Lipogenesis and Enzyme Activity in Rat and Mouse Epididymal Adipose Tissue. (30984)

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Numerous reports have demonstrated enhanced hepatic lipogenesis to be accompanied by increased activities of NADP malic dehydrogenase (malic enzyme) and hexose monophosphate shunt enzymes, namely, glucose-6-phosphate dehydrogenase (G-6-PD) and 6-phosphogluconate dehydrogenase (6-PGD). These reports have been discussed extensively in several review articles(1-4). Similarly, increases in enzyme activity have been observed to accompany accelerated lipogenesis in rat adipose tissue(5,6).

Rat adipose and mammary tissue can readily utilize aspartic and glutamic acids for fatty acid synthesis(6-8), suggesting that the transaminase activity of these tissues is high. Puchol and Carballido(9) have demonstrated a high activity of glutamic oxalacetic (GOT) and glutamic pyruvic (GPT) transaminases in rat adipose tissue.

Unpublished observations from this laboratory have suggested that the rate of lipogenesis is greater in mouse adipose tissue than in comparable tissue from the rat. Consequently, it was of interest to evaluate the relationship between rate of lipogenesis and dehydrogenase activity in adipose tissue of these two species and also to determine the normal levels of GOT and GPT activity. This report presents the results of such an investigation.

Materials and methods. Young male animals fed Purina rat chow were used for all experiments. Tissues were obtained from Holtzman rats weighing 285-485 g and Carworth Farms mice (strain CB) weighing 25-30 g. Rats were sacrificed by decapitation and mice by cervical dislocation, and the epididymal fat pads were quickly removed.

For the study of lipogenesis, pieces of adipose tissue weighing approximately 100 mg were incubated in 3.0 ml of calcium-free Krebs-Ringer bicarbonate buffer, pH 7.4, containing 30 μmoles of glucose and 0.3 units of insulin.* Incubations were carried out for 3 hours in an atmosphere of 95% O_2-5% CO_2 at 38°C in a metabolic shaker (90 strokes/min). At the completion of the incubation, fatty acid ^{14}C was isolated and radioactivity determined as previously outlined(10).

Epididymal fat pads from single rats and pooled tissue from 3-4 mice were used for preparation of homogenates for enzyme assay. The tissue was homogenized in cold 0.15 M KCl and centrifuged at 1000 $\times$ g for 20 minutes to remove unbroken cells, debris and lipid. The clear intermediate layer was used for enzyme assay and nitrogen determination. G-6-PD and 6-PGD were measured separately by the method of Horecker and Smyrniotis(11). NAD and NADP malic dehydrogenases were assayed as described by Ochoa(12). Transaminase activity was determined by a modification of the method of Wroblewski and LaDue(13) in which each cuvette contained, in a final volume of 2.8 ml, the following in addition to enzyme (μmoles): phosphate buffer, pH 7.4, 200; NADH, 0.18; α-ketoglutarate, 20; and L-alanine, 200 (GPT) or L-aspartic acid, 100 (GOT). The reaction was started by addition of α-ketoglutarate and NADH was omitted from the blank cell. Homogenate volumes used for transaminase assay contained 30-80 (GOT) or 3-8 μgN (GPT). All enzyme activities are expressed as the μmoles of substrate utilized per minute per mg N at 30°C. Nitrogen was determined by micro-Kjeldahl digestion followed by Nesslerization(14).

The data were statistically evaluated by means of the "t" test.

Results. The higher rate of lipogenesis from acetate-1-^{14}C by adipose tissue from mice as compared to that of rats is shown in Table I. Tissue from mice incorporated 29% more acetate-1-^{14}C into fatty acids than did rat adi-

* The glucagon-free porcine insulin in these studies was a gift of Dr. W. Bromer, Eli Lilly Research Laboratories, Indianapolis, Ind.

TABLE I. Fatty Acid Synthesis and Enzyme Activities in Rat and Mouse Epididymal Adipose Tissue.

	Tissue source		
	Rat	Mouse	P*
Acetate-1-^{14}C incorporation into fatty acids: †	278 ± 58‡	390 ± 65	<.025
Enzyme activities§			
G-6-PD	.157 ± .041	.563 ± .135	<.001
6-PGD	.111 ± .028	.237 ± .078	<.005
NAD MD	22.64 ± 3.68	22.55 ± 5.42	ns
NADP MD	.119 ± .063	.311 ± .110	<.001
GPT	2.491 ± .783	1.374 ± .408	<.050
GOT	.491 ± .110	.970 ± .483	<.050
GPT/GOT	5.1 ± .9	1.7 ± .8	<.010

* Probability of significance.
† mμmoles acetate-1-^{14}C incorporated into fatty acids/100 mg adipose tissue/3 hr.
‡ Mean for 6 animals ± standard deviation.
§ μmoles substrate utilized/mg N/min. Values are means ± SD for the following number of determinations: glucose-6-phosphate dehydrogenase (G-6-PD), 6-phosphogluconate dehydrogenase (6-PGD), NAD malic dehydrogenase (NAD MD) and NADP malic dehydrogenase (NADP MD), 7 and 10 assays for rat and mouse tissue, respectively; glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT), 6 assays.

pose tissue. The level of dehydrogenase activity in adipose tissue of these 2 species is also shown in Table I. Mouse adipose tissue exhibited significantly higher G-6-PD, 6-PGD and NADP malic dehydrogenase activities, while the activity of NAD malic dehydrogenase was identical in mouse and rat tissues. Mouse adipose tissue had significantly greater GOT activity but less GPT activity than did rat tissue (Table I). As a consequence of these differences in transaminase activity, the ratio of GPT/GOT activities was significantly higher in adipose tissue from the rat than in tissues from the mouse.

Discussion. The activity of several enzymes has been shown to be inversely related to body size or positively correlated to metabolic rate. Thus, the hepatic activity of a number of oxidative enzymes(15-18) and glutamic-pyruvic transaminase(19) has been shown to be inversely correlated to body size. However, this does not imply that all enzymatic activity bears such a relationship to body size or metabolic rate; the activities of liver glutamic dehydrogenase, fumarase, NAD malic dehydrogenase and lactic dehydrogenase do not show such a relationship (20). It would appear, therefore, that only those enzymes essential to the maintenance of elevated levels of metabolic activity are inversely related to body size. Consequently, activities of G-6-PD, 6-PGD and malic enzyme observed in mouse adipose tissue are undoubtedly related to the higher rate of lipogenesis in the mouse as compared to the rat, rather than to the difference in body size *per se*.

Numerous reports have implied that HMP shunt activity regulates lipogenesis by generating NADPH for reductive lipogenesis(1,2). However, not all of the evidence is in agreement with this concept, and considerable evidence is accumulating which would argue against such a control mechanism(3). A more plausible explanation may be found in the need to maintain the known excess of NADPH over NADP and of NAD over NADH in the cytoplasm(21). Schemes involving malic dehydrogenase and malic enzyme in a transhydrogenation from NADH to NADP have recently been proposed(5,6,22). Such a pathway of metabolism can explain the increased malic enzyme activity in enhanced lipogenesis and provides a means of maintaining cytoplasmic reduced to oxidized ratios of both NAD and NADP. The higher activities of HMP shunt enzymes and malic enzyme in mouse adipose tissue as compared to rat tissue would therefore be anticipated in view of the higher rate of fatty acid synthesis observed in mouse tissue. The lack of a difference in malic dehydrogenase activity be-

tween these two species is also not surprising in view of the very high activity of this enzyme.

The differences in transaminase activities observed in rat and mouse tissue are not readily explainable. Cornelius(19) has reported that in 21 species the hepatic GPT activity was inversely related to body size; however, the species studied did not include either the rat or mouse. Nevertheless, one might have anticipated that the activity of GPT would be higher in mouse as compared to rat adipose tissue; however, the reverse was true although the GOT activity was higher in mouse tissue. Puchol and Carballido(9) studied GOT and GPT activities in rat adipose tissue. The ratio of GOT/GPT activities reported by these workers is in fair agreement with that observed for rat adipose tissue in this study when the differences in breed, animal size and methodology are considered.

It is of interest to note the report of Waldorf *et al*(23) demonstrating that rat hepatic GOT and GPT activity can be increased by feeding a high protein diet. The adaptability of these adipose tissue enzymes to dietary manipulation is being investigated.

Summary. The ability of rat and mouse epididymal adipose tissue to incorporate acetate-1-^{14}C into fatty acids was studied. The activity of glucose-6-phosphate dehydrogenase (G-6-PD), 6-phosphogluconate dehydrogenase (6-PGD), NADP malic dehydrogenase (malic enzyme), NAD malic dehydrogenase, glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) in these tissues was also determined. Mouse adipose tissue incorporated significantly more acetate-1-^{14}C into fatty acids and exhibited higher G-6-PD, 6-PGD and malic enzyme activity than comparable tissue from rats. The malic dehydrogenase activity of rat and mouse adipose tissue was not different but was considerably higher than the activity of the other dehydrogenases studied. The activity of GPT was higher in rat adipose tissue, and that of GOT was higher in tissue from mice. The GPT/GOT ratio was higher in rat than in mouse adipose tissue (5.1 *vs* 1.7).

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