < .001$ *v* BBc rats fed NIH.

§$P < .005$ *v* BBdp rats fed NIH.

NIH diet. Despite a trend for higher values in BBdp rats fed HC compared with BBc rats fed the same diet, the difference was not significant.

In animals fed the NIH diet, no significant difference between control and diabetes-prone rats was found for D-[U-^{14}C]glucose oxidation or conversion to ^{14}C-labeled amino acids and for the ratio between D-[U-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization. The net generation of ^{14}C-labeled acidic metabolites from D-[U-^{14}C]glucose was lower ($P < .005$) in control rats compared with diabetes-prone animals. In the control rats, the oxidation of D-[U-^{14}C]glucose, its conversion to acidic metabolites and amino acids, and the paired ratio between D-[U-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization were all higher ($P < .001$ or less) in animals fed the HC diet compared with the NIH diet. In BBdp animals, this increase was significant ($P < .005$) in the case of D-[U-^{14}C]glucose oxidation.

Taken as a whole, these data suggest that there is little difference between BBc and BBdp rats in terms of L-glutamine and D-glucose catabolism in the spleen when the animals are fed the NIH diet. The HC diet tended to increase the mean values for the variables of D-glucose, but not L-glutamine, catabolism in splenocytes from both control and diabetes-prone rats. However, this change often did not reach statistical significance, especially in diabetes-prone rats.

MLN Cell Metabolism

The oxidation of L-[U-^{14}C]glutamine (1.0 mmol/L) was twice as high ($P < .005$) in cells from diabetes-prone rats compared with control animals regardless of diet (Table 5). It was decreased 2-fold ($P < .001$) in animals fed the HC diet compared with the NIH diet. As a result of the opposite effects of animal type (BBdp *v* BBc) and the change from the NIH diet compared with the HC diet on L-[U-^{14}C]glutamine oxidation, the results obtained in diabetes-prone rats fed the HC diet were virtually identical to those obtained in control animals fed the NIH diet.

A similar situation was observed in the case of D-[5-^{3}H]-glucose utilization and D-[U-^{14}C]glucose conversion to radioactive acidic metabolites. Thus, the mean generation of ^{3}HOH was higher ($P < .02$) in lymphocytes of diabetes-prone rats compared with control rats, and in both cases, it was lower in animals fed the HC diet ($P < .09$ in BBc and $P < .001$ in BBdp animals). Hence, the utilization of D-[5-^{3}H]glucose was again comparable in control rats fed the NIH diet and diabetes-prone animals fed the HC diet. Similarly, the net generation of ^{14}C-labeled acidic metabolites from D-[U-^{14}C]glucose was higher ($P < .001$) in diabetes-prone rats versus control animals fed the NIH diet, and was decreased by feeding the HC diet. The

Table 5. MLN Cell Metabolism in BBc and BBdp Rats by Diet

Parameter	BBc		BBdp	
	NIH	HC	NIH	HC
L-[U-^{14}C]glutamine oxidation*	0.84 ± 0.04 (18)	0.41 ± 0.07 (24)‡	1.78 ± 0.15 (23)	0.81 ± 0.09 (23)‡
D-[5-^{3}H]glucose utilization*	4.30 ± 0.21 (17)	3.28 ± 0.49 (20)	7.91 ± 0.59 (20)	4.73 ± 0.27 (23)‡
D-[U-^{14}C]glucose oxidation*	0.32 ± 0.04 (18)	0.41 ± 0.07 (24)	0.53 ± 0.02 (23)	0.57 ± 0.02 (24)‡
D-[U-^{14}C]glucose conversion to acidic metabolites*	2.99 ± 0.30 (18)	2.21 ± 0.41 (24)	4.88 ± 0.31 (24)	2.88 ± 0.20 (24)‡
D-[U-^{14}C]glucose conversion to amino acids*	0.13 ± 0.02 (18)	0.19 ± 0.05 (22)	0.28 ± 0.03 (24)	0.31 ± 0.03 (24)
D-[U-^{14}C]glucose oxidation/D-[5-^{3}H]glucose utilization†	7.28 ± 0.72 (17)	18.08 ± 2.46 (20)‡	7.63 ± 0.76 (20)	11.83 ± 0.38 (22)‡

NOTE. The number of MLN cell cultures is shown in parentheses.

*Results expressed as pmol of L-glutamine or D-glucose equivalents/10^3 cells per 120 min.

†Paired ratio expressed as percent.

‡$P < .001$ *v* rats fed NIH.

latter effect was significant only in diabetes-prone rats ($P < .001$).

In animals fed the NIH diet, the oxidation of D-[U-^{14}C]-glucose and its conversion to radioactive amino acids were also higher in BBdp rats ($P < .001$). However, no decrease in these metabolic variables was observed when comparing animals fed the HC diet versus NIH diet, the trend being invariably toward an increase in D-[U-^{14}C]glucose oxidation and conversion to amino acids in rats fed the HC diet rather than the NIH diet (Table 5). The paired ratio between D-[U-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization was also significantly higher ($P < .001$) in rats fed the HC diet, whether in control or diabetes-prone animals.

Thus, as judged from either D-[5-^{3}H]glucose utilization or the conversion of D-[U-^{14}C]glucose to ^{14}C-labeled acidic metabolites (eg, lactic acid), the HC diet decreased the rate of glycolysis in MLN lymphocytes; this effect was most marked in diabetes-prone animals, in contrast to the unaltered generation of both ^{14}CO and ^{14}C-labeled amino acids through the mitochondrial metabolism of glucose-derived pyruvate.

The metabolic behavior of MLN cells differed from splenocytes. There was a much greater increase in metabolic flux when comparing NIH-fed diabetes-prone and control rats and a greater response to the dietary manipulation. This was most apparent in the case of L-[U-^{14}C]glutamine oxidation in MLN lymphocytes, which was markedly increased in BBdp compared with BBc rats and markedly decreased in animals fed the HC diet compared with the NIH diet. By marked contrast, the metabolic flux in splenocytes was less affected by rat type or diet.

DISCUSSION

The degree of protection observed in HC-fed animals is substantial, resulting in 50% to 80% fewer cases of diabetes and a more benign inflammatory process in the pancreas.[1] It was proposed recently that this dietary modification begins in the period before classic insulitis as demonstrated by increased islet area[3] and decreased expression of β-cell MHC class I molecules.[4] These results suggested diet-induced changes in islet homeostasis and antigenicity occur well before substantial numbers of mononuclear cells and macrophages infiltrate the pancreas, producing a periislet and intraislet inflammation called insulitis. A few weeks later, by 70 days of age, the dampened insulitis lesion in the pancreas of HC-fed rats is characterized by fewer mononuclear cells. The phenotype of the cells that are present is changed so that the cytokine profile in the pancreas is predominantly Th2 and Th3 cytokines, as reflected by increased levels of IL-10 and TGF-β mRNA along with low levels of mRNA for the Th1 cytokine IFN-γ,[3] that normally predominates in BBdp rats fed cereal-based diets. The origin of these immunocytes, the reason they are activated and attracted into the islets, and the temporal relationship of autoimmune cell reactivity and altered target tissue homeostasis and MHC class I expression remain open questions. In considering how feeding the HC diet protects BB rats from developing diabetes, one possibility involves an alteration of the metabolism of target islet cells and/or selected cells of the immune system. The present studies using cells cultured in vitro were undertaken because data are lacking regarding the effect of the HC diet on β-cell metabolism in parallel with changes in spleen and MLN cells in the pre-insulitis period.

Previous studies were often conducted in slightly older BB rats, the diet was not a test variable, and the metabolism and function of isolated islets was not examined. To our knowledge, there is one study on the effect of dietary modification per se of BB rat nutrient metabolism, and this was focused on spleen and MLN cells but not isolated islets.[17] Field reported that BBdp rats fed a casein-based diet had lower rates of glutamine metabolism in spleen cells, whereas the metabolism of glucose, but not glutamine, was decreased in MLN cells compared with cells from animals fed a cereal-based diet.[17] In the present study, the energy metabolism of MLN cells was clearly increased in BBdp rats compared with BBc rats. When BBdp rats were fed the HC diet, the energy metabolism of MLN cells was decreased, an effect that was more marked than that observed in either MLN cells from BBc rats or splenocytes from BBdp rats fed the same diet (Tables 4 and 5). This is consistent with decreased immune activation in BBdp rats fed the HC diet. When comparing the present results with those reported by Field, two caveats should be borne in mind: (1) Animals in the study by Field were fed diets containing intact casein, whereas the present studies were performed in animals fed an HC-based diet. Although previous reports suggest that casein-based diets also protect BBdp rats from diabetes, there is currently some controversy regarding the diabetogenicity of casein.[1,18-20] It is now generally accepted that feeding HC-based diets from weaning protects a majority of BBdp rats and NOD mice from developing diabetes, and for this reason, we used the HC diet in the present studies. (2) It is not possible to predict which BBdp animals are destined to develop diabetes. Thus, young BBdp rats, as is the case with NOD mice or humans at risk, are from a heterogeneous population with different disease risk and kinetics of disease progression. The latter point probably is most relevant in understanding these contrasting results.

In the present study, pancreatic islet composition, metabolism, and function differed in BBdp rats compared with control animals, as shown by a slightly higher plasma glucose concentration, low plasma insulin concentration and insulinogenic index, low protein and insulin content of isolated pancreatic islets, low rate of insulin release in islets incubated in the presence of 16.7 mmol/L D-glucose, and an apparently decreased biosynthetic response of the islets to an increase in the hexose concentration from 2.8 to 16.7 mmol/L. This coincided with low rates of D-[5-^{3}H]glucose utilization, D-[U-^{14}C]glucose oxidation, and conversion of D-[U-^{14}C]glucose to both acidic metabolites and amino acids in islets incubated at either low or high D-glucose concentration. Young diabetes-prone rats differed from control animals in having higher rates of L-[U-^{14}C]-glutamine oxidation, D-[5-^{3}H]glucose utilization, and D-[U-^{14}C]-glucose conversion to $^{14}CO_2$ and radioactive acidic metabolites and amino acids in MLN lymphocytes, but not in splenocytes.

Evidence is accumulating that the islet tissue may participate in its own demise in the period before classic insulitis.[3,4,6,7,21] It was reported by Svenningsen et al[22] that compared with diabetes-resistant BB rats, BBdp rats as young as 30 days of age showed decreased islet mass and insulin content; both groups

were fed standard (cereal-based) rodent diets. The increase in the insulin content of isolated islets by 13% in BBdp and 23% in BBc rats fed HC in the present study suggests that the protective effect of the HC diet involves some recovery or partial reversal of diminished pancreatic insulin content. This is consistent with the increased islet area observed in HC-fed BBdp rats.[3] In rats fed the HC diet, there was also a trend for increased catabolism of D-glucose in pancreatic islets and splenocytes, but this change was most obvious in control animals. Although a direct role for individual immunocytes infiltrating the pancreas cannot be excluded, classic insulitis is rare in animals aged 50 days or less in our colony.

Diet-related changes in the systemic immune system of BBdp rats have been difficult to demonstrate in peripheral blood or spleen cells,[23] but seem more apparent in the local environment of the pancreatic mononuclear cell infiltrate and proximal tissues of the gut immune system such as the MLN.[3,17] The major protective effect of feeding a nondiabetogenic diet such as the HC diet in both rodent models of diabetes suggests a priori an important role for the gut in the pathogenesis of diabetes. The gut-associated lymphoid tissue is where dietary antigens have their first major encounter with the immune system. Antigens from the gut lumen are transported into the Peyer's patches and villus epithelium, where they encounter antigen-presenting cells that process and present antigens. These cells then enter the systemic circulation via the MLNs and thoracic duct. A key question arises as to the relative importance of events at the site of the autoimmune attack within the pancreas versus metabolic changes reflecting immune cell activation within the gut immune system.[24-26]

In yet another study, newly diagnosed diabetic BB rats were found to display a low pancreatic insulin content and a severely decreased secretory response of the isolated perfused pancreas to D-glucose.[27] Information on nutrient metabolism in cells of the immune system was reported in studies using splenocytes obtained from 115 to 140-day-old diabetic BB rats and control, non–diabetes-prone rats.[26] This laboratory reported that oxidation of L-[U-^{14}C]glutamine (1.0 mmol/L) was increased 2-fold in BBdp compared with control rats, while this anomaly was not observed in streptozotocin-induced diabetes in BBn rats.[26] This was one report in a detailed series of studies demonstrating that enhanced glutamine metabolism reflects the activation of selected immune cells. In further studies, this group was able to almost completely prevent diabetes in BBdp rats by administering the glutamine antimetabolite, acivicin, further demonstrating the importance of the observed increase in glutamine metabolism in immunocytes.[25]

The present findings are compatible with the view that the lower incidence of autoimmune diabetes in diabetes-prone BB rats fed the HC diet involves decreased nutrient catabolism in MLN lymphocytes as measured by decreased oxidation of L-glutamine. Under physiologic conditions, the catabolism of this amino acid provides the major fraction of basal energy needs in several cell types[25,26,28] and may reflect decreased immune activation in MLN cells. It remains to be investigated whether the metabolism of L-glutamine in pancreatic islets is also affected by the dietary manipulation. When considering the diet-induced changes in MLN lymphocyte metabolism, it also remains to assess the respective roles of a direct effect of dietary factors on such cells and an indirect effect mediated by changes in β-cell antigenicity.[4] That the HC diet increased the insulin content of isolated islets, partially reversing a deficit previously observed in young BBdp rats, is consistent with an enhanced islet area observed in young HC-fed BBdp rats.[3] The concomitant decrease in glutamine oxidation in MLN cells around the same period, possibly reflecting decreased immune cell activation, leads us to suggest that there are diet-induced changes in the metabolism of energy substrates occurring in MLN cells that drain the gastrointestinal tract, with smaller HC-related increases in glucose metabolism in the target β cells, along with some increase in insulin content. The BBdp rat has been reported to have a mildly inflamed gastrointestinal tract, and this is associated with gut leakiness.[29] The finding that MLN cell metabolism is decreased in HC-fed rats could reflect changes in gut inflammation and/or avoidance of stimulatory food antigens.

In conclusion, the present findings provide novel information on the simultaneous regulation of nutrient catabolism in cells of both the immune system and the endocrine pancreas and on the secretory behavior of isolated pancreatic islets in diabetes-prone BB rats examined before classic insulitis is a prominent feature. These studies set the scene for further investigations on the precise sequence of events responsible for diet-induced changes in the autoimmune process that leads to the destruction of islet β cells in this animal model of type 1 diabetes mellitus.

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REFERENCES

1. Scott FW: Food-induced type 1 diabetes in the BB rat. Diabetes Metab Rev 12:341-359, 1996

2. Malkani S, Nompleggi D, Hansen JW, et al: Dietary cow's milk protein does not alter the frequency of diabetes in the BB rat. Diabetes 46:1133-1140, 1997

3. Scott FW, Cloutier HE, Kleeman R, et al: Potential mechanisms by which certain foods promote or inhibit the development of spontaneous diabetes in BB rats: Dose, timing, early effect on islet area, and switch in infiltrate from Th1 to Th2 cells. Diabetes 46:589-598, 1997

4. Li XB, Scott FW, Park YH, et al: Low incidence of autoimmune type I diabetes in BB rats fed a hydrolysed casein-based diet associated with early inhibition of non–macrophage-dependent hyperexpression of MHC class I molecules on beta cells. Diabetologia 38:1138-1147, 1995

5. Field CJ, Chayoth R, Montambault M, et al: Enhanced 2-deoxy-D-glucose uptake and metabolism in splenocytes from diabetic and diabetes-prone BB rats. J Biol Chem 266:3675-3681, 1991

6. Homo-Delarche F, Boitard C: Autoimmune diabetes: The role of the islets of Langerhans. Immunol Today 17:456-460, 1996

7. Gotfredsen CF, Buschard K, Frandsen EK: Reduction of diabetes

incidence of Wistar rats by early prophylactic insulin treatment of diabetes-prone animals. Diabetologia 28:933-935, 1985

8. Bergmeyer HU, Berndt E: Measurement of glucose with glucose oxidase and peroxidase, in Bergmeyer HU (ed): Methods of Enzymatic Analysis. New York, NY, Academic, 1974, pp 1205-1215

9. Leclercq-Meyer V, Marchand J, Woussen-Colle MC, et al: Multiple effects of leucine on glucagon, insulin and somatostatin secretion from the perfused rat pancreas. Endocrinology 116:1168-1174, 1985

10. Malaisse-Lagae F, Malaisse WJ: Insulin release by pancreatic islets, in Larner J, Pohl SL (eds): Methods in Diabetes Research. New York, NY, Wiley, 1984, pp 147-152

11. Lowry OH, Rosebrough NH, Farr A, et al: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275, 1951

12. Malaisse WJ, Sener A: Hexose metabolism in pancreatic islets. Feedback control of D-glucose oxidation by functional events. Biochim Biophys Acta 971:246-254, 1988

13. Malaisse WJ, Giroix M-H, Malaisse-Lagae F, et al: 3-*O*-methyl-D-glucose transport in tumoral insulin-producing cells. Am J Physiol 251:C841-C846, 1986

14. Sener A, Malaisse WJ: Stimulation by D-glucose of mitochondrial oxidative events in islet cells. Biochem J 246:89-95, 1987

15. Keen H, Seels R, Jarrett RJ: A method for the study of the metabolism of isolated mammalian islets of Langerhans and some preliminary results. Diabetologia 1:28-32, 1965

16. Hudson L, Hay F: The basic techniques, in Hudson L, Hay F (eds): Practical Immunology. Oxford, UK, Blackwell Scientific, 1989, p 23

17. Field CJ: A diet producing a low diabetes incidence modifies immune abnormalities in diabetes-prone BBdp rats. J Nutr 125:2594-2603, 1995

18. Elliott RB, Bibby N, Reddy S: Casein peptide precipitates diabetes in the non-obese diabetic mouse and possibly humans, in Laron Z, Karp M (eds): Genetic and Environmental Risk Factors for Type I Diabetes (IDDM) Including a Discussion on the Autoimmune Basis. Tel Aviv, Israel, Freund, 1992, pp 57-62

19. Cavallo MG, Fava D, Monetini L, et al: Cell-mediated immune response to beta casein in recent-onset insulin-dependent diabetes: Implications for disease pathogenesis. Lancet 348:926-928, 1996

20. Ellis TM, Ottendorfer E, Jodoin E, et al: Cellular immune responses to beta casein: Elevated in but not specific for individuals with type I diabetes mellitus. Diabetologia 41:731-735, 1998

21. Huang X, Hultgren B, Dybdyl N, et al: Islet expression of interferon-α precedes diabetes in both the BB rat and streptozotocin-treated mice. Immunity 1:469-478, 1994

22. Svenningsen A, Dyrberg T, Markholst H, et al: Insulin release and pancreatic insulin is reduced in young prediabetic BB rats. Acta Endocrinol (Copenh) 112:367-371, 1986

23. Scott FW, Sarwar G, Cloutier HE: Diabetogenicity of various protein sources in the diet of the BB rat. Adv Exp Biol Med 246:277-285, 1988

24. Bellmann K, Kolb H, Hartmann B, et al: Intervention in autoimmune diabetes by targeting the gut immune system. Int J Immunopharmacol 19:573-577, 1997

25. Misra M, Duguid WP, Marliss EB: Prevention of diabetes in the spontaneously diabetic BB rat by the glutamine antimetabolite acivicin. Can J Physiol Pharmacol 74:163-172, 1996

26. Wu G, Field CJ, Marliss EB: Elevated glutamine metabolism in splenocytes from spontaneous diabetic BB rats. Biochem J 274:49-54, 1991

27. Pederson RA, Curtis SB, Chisholm CB, et al: Insulin secretion and islet endocrine cell content at onset and during the early stages of diabetes in the BB rats: Effect of the level of glycemic control. Can J Physiol Pharmacol 69:1230-1236, 1991

28. Sener A, Malaisse-Lagae F, Malaisse WJ: The stimulus-secretion coupling of glucose-induced insulin release. LII. Environmental influences on L-glutamine oxidation in pancreatic islets. Biochem J 202:309-316, 1982

29. Donegan L, Hardin JA, Gall DG: Mucosal inflammation in a genetic model of type 1 diabetes. Gastroenterology 114:A967, 1998 (abstr)