Insulin and Enzyme Responses in Three Strains of Rats (35643)

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(Introduced by D. L. Trout)

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Previous reports from these laboratories have indicated important strain differences in the metabolism of carbohydrates (1–5). It has also been shown that there are strain differences in the level of serum immunoreactive insulin (IRI) with age (6). Other investigators (7, 8) have observed strain differences in the activities of key glycolytic enzymes and in the rate of hepatic lipogenesis. Since insulin plays an important role in the activation and/or synthesis of certain enzymes of carbohydrate metabolism (9, 10), the current studies were undertaken in an attempt to determine the relationship of IRI, enzyme activity, and the lipid content of the blood, liver, and carcass in animals of the Wistar, BHE,[1] and a substrain of the BHE[2] strain.

Experimental Methods. Three groups of 12 male weanling rats of the Wistar, BHE, and the inbred BHE (designated IN-BHE) strains were used for these studies. Rats were housed individually in wire mesh cages with raised floors in a temperature–humidity controlled room. The animals had access to food and water *ad libitum*. A diet[3] containing 6% corn oil, 45% carbohydrate, and 40% protein was used to promote good growth. Food intakes and body weight gains were determined weekly.

At 100 days of age, half the animals were killed without prior fasting while the remaining animals of each group were killed after a 24-hr fast. The animals were anesthetized by an intraperitoneal injection of 60 mg/kg of amobarbital; the thoracic cavity was opened, and blood was drawn by heart puncture. A 0.2-ml aliquot of blood was deproteinized and the glucose concentration was determined (12). The serum from the remaining blood was collected after centrifugation (30 min, 3000 rpm) in a refrigerated centrifuge. Serum IRI (13), cholesterol (14), and triglycerides (15) were determined. The livers were excised, blotted, and weighed. A 1-g sample was taken for enzyme analysis, while the remaining tissue was used for the determination of total lipid (16) and cholesterol (14). Enzyme activities were determined in a 10% liver homogenate prepared in a Potter Elvehjem homogenizer with ice-cold 0.14 *M* KCl (pH 7.4). Glucose-6-phosphatase activity was determined by measuring the amount of inorganic phosphate liberated in 15 min at 37° (17). The crude homogenate was centrifuged at 0° for 30 min at 15,000 rpm. The clear supernatant was used for the determination of soluble liver protein (18), glucose-6-phosphate dehydrogenase (17), malic enzyme (17), isocitrate dehydrogenase (19), 6-phosphogluconate dehydrogenase (17), citrate cleavage enzyme (20), and L-α-glycerophosphate dehydrogenase (17).

Enzyme activity is expressed as units per 100 g of body weight. The rationale for expressing enzyme activity per standard body weight has been previously discussed (17).

The ingesta-free carcass was autoclaved (15 lb/15 min) and ground in a Waring blender with crushed ice (carcass:ice, 1:2). Aliquots of this homogenate were taken for total carcass lipid determination (16). Differ-

[1] The BHE strain is a strain resulting from a cross between the Pennsylvania State College strain and the Osborne-Mendel Strain (also called the Yale strain).

[2] The IN-BHE animal, developed by Mary W. Marshall of these laboratories, is characterized by increased levels of lipid in the carcass and have been described elsewhere as line 3 (11).

[3] Composition of this diet: 1:1 casein/lactalbumin, 40%; corn oil, 6%; fiber, 3%; dextrin, 5%; Jones-Foster salt mix, 5%; A.O.A.C. vitamin mix, 1%; and 1:2:5 glucose/sucrose/cornstarch, 40%.

TABLE I. Serum Immunoreactive Insulin and Blood Glucose Levels in Three Strains of Rats.

Group	Strain	Insulin (μU/ml)		Glucose (mg/100 ml of whole blood)	
		Fasting	Nonfasting	Fasting	Nonfasting
1	IN-BHE	29 ± 3^{ab}	49 ± 4^{bc}	104 ± 3^{b}	111 ± 6^{b}
2	BHE	26 ± 5^{b}	52 ± 4^{bc}	101 ± 0^{b}	118 ± 6^{bc}
3	Wistar	43 ± 5	79 ± 5^{c}	84 ± 3	96 ± 3^{c}

[a] Standard error of the mean of six rats.

[b] Significant differences between the values from animals of the IN-BHE or BHE strain compared to the Wistar strain ($p < .05$).

[c] Significant differences between the values from fasting and nonfasting animals in the same group ($p < .05$).

ences between the means of each group for each determination were evaluated by the Student's t test and by an analysis of variance.

Results. Fasting and nonfasting serum IRI and whole blood glucose values are given in Table I. Fasting reduced all values; however, BHE and IN-BHE rats had lower serum IRI and higher blood glucose values than animals of the Wistar strain. As previously reported (1–5), the BHE animals have higher liver cholesterol levels than Wistar animals (Table II); however, IN-BHE animals differed from their parent strain in that their fasting liver cholesterol levels were not nearly as high. In contrast, both fasting and nonfasting serum cholesterol levels of the IN-BHE animals were the highest of all three strains. Wistar animals had higher levels of serum triglycerides, both in the fasting and nonfasting state, than animals of the BHE and IN-BHE strains (Table III). Wistar animals also had less liver lipid, although these differences were significant only in the nonfasting state. No significant differences in carcass lipid were observed. No strain differences were observed in the food intakes or body weight gains although the Wistar animals were consistently lighter than the BHE and IN-BHE animals. The average final body weights were 297, 310, and 300 g for the Wistar, BHE, and IN-BHE animals, respectively. The average food intakes for these animals were similar; approximately 10 g/day was consumed initially with a gradual increase to 21 g/day.

Finally, the activities of the liver enzymes, including glucose-6-phosphatase (G6Pase), glucose-6-phosphate dehydrogenase (G6PD), malic enzyme (ME), 6-phosphogluconate dehydrogenase (6PGD), citrate cleavage (CE), L-α-glycerophosphate dehydrogenase (L-α-GolPD), and isocitrate dehydrogenase (ICD) are summarized in Tables IV and V. As expected, the activity of the gluconeogenic enzyme G6Pase increased with fasting thus

TABLE II. Liver and Serum Cholesterol Levels of Three Strains of Rats.

Group	Strain	Serum cholesterol (mg/100 ml)		Liver cholesterol (mg/g)	
		Fasting	Nonfasting	Fasting	Nonfasting
1	IN-BHE	216 ± 20^{a}	311 ± 23^{bc}	7.27 ± 42^{b}	$8.15 \pm .25^{b}$
2	BHE	154 ± 31	217 ± 25^{c}	9.77 ± 45^{b}	$6.67 \pm .63^{bc}$
3	Wistar	166 ± 6	189 ± 7^{c}	$5.68 \pm .39$	$4.51 \pm .50$

[a] Standard error of the mean of six rats.

[b] Significant differences between the values from animals of the IN-BHE or BHE strain compared to the Wistar strain ($p < .05$).

[c] Significant difference between the values from fasting and nonfasting animals of the same strain ($p < .05$).

TABLE III. Serum Triglyceride Level and Lipid Content of the Livers and Carcasses of Three Strains of Rats.

Group	Strain	Serum triglycerides (mg/100 ml)		Liver lipids (mg/100 g of body wt)		Carcass lipid (g/100 g of body wt)	
		Fasting	Nonfasting	Fasting	Nonfasting	Fasting	Nonfasting
1	IN-BHE	43 ± 18[a]	58 ± 14[b]	140 ± 6	171 ± 6[b,c]	14 ± 2	16 ± 1
2	BHE	29 ± 13	87 ± 19[b,c]	131 ± 5	149 ± 5[c]	13 ± 3	13 ± 2
3	Wistar	65 ± 39	233 ± 40[c]	127 ± 2	137 ± 4[c]	13 ± 1	17 ± 1

[a] Standard error of the mean of six rats.

[b] Probability of differences between the values from animals of the IN-BHE or BHE strain compared to the Wistar strain ($p < .05$).

[c] Significant differences between the values from fasting and nonfasting animals of the same strain ($p < .05$).

providing the animals with an increased ability to produce glucose during the fasting period. The exception to this expectation was seen in the IN-BHE animal; the activity of G6Pase tended to decrease with fasting, a response typical of diabetes mellitus (21). In addition, these animals had the greatest G6Pase activity of the three groups studied. An analysis of variance of the G6Pase data showed strain differences in the activity of this enzyme and a strain/fasting interaction.

Citrate cleavage activity decreased with fasting in all three strains indicating decreased lipogenic activity. However, the fasting level of CE in both the BHE and IN-BHE animals was nearly twice that of the Wistar animals. While only a slight and nonsignificant difference between the fed and fasting levels of this enzyme was observed in the IN-BHE animals, marked differences were seen in the BHE and Wistar animals with the BHE animals having the greatest nonfasting CE activity. This probably indicates a greater lipogenic capacity of the BHE animals as compared to the Wistar and IN-BHE animals. The activity of L-α-GolPD was greater in nonfasted BHE and IN-BHE compared to the Wistar animals and was significantly reduced by fasting in these two strains.

Strain differences in the activities of the hepatic enzymes involved in the generation of NADPH was observed (Table V). The activities of these enzymes were reduced by fasting in the parent BHE strain but only the activities of 6PGD and ICD were reduced by fasting in the other two strains. The activities of G6PD and ME were highest in the nonfasting BHE strain and were less in the IN-BHE and Wistar strains.

Discussion. The results of these studies show that animals from different strains with similar food intakes and body weight gains up to 100 days of age have different patterns of intermediary metabolism. This was particularly evident when the responses of these animals to a 24-hr fast were examined in detail. Strain differences in response to fasting were observed in the levels of serum triglycerides, serum and liver cholesterol, and liver lipid. This may indicate strain differences in the choice of substrate used for energy during the fasting period which preceded the killing of the animals. The data also suggest that the strains may differ in the relative importance of the liver, compared to the adipose tisse, for the synthesis of fatty acids from carbohydrate and amino acids. In the fed state, animals of the parent BHE strain showed the highest levels of G6PD and ME, two of the chief enzymes involved in the regeneration of the reduced form of extramitochondrial NADP. These animals also showed the highest levels of hepatic L-α-GolPD, a key enzyme in the production of glyceryl moiety of fatty acid esters.

A comparison of the enzyme levels in the fasting and nonfasting state suggests, in addition, that the internal controls which regulate the activities of these enzymes may differ significantly among the three strains. Specifically, the activities of G6PD and ME were

TABLE IV. Activities of Glucose-6-Phosphatase, Citrate Cleavage Enzyme, and L-α-Glycerol Phosphate Dehydrogenase in the Livers of Fasted and Nonfasted Rats of Three Strains.

Group	Strain	Soluble liver protein (mg/100 g of body wt)		Enzyme[a] (units[b]/100 g of body wt)					
				G6Pase		CE		L-α-GolPD	
		Fasting	Nonfasting	Fasting	Nonfasting	Fasting	Nonfasting	Fasting	Nonfasting
1	IN-BHE	328 ± 17[c]	405 ± 12[de]	66.3 ± 5	76.0 ± 3.7[e]	1.21 ± 0.09[e]	1.33 ± 0.18[e]	155 ± 15	220 ± 14[de]
2	BHE	288 ± 9	352 ± 6[d]	58.7 ± 5	51.1 ± 3.2	1.25 ± 0.13[e]	2.60 ± 0.31[d]	193 ± 5[e]	243 ± 6[de]
3	Wistar	311 ± 14	361 ± 13[d]	62.3 ± 1.8	55.4 ± 4.9	0.68 ± 0.04	1.95 ± 0.27[d]	170 ± 6	172 ± 13

[a] Abbreviations used: G6Pase = glucose-6-phosphatase; CE = citrate cleavage enzyme; L-α-GolPD = L-α-glycerol phosphate dehydrogenase.
[b] One unit of enzyme activity = amount of enzyme which can produce 1 μmole of measured product/min under the conditions of the assay.
[c] Standard error of the mean of six rats.
[d] Significant differences between the values from fasting and nonfasting animals of the same strain ($p < .01$).
[e] Significant differences between the values from animals of the IN-BHE or BHE strains compared to the Wistar strain ($p < .05$).

TABLE V. Liver NADP-Linked Dehydrogenase Activities of Three Strains of Rats.

Group	Strain	Enzyme[a] (units[b]/100 g of body wt)							
		G6PD		ME		ICD		6PGD	
		Fasting	Nonfasting	Fasting	Nonfasting	Fasting	Nonfasting	Fasting	Nonfasting
1	IN-BHE	8.65 ± 1.54[cd]	7.76 ± 0.97	4.23 ± 0.43[d]	3.49 ± 0.32[d]	39.2 ± 1.1[d]	53.7 ± 1.7[de]	9.09 ± 0.89[d]	12.9 ± 1.16[de]
2	BHE	8.55 ± 1.23[d]	16.8 ± 1.6[de]	4.41 ± 0.47[d]	6.60 ± 0.40[de]	32.0 ± 1.0	44.4 ± 1.5[de]	6.54 ± 0.58[d]	9.21 ± 0.36[de]
3	Wistar	6.08 ± 0.35	7.71 ± 1.07	2.62 ± 0.68	2.44 ± 0.28	29.3 ± 1.8	38.1 ± 1.3[e]	4.79 ± 0.26	6.22 ± 0.35[de]

[a] Abbreviations used: G6PD = glucose-6-phosphate dehydrogenase; ME = malic enzyme; ICD = isocitrate dehydrogenase; 6PGD = 6-phosphogluconate dehydrogenase.
[b] One unit of enzyme activity = amount of enzyme which can produce 1 μmole of measured product/min under the conditions of the assay.
[c] Standard error of the mean of six rats.
[d] Significant differences between the values from animals of the IN-BHE or BHE strains compared to the Wistar strain ($p < .05$).
[e] Significant differences between the values from fasting and nonfasting animals of the same strain ($p < .01$).

much higher in nonfasting BHE animals compared to the other two strains. The activities of these two enzymes were greatly reduced by fasting. The Wistar animals were unique in that there was no effect of fasting on the level of L-α-GolPD. Similarly, the activity of CE was unaffected by fasting in the IN-BHE animals. It is possible that in the IN-BHE animal ICD (NADP-dependent) could serve as a more important source of reducing equivalents for fatty acid synthesis than G6PD or perhaps ME. However, in the normal animal this is unlikely since ICD would probably operate in the reverse direction providing citrate for citrate cleavage (22).

Since insulin is one of the key regulators of intermediary metabolism, differences in insulin status may account for the observed differences in metabolism. Previously we reported a hyperinsulinemia (IRI values above 100 μU/ml) in 50-day-old male BHE and IN-BHE animals which subsided by 100 days of age (6). In the present study IRI levels of BHE and IN-BHE rats were observed to be similarly elevated at 50 days of age (data not shown) and to have fallen by 100 days of age to below the levels observed in the Wistar animals. The enzyme data from the current study suggest that this early hyperinsulinemia may have had a long-term effect on the activity of the pentose shunt enzymes as well as the synthesis of liver lipid and cholesterol. This suggestion appears to be supported in part by earlier studies using the starve-refeed technique (23). These studies showed that in refed rats the overshoot in ME and G6PD was associated with an increase in liver lipid and that both the increase in enzyme activity and liver lipid was preceded by an increase in IRI. Further studies of the "insulin dependency" of the pentose shunt enzymes have been conducted and are reported elsewhere (24).

Summary. Three groups of male weanling rats of the BHE, Wistar, and inbred BHE strains were fed a 6% corn oil, 40% protein, 45% carbohydrate diet until they were 100 days old. Half of the animals were killed after a 24-hr fast while the remaining animals were killed without prior fasting. Animals of the BHE and IN-BHE strains had lower levels of IRI and higher blood glucose levels than animals of the Wistar strain. Strain differences in the fasting and nonfasting activities of the hepatic enzymes and in the lipid levels of the blood and liver appeared to be related in part to the insulin status of these animals. However, the data also suggested that different mechanisms of internal control of intermediary metabolism exist for each of these strains.

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