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Serum Glycerol and Hepatic Glycerokinase Activity in the Carbohydrate-Sensitive BHE Strain of Rat[1] (39501)

LILLY B. GARDNER AND SHELDON REISER

NUSC, University of Maryland, College Park, Maryland 20742, and Nutrition Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705

The carbohydrate-sensitive BHE[2] strain of rat is presently being used by this laboratory as a model of the interrelationship between carbohydrate and lipid metabolism. Rats of this strain have been shown to develop hyperlipemia and to exhibit increases in synthesis and storage of carcass and liver lipid when fed a high carbohydrate diet (1). BHE rats also respond to different kinds and amounts of dietary carbohydrate with substantial increases in hepatic lipogenic enzyme activity before the actual onset of hyperlipemia. Recent studies indicate that the nonfasted rats of this strain also exhibit elevated serum free glycerol levels (2). Similar elevations in serum free glycerol have also been observed in Zucker "fatty" rats during *in vitro* fat mobilization studies (3). These increases in glycerol in the Zucker rats have been attributed to excessive adipose tissue lipolysis. This does not seem to be the case with the BHE strain, however, since data recorded for this strain suggest that the metabolic error resides in the liver rather than the adipose tissue (1, 4).

Uptake of glycerol by the tissues is regulated by several factors including the concentration of glycerol in the blood (5), the nutritional state of the animal (6), and the simultaneous metabolism of other substrates (7). Glycerokinase (EC 2.1.1.30), which is found primarily in the liver and kidney, is involved in the phosphorylation of glycerol to α-glycerophosphate by ATP. Glycerokinase is thus directly involved in the regulation of glycerol uptake and utilization by these tissues (8).

This study was undertaken to determine whether the elevated serum free glycerol found in the nonfasted BHE rats was related to hepatic glycerokinase activity.

Materials and methods. For each of two experiments, 45-day-old male BHE and Wistar (Grand Island Biological Laboratory, Madison, Wisconsin) rats were housed individually in wire mesh cages in a temperature-humidity controlled room. Light was regulated so as to provide equal periods of light and dark. Animals were fed a laboratory chow diet (Purina laboratory rat chow, Ralston Purina Co., St. Louis, Missouri) *ad libitum*. At 75 days of age the animals in a nonfasted state were sacrificed after anesthesia with 60 mg of sodium amobarbital/kg body weight, the thoracic cavity was opened, and blood was drawn by heart puncture. The sera collected after centrifugation (4°, 15 min, 3000 *g*) were used for the determination of free glycerol (9). The livers were quickly removed, placed in preweighed bags, fast-frozen in methanol and dry ice, and stored at −80° for not more than 3 days. Sections of the fast-frozen tissue were homogenized in 2 vol of cold 1% (w/v) KCl-EDTA. Glycerokinase activity was assayed by the radiochemical method of Newsholme *et al.* (10), which is based on the conversion of ^{14}C glycerol (glycerol-1-^{14}C, sp act 30 mCi/mmole, Amersham/Searle Corporation, Arlington Heights, Illinois) into L-[3-^{14}C]glycerophosphate and adsorption of the latter on DEAE cellulose paper disks. Supernatants prepared from liver homogenates by ultracentrifugation (105,000 *g*) were used as the source of glycerokinase in the first experiment. For the second experiment, glycerokinase activity was deter-

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[2] The BHE is a strain of rat resulting from a cross between the Pennsylvania State College strain and the Osborne-Mendel (also called Yale) strain. These animals are currently available from Floco Laboratories, Dublin, Virginia. (Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.)

mined from crude liver homogenates without further treatment (10) L-α-glycerophosphate dehydrogenase (EC 1.1.1.8, L-GPD) activity was determined from supernatants of 1-g liver samples homogenized in ice-cold 0.14 *M* KCL (11) and centrifuged at 4° at 30,000*g* for 25 min.

Correlation coefficients of relationships were determined and pairs of means were compared by the Student's *t* test (12).

Results. Comparisons of body weights, liver weights, relative liver size, serum free glycerol, and hepatic glycerokinase activity of 75-day-old, nonfasted, male Wistar and BHE rats used in Experiment 1 are shown in Table I. Serum free glycerol ($P < 0.01$) levels and liver glycerokinase activity measured in supernatant fractions ($P < 0.05$) were significantly higher in BHE rats than in Wistar controls. No significant differences were observed in body weight, liver weight, or relative liver size. Correlation coefficients between glycerokinase activity and serum free glycerol levels or liver weights were not significant.

Table II compares body weights, liver weights, relative liver size, serum free glycerol, and hepatic glycerokinase and L-α-glycerophosphate dehydrogenase activity for 75-day-old, nonfasted Wistar and BHE rats in Experiment 2. Although significant differences were observed in body weights ($P < 0.05$) and liver weights ($P < 0.01$), no differences were noted in relative liver size. Body weights and liver weights in Experiment 2 were also different from those observed in Experiment 1, despite the fact that all animals received similar treatment. Thus, in order to permit comparisons of enzyme activity, results are expressed as per 100 g body weight. Glycerokinase activity measured in crude homogenates in Experiment 2 was also slightly higher than glycerokinase activity measured in Experiment 1.

TABLE I. COMPARISONS OF BODY WEIGHTS, LIVER WEIGHTS, RELATIVE LIVER SIZE,[a] SERUM FREE GLYCEROL, AND HEPATIC TISSUE GLYCEROKINASE ACTIVITY OF 75-DAY-OLD, NONFASTED, MALE WISTAR AND BHE RATS IN EXPERIMENT.

Strain	Final body weight (g)	Liver weight (g)	Relative liver size	Serum free glycerol (μmole/100 ml)	Glycerokinase activity (μmole α-glycerophosphate produced/min/100 g body weight)
Wistar (6)[b]	248 ± 9[c]	10.71 ± 0.43	4.30 ± 0.05	3.8 ± 0.75	2.7 ± 0.5
BHE (8)	280 ± 5	11.43 ± 0.39	4.06 ± 0.11	7.4 ± 1.60[d]	7.6 ± 1.6[e]

[a] Relative liver size = (liver weight/body weight × 100.
[b] Indicates number of animals in group.
[c] Mean ± SEM.
[d] Values significantly different from BHE at $P < 0.01$.
[e] Values significantly different at $P < 0.05$.

TABLE II. COMPARISONS OF BODY WEIGHTS, LIVER WEIGHTS, RELATIVE LIVER SIZE,[a] SERUM FREE GLYCEROL, LIVER GLYCEROKINASE ACTIVITY, AND LIVER -α-GLYCEROPHOSPHATE DEHYDROGENASE ACTIVITY OF 75-DAY-OLD, NONFASTED, MALE WISTAR AND BHE RATS IN EXPERIMENT 2.

Strain	Final body weight (g)	Liver weight (g)	Relative liver size	Serum free glycerol (μmole/100 ml)	Glycerokinase activity (μmole α-glycerophosphate produced/min/100 g body weight)	L-α-Glycerophosphate dehydrogenase activity (μmole/100 g body weight)
Wistar (12)[b]	228 ± 4[c,f]	8.83 ± 0.24[c]	3.85 ± 0.02	1.06 ± 0.15[d]	6.3 ± 0.9[e]	139 ± 9
BHE (12)	203 ± 3	7.65 ± 0.24	3.80 ± 0.04	4.05 ± 0.23	9.9 ± 0.7	141 ± 6

[a] Relative liver size = liver weight/body weight × 100.
[b] Indicates number of animals in group.
[c] Mean ± SEM.
[d] Values significantly different from BHE at $P < 0.001$.
[e] Values significantly different from BHE at $P < 0.01$.
[f] Values significantly different from BHE at $P < 0.05$.

Homogenates are usually preferred for measuring maximum glycerokinase activity since there is less chance of losing activity during preparation (10). Correlation coefficients between glycerokinase activity and serum free glycerol, liver weights, or L-α-glycerophosphate dehydrogenase for Experiment 2 were not significant.

Discussion. The data for the two experiments show that free glycerol levels in the BHE rats remain elevated despite increased glycerokinase activity.

At physiological concentrations, i.e., <1 m*M*, glycerol entry into the hepatocyte is proportional to the extracellular glycerol concentration and is first order (8, 13, 14). As extracellular glycerol concentration increases, glycerol uptake is inhibited by intracellular α-glycerophosphate. Glycerokinase activity is also inhibited by α-glycerophosphate, and this inhibition is competitive with glycerol (6). High ATP concentrations activate glycerokinase when glycerol concentrations are high (5, 7). Inhibition occurs when concentrations of ADP (8) and AMP (6) are high. Thus, at least two possible explanations exist for the presence of the elevated serum free glycerol observed in nonfasted BHE rats: (1) an insufficient glycerol uptake resulting from competitive inhibition by intracellular α-glycerophosphate, or (2) a reversible movement of unphosphorylated glycerol from the hepatocyte into the blood stream. The presence of strain differences in the amount of α-glycerophosphate produced by the liver homogenates used in the experiment indicates that a potential for increased phosphorylation of free glycerol exists in the BHE rats. This increased enzyme activity does not eliminate this step as the possible source of the serum free glycerol, however, since the assay incubation medium used to estimate glycerokinase activity requires a high concentration of ATP. In an *in vivo* situation, reversible movement of glycerol could result from an insufficient supply of ATP within the hepatocyte. A recent study dealing with hepatic tissue cyclic-AMP levels in the BHE strain alluded to such an insufficiency (15). In this experiment, a rapid decrease in cyclic-AMP levels was noted when the animals were subjected to stress. It was suggested that this situation may have been the result of the animals' attempts to conserve ATP for energy rather than cyclic-AMP production.

The glycerophosphate that is derived from glycerol can be a direct precursor for gluconeogenesis or as a substrate for glyceride synthesis (14), a process that does not involve L-α-glycerophosphate dehydrogenase. L-α-Glycerophosphate dehydrogenase has recently been implicated in gluconeogenesis from glycerol (13, 14). Unless the enzyme was operating at maximum velocity, the lack of strain differences in this enzyme activity probably indicates that any excess α-glycerophosphate produced by the BHE rat is not being used for gluconeogenesis.

Summary. Serum free glycerol and hepatic glycerokinase and L-α-glycerophosphate dehydrogenase were measured in nonfasted, 75-day-old Wistar and carbohydrate-sensitive BHE rats. Serum free glycerol levels and glycerokinase activity were found to be elevated in the BHE strain. These results suggest that hepatic glycerol utilization by the BHE rat is being determined by factors other than the kinetics of the first glycerol-metabolizing step.

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