Adipose and Liver Tissue Enzyme Profiles in Obese Hyperglycemic Mice[1] (36997)

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Lipid metabolism of the genetic obese mouse (obob) has been extensively investigated over the past 20 yr by a number of investigators. In general, their studies indicate that lipogenesis is enhanced in both liver (1) and adipose tissue (2, 3) and that the enzymes associated with hyperlipogenic states are also elevated (4, 5). One of the difficulties in interpreting data from studies of genetic obesities is the fact that hyperphagia usually accompanies these syndromes (6). Hyperphagia in the normal animal will cause obesity and influence lipid metabolism (7). Therefore, it is important to determine the tissue enzyme pattern in the genetic strain of obese mice in the absence of hyperphagia, since it has been shown through pair feeding experiments that hyperphagia is not essential for excess deposition of lipid in the obob mouse (8). It has also been shown that the obob mice are less active than their lean littermates (9). This could complicate the enzyme profile since Mole and Holloszy (10) have found that physical activity can influence tissue lipid metabolism.

The objective of this study was to determine the enzyme profile of liver and adipose tissue from obese mice whose body weight gain was controlled by regulating food intake and physical activity.

Methods. Obese male mice (C57Bl/6J-ob, Bar Harbor) and lean male mice were purchased and placed on experiment at approximately 5 wk of age. These obese mice were divided into two groups of six. One group (obese) received laboratory chow diet, *ad libitum*; the other group (obese) (control) was fed so that they gained weight at the same rate as the lean mice. The obese control group was also exercised by swimming to exhaustion. To prevent the obese control mice from consuming their chow in meals, the chow pellets were placed on the wire lid of the cages so that the mice had to eat the pellets slowly. It usually took 18 hr for mice to complete their feeding. After 4 wk on experiment, the mice were sacrificed by decapitation and the liver and adipose tissue were removed quickly. The liver and adipose tissues were homogenized (1:5, w/v) in 0.25 *M* sucrose containing 1 m*M* dithiothreitol with a motor driven Teflon–glass Potter Elvejhem apparatus. The homogenates were centrifuged at 27,000*g* for 20 min and the resulting supernatants were used for enzyme analysis. Liver homogenates used for assay of glucose-6-phosphatase (G6Pase) were prepared in 0.1 *M* citrate buffer, pH 6.5. The assay of G6Pase (EC 3.1.3.9.) was performed on the liver homogenates by measuring the release of phosphate from glucose-6-phosphate as described by Freedland and Harper (11). The following assays were determined on a Gilford 2400 recording spectrophotometer at 25°: hexokinase (EC 2.7.1.1.) by the method of Katzen (12); glucose 6-PO_4 dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) by the method of Glock and McLean (13); malic enzyme (EC 1.1.1.40) by the procedure of Ochoa (14); α-glycerol-PO_4 dehydrogenase (EC 1.1.1.8)

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TABLE I. Effects of Dietary Restriction and Exercise on Body Weight of Obese Mice.

Expt Group	No. of mice	Treatment	Body wt (gm)
Lean	10	ad lib. fed	23.1 ± 0.9[a]
Obese	6	ad lib. fed	35.8 ± 1.8[b]
Obese control	6	Restricted intake and exercised	22.8 ± 0.5[a]

[ab] Means ± standard error in column with different superscript are different, $p < .01$.

as described by Fitch and Chaikoff (15); isocitrate dehydrogenase (EC 1.1.1.42) and malate dehydrogenase (EC 1.1.1.37) by the methods of Baldwin and Milligan (16); aspartate aminotransferase (EC 2.6.1.10) by the method of Roswell (17); alanine aminotransferase (EC 2.6.1.2) by the procedure of Segal and Matsuzawa (18); serine dehydratase (EC 4.2.1.15) as described by Freedland and Harper (11). All enzyme assays were linear with time and enzyme concentration. Adipose tissue enzyme activities were expressed as nanomoles of product formed per milligram of protein. Protein was determined by the method of Lowry *et al.* (19). Liver tissue enzymes were expressed as micromoles of product formed per 25 g body weight. Liver DNA values were determined by the procedure of Cerreotti (20).

Results. Body weights of mice 9 wk of age are presented in Table I. By dietary restriction and exercise, body weight gain of the obese mice was successfully maintained with that of the lean mice. Observation of the fat deposits of the obese control group revealed that these mice were still fatter than the lean mice even though weight gain was regulated. Adipose tissue enzyme levels are presented in Table II. Enzymes involved in the uptake and conversion of glucose to lipid (hexokinase, glucose-6-PO_4 dehydrogenase, 6-phosphogluconate dehydrogenase, and α-glycerol-PO_4 dehydrogenase) were elevated in adipose tissue extracts from obese mice when compared to lean mice. There were no significant differences in these same enzyme levels between *ad libitum* fed obese mice and restricted obese control mice. Isocitrate dehydrogenase, malic enzyme, and malate dehydrogenase were not significantly different in the three experimental groups. Soluble protein concentration was significantly higher in adipose tissue of lean mice, indicating greater cellular hypertrophy in both groups of obese mice (Table II).

Liver tissue enzyme levels are presented in Table III. Again, increases in the enzymes involved in glucose uptake and conversion to lipid (hexokinase, malic enzyme, α-glycerol-PO_4 dehydrogenase) were observed in the obese mice. However, these enzyme levels were not elevated in the obese control group indicating that the adaptive responses observed in these liver enzymes were probably the result of hyperphagia or inactivity. One exception to these general patterns is the similarity of liver glucose-6-PO_4 dehydrogenase levels in all three experimental groups.

TABLE II. Effects of Dietary Restriction and Exercise on Adipose Tissue Enzyme Levels in Obese Mice.

Enzyme	Expt groups (nmoles/min/mg protein)		
	Lean	Obese	Obese control
Hexokinase	55.6 ± 3.2[a]	71.5 ± 6.9[b]	73.7 ± 5.3[b]
Glucose-6-PO_4 dehydrogenase	95.8 ± 8.0[a]	286.2 ± 31.1[b]	309.1 ± 16.5[b]
6-P-gluconate dehydrogenase	45.1 ± 4.6[a]	120.2 ± 9.8[b]	118.1 ± 8.7[b]
Malic enzyme	107.2 ± 10.9[a]	91.0 ± 30.1[a]	111.2 ± 12.0[a]
α-Glycerol-P-dehydrogenase	393.1 ± 20.9[a]	944 ± 24.2[b]	883 ± 34.2[b]
Isocitrate dehydrogenase	99.5 ± 8.2[a]	132.5 ± 11.0[a]	110 ± 12.1[a]
Malate dehydrogenase	443.4 ± 51.3[a]	659.8 ± 49.8[a]	542.1 ± 67.4[a]
		(mg/g tissue)	
Soluble protein	12.1 ± 1.5[a]	3.2 ± 0.3[b]	4.4 ± 0.4[b]

[ab] Means ± standard error in horizontal lines with different superscripts are different $p < .01$.

TABLE III. Effect of Dietary Restriction and Exercise on Liver Enzyme Levels in Obese Mice.

Enzymes	Expt groups (μmoles/min/25 g body wt)		
	Lean	Obese	Obese control
Hexokinase	0.89 ± 0.06[a]	2.22 ± 0.15[b]	1.18 ± 0.08[a]
Glucose-6-P dehydrogenase	0.25 ± 0.03	0.26 ± 0.02	0.29 ± 0.05
6-P-gluconate dehydrogenase	0.50 ± 0.04[a]	1.41 ± 0.19[b]	0.61 ± 0.11[a]
Malic enzyme	1.25 ± 0.21[a]	8.67 ± 1.26[b]	2.13 ± 0.40[a]
α-glycerol-P-dehydrogenase	8.0 ± 1.2[a]	17.2 ± 1.3[b]	10.0 ± 1.0[a]
Isocitrate dehydrogenase	10.4 ± 1.1[a]	24.1 ± 1.7[b]	22.5 ± 1.2[b]
Glucose-6-Pase	22.0 ± 0.71[a]	41.4 ± 3.7[b]	27.9 ± 1.8[a]
Serine dehydratase	0.44 ± 0.02[a]	0.86 ± 0.07[b]	0.49 ± 0.05[a]
Aspartate aminotransferase	30.6 ± 2.4[a]	37.8 ± 2.1[b]	23.9 ± 3.2[a]
Alanine aminotransferase	14.4 ± 1.2[a]	23.9 ± 3.2[b]	16.9 ± 0.8[a]
DNA (mg/g tissue)	5.5 ± 0.4	4.8 ± 0.6	4.8 ± 0.4

[ab] Means ± standard error in horizontal lines with different superscripts are different $p < .01$.

Levels of enzymes normally associated with increased rates of gluconeogenesis and amino acid catabolism (G6Pase, SDH, GOT and GPT) were higher in the livers of *ad libitum* fed obese mice (Table III). However, these enzyme levels were essentially the same in the obese control and lean mice, indicating that the shift in the liver gluconeogenic enzyme profile observed in *ad libitum* fed obese mice was primarily caused by either hyperphagia or inactivity. Liver DNA concentrations in the three groups of mice were essentially the same (Table III).

Discussion. Tissue enzyme levels can be influenced by age (21), hormonal status (22), exercise (23), diet composition (24) and dietary intake pattern (25). Therefore, when attempting to determine specific shifts in enzyme profiles due to a genetic lesion, as many of these variables as possible should be controlled. This study has shown that some changes in liver enzyme profiles observed in obob mice fed *ad libitum* were due to the higher levels of dietary intake and/or lower levels of physical activity. Adipose tissue lipogenic enzyme levels were elevated in both groups of obese mice and were not apparently influenced by dietary intake and/or physical activity. The levels of obob mouse adipose tissue G6PD and 6PGD have been shown to be elevated and fasting does not depress the levels of these enzymes as it does in lean littermates (26). On the other hand, levels of liver tissue G6PD and 6PGD have been found to be depressed when subjected to a 24-hr fast in both obob mice and their lean controls (27). Hepatic citrate cleavage enzyme levels are also reduced by fasting in obese and lean mice (28). It appears that the liver tissue enzyme profile follows dietary intake more closely than does the same enzyme profile of adipose tissue from the obob mouse.

Mayer (29) was the first to observe the refractory nature of obob mouse adipose tissue to dietary restriction. Rates of both lipogenesis (2) and lipolysis (30) were reported to be resistant to change during fasting of obob mice. This apparent abnormality could be related to the elevated levels of insulin found in both fed and fasted obob mice (31). The altered adipose tissue enzyme profiles of obob mice are apparently a secondary phenomena related to the extremely high levels of insulin found in obese mice. Two types of evidence support this theory. By transplanting adipose tissue from lean mice into obese mice and vice versa, it was found that the transplanted adipose tissue takes on the characteristics of the host animal (32). Strautz (33) has shown that by transplanting pancreatic islets from normal mice into obob mice, the hyperglycemia, hyperinsulinemia and excessive weight gain is reduced to normal levels. In the latter study, the adipose mass did not continue to expand after insulin levels were reduced to normal.

Another metabolic abnormality observed in obese rodents is the decrease in the synthesis

of muscle protein (6). This may be the result of increased utilization of amino acids for glucose synthesis in the liver and the subsequent conversion of glucose to lipid by adipose tissue. We have found that hepatic enzymes associated with amino acid catabolism and gluconeogenesis were elevated in obob mice when fed *ad libitum*. Other investigators have demonstrated elevated levels of gluconeogenic enzymes in the obob mice (34, 35). Herberg *et al.* (37) found that gluconeogenesis in the obob mouse was greatest during the development stage of the obese condition, and tended to decrease as the mice reached maximum body weight. Willms, Ben-Ami and Soling (36) did not detect any differences in G6Pase of FDPase in livers of NZO and obob mice after maximum weight had been achieved. In pair-feeding experiments, glucose production in isolated perfused livers of obese mice was similar to lean control (38). Furthermore, Seidmann, Horland and Teebor (35) reported that G6Pase and FDPase were elevated in both obob mice and gold thioglucose treated hyperphagic mice. Our studies and those of Seidmann, Horland and Teebor (35) indicate that the shift in the gluconeogenic enzyme profile of obese mice was primarily related to excessive dietary intake and not directly associated with a primary genetic lesion.

Summary. In order to determine metabolic abnormalities associated with obesity in the obese hyperglycemic mouse, enzyme levels in adipose and liver tissue were measured in three groups of mice. One lean group and one obese group were fed *ad libitum*. The third group consisted of obese mice subjected to weight gain control by dietary restriction and exercise. Levels of adipose tissue enzymes associated with lipogenesis were higher in obese mice than the lean mice. The data also indicated that obob mouse adipose tissue enzymes were not appreciably affected by diet restriction and exercise. However, liver tissue enzymes associated with lipogenesis and gluconeogenesis, which are normally elevated in obese mice, were restored to normal levels when the obese mice were on a weight gain control schedule. These data indicate that some differences in enzyme profiles observed in a genetically obese mouse are secondary adaptations caused by changes in either dietary intake or spontaneous activity.

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