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ADAPTIVE ENZYME RESPONSES IN LIVER AND ADIPOSE TISSUE OF OBESE HYPERGLYCEMIC MICE

by

Murray L. Kaplan

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1972

This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

ADAPTIVE ENZYME RESPONSES IN LIVER AND
ADIPOSE TISSUE OF OBESE
HYPERGLYCEMIC MICE

by

Murray L. Kaplan

Adviser: Professor George H. Fried

Effects of fasting-refeeding regimens were studied in genetically obese hyperglycemic mice and their thin littermates to ascertain the possible existence of a differential response. Parameters studied were body weight, liver and epididymal fat pad weights, glycogen content, and activities of glucose-6-phosphate dehydrogenase, malic enzyme, alpha-glycerophosphate dehydrogenase, glycerol kinase, lactic dehydrogenase, and glucose-6-phosphatase. Animals were killed after a 48 hr fast and after a 48 hr fast followed by 24, 48, and 72 hr of refeeding with laboratory pellets plus 15% glucose in the drinking water; and 72 hr refeeding with laboratory pellets plus 15% glycerol in the drinking water. In addition, obese mice were fasted 96 hr followed by 144 hr refeeding.

Obese mice lost relatively less weight on fasting and gained at a slower rate than the thins on refeeding. The hepatosomatic index was higher in the obese and was sharply reduced in both thin and obese mice with fasting. Only the thin mice showed a slight increase in hepatosomatic index after refeeding.

In adipose tissue of fasted-refed thin mice, activities of glucose-6-phosphate dehydrogenase, malic enzyme, alpha-glycerophosphate dehydrogenase, lactic dehydrogenase, and also glycogen content were increased over control values. In obese mice, neither significant changes in the activities of these enzymes nor glycogen content were observed. In alloxan-treated thin mice, adipose tissue glucose-6-phosphate dehydrogenase activity was decreased, while in identically-treated obese animals, only alpha-glycerophosphate dehydrogenase activity was increased.

In the liver of fasted-refed thin mice, activities of glucose-6-phosphate dehydrogenase, malic enzyme, alpha-glycerophosphate dehydrogenase, and glycerol kinase were increased over control values. In identically-treated obese animals, large increases were seen only in the activities of alpha-glycerophosphate dehydrogenase and glycerol kinase. In alloxan-treated thin mice, large changes in enzyme activity were not observed. Alloxan-treated obese mice showed a decrease in glucose-6-phosphate dehydrogenase and an increase in alpha-glycerophosphate dehydrogenase activities.

These data indicate a differential response to fasting-refeeding regimens in the obese mouse and are consistent with the major lipogenic role assigned to the liver in obesity. These data also support the concept of an impaired "adaptive hyperlipogenic" response in the obese hyperglycemic mouse.

DEDICATION

This is dedicated to the ones I love, my dearest wife, Helene Joyce, and my two daughters, Alissa Deborah and Cheryl Lynn. They have been an almost constant source of joy, making possible my survival in the not always so joyous world of graduate student life. Without them, I surely would never have accomplished anything.

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INTRODUCTION

Obesity is now recognized as a major health hazard in the United States (14, 53, 63, 76). It can be considered a state of malnutrition (63) marked by an excessive accumulation of adipose tissue. It leads to a variety of physiological as well as psychological and social aberrations among afflicted individuals. Among the physiological deviations are atherosclerosis, arthritis, coronary heart disease, cholecystitis, hypertension, diabetes mellitus and renal disorders (14, 63).

Predisposition to obesity may have a genetic basis in man. A close correlation between obesity and an endomorphic somatotype during adolescence, together with evidence from twin studies favor this concept (76). But cultural differences may affect genetic expression of obesity. The inappropriateness of the usual tools of the geneticist, such as parent-offspring and sibling-sibling matings, in human genetic studies mandates the use of appropriate animal models.

These animal models have been classified by Mayer (76) into regulatory and metabolic obesities. The regulatory obesities include syndromes which generally affect appetite control, e.g., goldthioglucose obese mice and

stereotaxic hypothalamic lesioned rats. Examples of metabolic obesities include syndromes in which there are probably some primary metabolic defects, such as yellow obesity, New Zealand obesity, and the obese hyperglycemic syndrome. This simple classification may no longer be valid in that more recent work has demonstrated that both primary metabolic defects and problems in regulation are encountered in most animal models.

The genetically obese hyperglycemic mouse (C57BL/6J ob ob) has been most extensively characterized. This obese syndrome segregates as a simple Mendelian autosomal recessive trait (49). In addition to the obesity and hyperglycemia, other major differences between obese and thin mice have been found. Paradoxically, hyperinsulinemia coexists with the hyperglycemia and the animals are also resistant to exogenous insulin as measured by blood glucose responses (10, 37, 38, 39, 103, 104, 105, 125). Specifically, adipose tissue and skeletal muscle appear to be the resistant tissued (20, 23, 64, 103, 105). The liver, pancreatic islets, and the adrenal cortex are enlarged (46) and obese animals have a high rate of sterility (44). Total oxygen consumption of young adult obese mice is approximately one-half that of their thin litter mates (33) which is consistent with their diminished voluntary activity (49). Concomitant differences in carbohydrate

and lipid metabolism exist, as evidenced by dissimilarity between obese and thin hepatic and adipose tissue enzyme activity profiles, incorporation of various substrates into lipid components, and rates of lipogenesis and lipolysis.

Although the rates of lipogenesis in adipose tissue of obese mice are higher than those in thin mice, most of the *de novo* synthesized fatty acids are probably made in the liver and subsequently transported to adipose tissue storage sites (25, 50, 97, 98). *In vitro* incorporation of C^{14}-glucose into hepatic lipid is higher in the obese (45), while incorporation into adipose tissue lipid is lower in the obese than in the thins (23, 50, 64, 97). However, *in vitro* incorporation of C^{14}-acetate into lipid is increased considerably over values for the thins in both tissues (24, 25, 126). Apparently, some block to glucose entry may exist in the adipose cell of the obese mouse. Insulin does not augment incorporation of glucose into the obese fat cell (64), which differs markedly from the situation in normal rodents. These results are difficult to reconcile and suggest closer examination of the metabolic profile in the obese state.

Winand *et al*. (126) indicate that obese liver has an increased capacity to synthesize long-chain monounsaturated fatty acids. *In vitro* experiments, measuring the incorporation of C^{14}-acetate, C^{14}-palmitate and

C^{14}-stearate, indicate that the pools of non-esterified fatty acids in both liver and adipose tissue are heterogeneous (25, 126). In the liver two pools presumably exist, one from lipolysis and the second from *de novo* synthesis (25). In epididymal adipose tissue three pools of non-esterified fatty acids may exist: one from *de novo* synthesis, a second from lipolysis of depot fat, and the third, by far the largest pool, that which is taken up from the extracellular media (25) (presumably derived from the liver). The hyperinsulinism of the obese mouse has more dramatic effects on hepatic than on epididymal adipose tissue lipogenesis.

In vivo studies of Jansen *et al*. (50) confirmed the conclusions of others (25, 50, 97, 98, 126) that the liver may play the major role in lipogenesis in the obese state. The incorporation of C^{14}-glucose into fatty acids was much greater in livers of the obese than in those of the thins. In contrast, incorporation of a single dose of C^{14}-glucose into fatty acids by extrahepatic tissues (everything remaining following removal of the liver) of obese mice was one-half that of thin litter mates. Shigeta and Shreeve (93) agree that rates of glucose incorporation into total hepatic fatty acids are at least ten times higher in the obese than in the thins. Unlike Jansen *et al*. (50) they found two to four times as much glucose

incorporation into total fatty acids of the extrahepatic tissues of the obese as that of thin siblings. Despite the emphasis assigned to the liver in obesity by both groups of investigators, the adipose tissue is not simply relegated to the role of a lipid storage depot.

The impaired glucose utilization (23, 104) and decreased incorporation of glucose into glyceride-glycerol, glycogen and fatty acids (23, 64) of obese adipose tissue are difficult to reconcile with an increased synthesis of fatty acids from acetate (24). Clearly, the pentose shunt is not providing the reducing power required for fatty acid synthesis as indicated by the poor utilization of glucose-1-H^3 by obese adipose tissue (93). Although Katz *et al*. (52) have indicated that the pentose cycle provides only 59 to 88% of the required reducing power for fatty acid synthesis, few workers have attempted to measure relative contributions of the pentose pathway and the malic enzyme system in altered metabolic states, such as obesity. Lamdin *et al*. (60) found a three-fold increase in the incorporation of malate-2-H^3 into fatty acids of the obese mice as compared to thins. This tends to lend credibility to the idea of Leveille and Hanson (65) and Ball (6) that malic enzyme and malate dehydrogenase function in a trans-hydrogenation system in adipose tissue. Thus, where glucose utilization is impaired, sources of reducing power other than glucose are available in adipose tissue.

Even if one concedes that the adipose tissue of obese mice may not be highly lipogenic but functions primarily as a depot for fatty acids that are synthesized in the liver (25, 126), a problem exists in terms of the source of the alpha-glycerophosphate required for esterification (55, 117). The impaired incorporation of glucose into glyceride-glycerol in obese adipose tissue (23, 64) raises the possibility of an alternate source of alpha-glycerophosphate. For many years, it was generally believed that white adipose tissue lacked a glycerol kinase and therefore glycolysis was the only source of alpha-glycerophosphate. Recently, a low but detectable level of activity of glycerol kinase was found in white adipose tissue (68, 84, 116). The levels in white adipose tissue of the obese mice are twice as high as those in their thin litter mates and may provide a mechanism for generation of some of the required alpha-glycerophosphate (116).

Another three carbon fragment, pyruvate, has been shown to be a precursor of glyceride-glycerol in rat adipose tissue (17, 83). This occurs through reversal of glycolysis via an abbreviated dicarboxylic acid shuttle involving pyruvate carboxylase and phosphoenol pyruvate carboxykinase. In obese mice, however, glyceride-glycerol synthesis from pyruvate is decreased (17, 24) which places

more importance on the elevated glycerol kinase pathway. The relative roles of the three alternate pathways for generation of alpha-glycerophosphate (glycolysis, glyceroneogenesis, and direct phosphorylation of free glycerol) have not been completely established in the obese state.

Alterations in lipolysis, such as decreased basal rates, reduced lipase activity (68, 106), and selective mobilization of palmitate rather than linoleic (42, 106) from depot fat may be another contributing factor to obesity; i.e., there is a decrease in the rate of lipid turnover by adipose tissue of obese animals (64, 108). The epididymal adipose tissue of these animals show a diminished lipolytic response to several hormones. Epinephrine does not increase the release of free fatty acids as it does in normal mice (64). Recently, in an extensive study, Steinmetz *et al.* (108) demonstrated that not only are the basal levels of lipolysis lower than normal but this tissue fails to respond to such lipolytic agents as epinephrine, norepinephrine, isoproterenol, corticotropin, theophylline, and N^6, O^2-dibutyrylcyclic adenosine-3', 5'-monophosphate. It has also been shown that the membrane of the normal adipose cell has a single catalytic unit of adenyl cyclase which is coupled to several independent hormone receptor sites (9). Since the actions of all these hormones are presumably mediated

through the adenyl cyclase system (109), the defect in lipolysis in the obese state probably involves the adenyl cyclase system.

Concomitant with increased rates of hepatic lipogenesis in obese mice, the activities of several hepatic enzymes are increased. These include phosphorylase (99), alpha-glycerophosphate dehydrogenase (34), glucose-6-phosphate dehydrogenase (34), glucose-6-phosphatase (88), glucokinase (88), fructose-1, 6-diphosphatase (88), pyruvic kinase (88), citrate lyase (102), acetyl-CoA carboxylase (18), and fatty acid synthetase (18). In general, activities of those hepatic enzymes which push in the direction of fatty acid and triglyceride synthesis are elevated, which is consistent with the major role assigned to the liver for total lipogenesis (25, 50, 93). The activities of enzymes of both the glycolytic and gluconeogenic pathways appear to be elevated, differing from the more usual findings of a reciprocal relationship between these pathways (120). Increased levels of glucose-6-phosphatase coexistent with increased glycogen levels support the concept of a simultaneous enhancement of both gluconeogenesis and glycolysis in obese mice. The common assumption that glycolysis and gluconeogenesis are mutually exclusive may be erroneous.

Distinctive adipose tissue enzyme activities in the obese mouse include increased levels of glucose-6-phosphate

dehydrogenase (34), glycerol kinase (68, 116), and pyruvate carboxylase (17). Cytoplasmic NAD dependent alpha-glycerophosphate dehydrogenase has not been found to be elevated in obese adipose tissue (34). Unlike some cases of human obesity (36) mitochondrial alpha-glycerophosphate dehydrogenase activity is not lower in the obese mouse (34). Levels of triglyceride lipase (68), monoglyceride lipase (106), and phospho-enolpyruvate carboxykinase (17) are decreased. These data are consistent with lower levels of lipolysis (42, 68, 106), increased rates of glycerol uptake (68, 116), and diminished glucose uptake (23, 64) in the adipose tissue of these animals. It is difficult, however, to reconcile the high levels of glucose-6-phosphate dehydrogenase in obese adipose tissue with the decreased glucose utilization (23, 64).

Many of the preceding differences can be reversed by dietary restriction, as summarized in part by Hellman (46, 47). When food intake was restricted to maintain normal body weights in the obese, liver (81), pancreatic islets (46, 81), and adrenal cortex (46) attained a normal appearance and weight and the hyperglycemia disappeared (46). Some obese males became fertile when maintained on a restricted diet (61), suggesting that hyperphagia and obesity may contribute to reduction of sexual function. But histological examination showed smaller than normal

Leydig cells (47), indicating that obesity per se may not provide the total explanation of sterility in this strain.

Maintenance of obese mice in the 30-40 gram weight range by restricted feeding also increased their longevity from 457 days in fed ad libitum mice to 795 days (62), demonstrating that overeating per se probably leads to a reduction of the life span. When body weight was not kept constant but food consumption restricted in the obese so as to be identical to that of fed ad libitum thins, the obese gained weight and increased their adipose tissue stores (21) despite the absence of excessive food intake. Obesity without hyperphagia has also been reported in hypothalamic lesioned rats (44), indicating that additional factors other than hyperphagia may contribute to development of obesity.

The hypothalamus is known to be involved in appetite control. Destruction of the ventromedial nuclei of the hypothalamus, either by electrolytic lesions or treatment with goldthioglucose, leads to hyperphagia (13). The opposite effect, aphagia, can be induced by bilateral destruction of the lateral hypothalamic areas. These lateral areas are viewed as "feeding centers" which can be inhibited by the ventromedial area. The ventromedial nuclei are beleived to function as satiety centers and are sensitive to glucose. Therefore, destruction of these

nuclei would represent a removal of inhibition on the "feeding centers." Since adrenergic stimulation of extrahypothalamic areas of the rat brain, such as the amygdala (100) and selected areas of the diencephalon (11) also induce eating behavior, the hypothalamus is probably not the only center for regulation of appetite. The ventromedial hypothalamic nuclei of obese mice appear to be relatively insensitive to insulin and resistant to destruction by goldthioglucose (5), perhaps indicating a defect in a feedback component of the hypothalamic appetite control system. One may assume that an insensitive hypothalamus in the obese mouse is only one contributing factor to the obesity, and may even be secondary to its development.

Considerations of the etiology of obesity have led to attempts to differentiate metabolic characteristics secondary to the development of obesity from those presumably causal factors that precede it. For approximately the first 23-26 days of life, no gross differences are noticed between obese and thin mice, including body weight (125). The occurrence of hyperglycemia, hyperinsulinemia and insulin resistance were first noted to coincide with the period of rapid weight gain (104, 105, 125).

Genuth _et al_. (38) followed plasma insulin and glucose levels in these mice and later noted which became

obese. From 13 to 24 days of age plasma insulin levels of future obese mice were indistinguishable from those of future thins. Increased plasma insulin levels in the obese group were first observed at 26 to 32 days of age. Hyperglycemia was absent at six weeks but present at nine weeks of age. By six weeks of age, insulin resistance, as measured by absence of insulin-stimulated incorporation of 2-deoxyglucose into diaphragm muscle, in vitro, was well developed. Genuth also suggested that insulin resistance by diaphragm muscle was absent in two week old future obese mice (38). He felt that the marked hyperinsulinemia of the obese groups of every age suggested a primary pancreatic defect (37, 38). He did not identify the future obese animals by any type of diagnostic test, such as decreased O_2 consumption (32) or glucosuria following intraperitoneal glucose injections (27, 47). Among a group of 36 mice, approximately two weeks old, he assumed that 25% or nine of these were future obese. Since he was dealing with small groups there is no assurance that any of these animals were genetically obese. Therefore, occurrence of insulin resistance at a very early age cannot be excluded.

Other evidence suggests that insulin resistance is present in genetically obese mice prior to the obesity. Administration of insulin to future obese at 27 days of age resulted in a slower decrease in blood glucose levels

than an equivalent dose in future thin mice. The future obese animals had the ability to resist insulin-induced convulsions and demonstrated glucosuria after an intraperitoneal glucose load (27), which may be a method for early detection (125). Epididymal adipose tissue homogenates of the obese displayed an eight-fold higher insulin degrading activity than homogenates from thin animals (124). Although this latter study was performed in five month old mice, the insulin resistance may be related to elevated insulin degrading activity.

Stauffacher and co-workers detected a decreased peripheral responsiveness to insulin at 4-5 weeks of age (103, 104, 105). By comparison, the diaphragm muscle was found to be more resistant to insulin than the adipose tissue in the younger genetically obese mice (103, 105). Tissues from goldthioglucose-induced obese mice did not show any resistance to insulin. Since the increased circulatory levels of insulin are unbound (39) in the genetically obese mice, Stauffacher's group postulated that the initial lesion is a muscular insulin resistance. This is followed by compensatory increased circulating insulin levels with increased lipogenesis by adipose tissue. Eventually an increased cell size of adipocytes results, which in turn leads to a further increase in circulating insulin. Large adipocytes are known to be more resistant,

in vitro, to insulin than smaller adipocytes (86). Consequently, increased lipid causes an increase in cell size and renders the tissue relatively more resistant to insulin. It is suggested that the increased peripheral demand for insulin synthesis is dependent upon an initial insulin resistance by muscle. This concept is consistent with the increased insulin tolerance in the pre-obese stage of these animals (27, 47, 125). A primary muscular lesion is also compatible with diminished voluntary activity (49) and a lower oxygen consumption at this stage (32).

An alternate hypothesis as to the sequence of events leading to the manifestation of the obese-hyperglycemic syndrome is that increased adiposity leads to insulin resistance and hyperglycemia. At three weeks of age, prior to detection of obesity by weight, offspring from heterozygote breeding pairs were killed and lipid to body weight ratios were determined. Two distinct groups were detected. One group, presumably obese, showed 9.6% body fat compared to 3.0% body fat for the other group (22). Neither blood glucose nor insulin levels were significantly different. This suggests that adiposity may precede the occurrence of significant hyperinsulinemia and insulin resistance. Consistent with this hypothesis is the finding that muscular and adipose tissue insulin resistance can be reversed following body weight reduction of obese mice on restricted

feeding schedules (10, 20). Further work is required to determine the sequence of events prior to pathological increases in body weight to ascertain the primary metabolic event in the development of obesity.

The occurrence of pancreatic enlargement, hyperinsulinemia, and hyperglycemia noted in this obesity mandates a closer examination of the pancreas. The enlargement can be reversed to normal weight, size, and appearance following weight reduction (46, 81). Degranulation of beta cells (127), consistent with increased secretion, accompanies the hyperplasia of the obese state. Reduction of caloric intake results in lower blood sugar levels (46), regranulation of beta cells, and normal blood insulin levels (46, 81).

Alloxan treatment lowers pancreatic insulin synthesis and secretion in normal animals (59, 78, 101). Attempts to lower insulin levels in obese mice by alloxan treatment have produced unexpected results. While alloxan-treated thins had elevated blood glucose levels and degranulation of the beta cells, similarly treated obese had decreased blood glucose levels and increased beta cell granulation (101). In an attempt to reveal the mechanism of this paradoxical finding, Kuftinec and Mayer (59) investigated the effects of caffeine which is structurally related to alloxan. Caffeine caused an effect opposite to that of alloxan in the obese, a prolonged elevation of

blood glucose levels and even more degranulation of the pancreatic beta cells than in untreated obese mice. There was no enhancement of sensitivity to insulin after caffeine treatment. Apparently, the effects of alloxan and caffeine are antagonistic (78) perhaps competing for the same receptor site on the beta cell.

At present, peculiar effects of these compounds in the obese mice cannot be explained. Nor have any investigations been conducted into their effects on adipose tissue or liver glycolytic enzyme activities in obese animals, some of which are known to be affected by alloxan treatment in normal rodents (82).

Blood insulin levels were not monitored during these experiments, but such information would certainly help in an interpretation of these phenomena. At present the mechanisms by which the pancreas affects obesity remains very poorly understood.

The exocrine pancreas has been examined from an enzymatic perspective. For a variety of reasons, Khayat *et al*. (56) suspected that the exocrine pancreas would show abnormalities. Insulin-induced hypoglycemia provokes a vagal excitation of the pancreas and a hypersecretion of hydrolases in normal man (35), in the dog (115), and the rat (1). But in human cases of diabetic plethora (syndrome of hyperinsulinism, hyperglycemia, and obesity)

the exocrine pancreas appears normal (19). The activities of lipase, amylase, chymotrypsinogen, typsinogen and ribonuclease were the same in both obese and thin mice. Therefore, whatever pancreatic pathology exists is probably limited to the endocrine pancreas, similar to the situation in human diabetic obesity.

It has been demonstrated that activities of hepatic and adipose tissue enzymes involved in fatty acid synthesis are increased under physiological conditions favoring lipogenesis and decreased under non-lipogenic conditions (4, 40). Parallel alterations in the activities of enzymes involved in generating the glyceride-glycerol moiety of lipids would also appear probable, since alpha-glycerophosphate (55) and dihydroxyacetone phosphate (43) have been established as alternative obligatory intermediates in the formation of phosphatides and glycerides. Work in this area, however, has been rather limited. The Teppermans (113) indicated that activities of rat liver alpha-glycerophosphate dehydrogenase decrease on fasting and increase after refeeding following a fast. They imply that alpha-glycerophosphate accumulation is synchronous with lipogenesis. It is also known that alpha-glycerophosphate stimulates the rate of lipogenesis (48).

Given the unique metabolic _milieu_ of the obese hyperglycemic mouse, a differential response to nutritional

alterations, such as fasting and fasting-refeeding would be expected. The failure of insulin to augment glucose incorporation into glycogen, glyceride-glycerol, and fatty acids in adipose tissue of obese mice (64) is difficult to reconcile with the high rates of fatty acid synthesis from acetate (24) and the high glucose-6-phosphate dehydrogenase activity (34). If, indeed, the liver is the major lipogenic organ in obesity (25, 50, 97, 98, 126), then one would expect greater adaptive changes in hepatic enzyme activities than those in adipose tissue of obese animals. A problem also exists as to both the role of glycolysis in the generation of alpha-glycerophosphate for esterification, and the involvement of the pentose shunt in providing reducing equivalents for fatty acid synthesis. The exploration of adaptive enzyme responses, which forms the basis of the present investigation, is one approach to elucidate the relative contributions of alternate pathways providing similar by products, e.g., the pentose shunt versus the malic enzyme pathway in the generation of NADPH for fatty acid synthesis. Furthermore, reports dealing with adaptive responses of alpha-glycerophosphate generating enzymes have been rather limited. The adaptive responses of glucose-6-phosphate dehydrogenase and malic enzyme are, to my knowledge, unknown in tissues of obese animals. To explore the influence of the hyperinsulinemia of the obese

mice on the nature of adaptive enzyme responses in liver and adipose tissue, the effects of alloxan on glycolytic enzyme activities were also determined in a peripheral group of experiments. Since alloxan is known to lower insulin synthesis and secretion in normal and obese animals (59, 78, 101), it may be a useful tool in indirect explorations of the effects of insulin levels on enzyme activity. The data to be reported here indicate a differential response to dietary manipulation in genetically obese hyperglycemic mice as compared to their thin littermates and support the concept of an imparied adaptive enzyme response in these animals as a significant aspect of the obese condition.

MATERIALS AND METHODS

Maintenance and Treatment of Animals

Obese-hyperglycemic mice, C57BL/6-J ob ob, and their thin littermates were obtained from the Jackson Laboratory at Bar Harbor, Maine. Animals were maintained in a temperature controlled room at 70-80 F. They were fed *ad libitum* with Rockland Complete Mouse/Rat Diet (Teklad, Inc., Monmouth, Illinois) and had free access to water. Heat-treated woodchips were used as bedding in solid bottom cages.

Groups of obese and thin mice, 4-6 months old, were fasted 48 hr and killed. Others were fasted 48 hr and killed after refeeding 24 hr, 48 hr, and 72 hr with laboratory chow plus 15% dextrose in the drinking water (preserved with 0.1% sodium benzoate). In another series animals were refed for 72 hr with laboratory chow plus 15% glycerol in the drinking water. In addition, a group of obese mice were fasted 96 hr and refed for 144 hr with the laboratory chow plus 15% dextrose in the drinking water.

To study the effects of alloxan on these animals, 5 mg of alloxan in sterile 0.9%-NaCl was injected intraperitoneally into obese and thin mice. The dose was 17 mg

per 100 g of body weight, based on the average body weight of 10 thin animals in the group. The dose for obese animals was the same as for thins because the obese lean body mass is not greater than that of the thins (75, 80). These animals were fed laboratory chow for 3 days and killed.

Homogenization of Tissues

Obese and thin mice were killed by decapitation and exsanguinated. The liver and the left and right epididymal fat pads were rapidly excised and the gall bladder was dissected free from the liver. The tissues were rinsed in ice-cold 0.9%-NaCl, gently blotted dry on paper towels and weighed. Nine milliliters of 0.25M-sucrose per gram of tissue were used to homogenize each liver sample. In most instances 4 ml and in others 9 ml (depending upon the amount of available epididymal fat pad) of 0.25M-sucrose per gram of tissue were used to homogenize the total combined epididymal fat pads. Tissues were homogenized at 4 C in glass tubes fitted with Teflon pestles driven by a "Tri-R" stirrer.

The homogenates were centrifuged at 4 C for 30 minutes at 12,000 x g. The fat cake that formed on top of the liver homogenates was removed by suction. The remaining supernatant fluid was removed with a Pasteur pipet. The supernatant fraction below the fat cake of epididymal fat

pad homogenates was removed with a syringe fitted with a 6 inch long 18 gauge needle. The hepatic and adipose tissue supernatant fractions were then frozen and stored at -20 C for enzyme assays, except for glucose-6-phosphatase. For the hepatic glucose-6-phosphatase studies, liver samples were homogenized in 9 ml of 0.15M-KCl per gram of tissue. The homogenates were centrifuged at 4 C for 10 minutes at 510 x g. The fat cakes that formed on the top were removed by suction and the remaining supernatant fractions were used for assay of glucose-6-phosphatase activity on the day of preparation.

Assay of Enzyme Activities

The activities of alpha-glycerophosphate dehydrogenase, glycerol kinase, glucose-6-phosphate dehydrogenase, lactic dehydrogenase, and malic enzyme were measured at room temperature (approximately 25 C) by following either an increase or decrease in absorbance of the reaction mixture over that of the blank at 340 nm in quartz cuvettes with 1 cm light path. One unit of enzyme activity is defined as that catalysing either the formation or disappearance of 1 micromole of NADH or NADPH per minute. A preliminary study indicated that the activity of these enzymes in crude extracts that were frozen for as long as 2 months did not significantly differ from those in freshly prepared extracts.

Alpha-glycerophosphate Dehydrogenase (E.C. 1.1.1.8)

For the assay of activity in liver homogenates, 60 mM sodium phosphate, pH 7.4, 0.1mM β-NADH, 1.5mM dihydroxyacetone phosphate (DHAP) and 0.01-0.02ml of 10% homogenate were mixed to a final volume of 3ml. For the assay of activity in epididymal fat pad homogenates, 54mM sodium phosphate, pH 7.4, 0.1mM β-NADH, 2.3mM- DHAP and 0.05-0.10ml of 10% or 20% homogenate were mixed to a final volume of 1.0ml. Everything was added except the DHAP. The "blanks" contained all the ingredients of the assay mixture except β-NADH and DHAP. The reaction mixtures were mixed and incubated for 15 minutes at room temperature. The reactions were started by the appropriate addition of DHAP; the cuvettes were mixed by inversion and absorbances were recorded at 15 second intervals for 2-3 minutes. The initial rates were used for calculation of enzyme activity.

The DHAP was prepared from the cyclohexylamine salt of dihydroxyacetone phosphate dimethyl ketal by treatment with Dowex 50 (H^+) ion exchange resin, hydrolysis, and neutralization to pH 4.5 according to the procedure of Ballou and Tomita (8). The DHAP was frozen and stored at -20 C until used. The β-NADH was prepared fresh each day in 0.1M sodium phosphate, pH 7.4.

Glycerol Kinase (E.C. 2.7.1.30)

For the assay in liver extracts, the reaction mixture contained 615mM hydrazine-123mM glycine buffer, pH 9.8, 30mM DL-cysteine, 37.4mM sodium fluoride, 2.5mM magnesium chloride, 1.4mM β-NAD, 3.7mM adenosine triphosphate (ATP), 2.32 units (25μg) alpha-glycerophosphate dehydrogenase, 37.4mM glycerol and 0.05-0.10 ml of 10% homogenate in a final volume of 2 ml. Everything was added except glycerol. The reaction mixture was incubated for 15 minutes. To start the reaction, glycerol was added to the reaction mixture, but not the "blank," which contained all the other ingredients. The contents of the cuvettes were mixed by inversion and absorbances were recorded for 30 minutes. The steady rate after the initial lag period was used for the calculation of enzyme activity. The ATP was prepared daily in water. Cysteine was also prepared on the day of use in 1.0N sodium hydroxide.

Glucose-6-phosphate Dehydrogenase (E.C. 1.1.1.49)

The assay mixture contained 65mM Tris-HCl, pH 7.4, 10mM magnesium chloride, 1.2mM-NADP, 3.3mM glucose-6-phosphate and 0.05-0.10 ml of homogenate in a final volume of 2 ml. All ingredients were mixed except glucose-6-phosphate. The mixture was incubated for 15

minutes. Glucose-6-phosphate was added to the reaction mixture, but not the "blank," to start the reaction. The contents of the cuvettes were mixed by inversion and absorbance was recorded at 15 second intervals for 2-3 minutes. The initial rate was used for calculation of enzyme activity. The NADP and G-6-P were prepared in water on the day of use.

Lactic Dehydrogenase (E.C. 1.1.1.27)

The assay mixture contained 95mM sodium phosphate, pH 7.4, 0.1mM β-NADH, 0.33mM pyruvate and 0.01-0.02 ml of either 1.0% liver or 20% epididymal fat pad homogenate in a final volume of 3 ml. All ingredients were mixed together except pyruvate. The mixture was incubated for 15 minutes. The reaction was initiated by addition of pyruvate and absorbance was recorded at 15 second intervals for 3 minutes. The "blank" contained all the ingredients of the assay mixture except β-NADH and pyruvate. The β-NADH and pyruvate were prepared daily in 0.1M sodium phosphate, pH 7.4.

Malic Enzyme (E.C. 1.1.1.40)

For the assay of activity in liver homogenates, 25mM Tris-HCl, pH 7.4, 1.0mM manganese chloride, 0.49mM NADP, and 0.05-0.10 ml of 10% homogenate were mixed to a final volume of 3 ml and incubated for 15 minutes. The

reaction was started by addition of 0.5mM L-malate to the assay mixture, but not the "blank." Absorbance was recorded at 15 second intervals for 3 minutes. For the assay of activity in epididymal fat pad homogenates, 25mM Tris-HCl, pH 7.4, 1.0mM manganese chloride, 0.51mM NADP, and 0.05-0.10 ml of 20% epididymal fat pad homogenate were mixed to a final volume of 1.0 ml and incubated for 15 minutes. The reaction was started by addition of 0.6mM L-malate to the assay mixture, but not to the "blank." Absorbances were recorded for 30 minutes. The initial rates were used for calculations of enzyme activities. NADP was prepared daily in water. The L-malate was prepared in 0.1M-Tris-HCl, pH 7.4, on the day of use.

Glucose-6-phosphatase (E.C. 3.1.3.9)

The reaction mixture contained 80mM to 70mM maleic acid buffer, pH 6.5, 0.01-0.05 ml of 10% liver homogenate, and 24mM D-glucose-6-phosphate in a final volume of 0.5 ml. "Blanks" without D-glucose-6-phosphate and without homogenate, respectively, were also carried through the procedure. The reaction mixtures were incubated for 15 minutes at 37 C. The reaction was stopped by addition of 1.0 ml of 10% trichloroacetic acid (TCA), chilled for 5 minutes at 4 C followed by addition of 1.0 ml of water, and

centrifuged for 10 minutes at 3000 RPM. Two ml portions of the supernatant fraction were used for the determination of inorganic phosphate by the method of Lowry and Lopez (71).

To each volume of TCA extract, 0.1 volume of 10 mg per ml of L-ascorbic acid (prepared fresh daily) and 0.1 volume of 10 mg per ml of ammonium molybdate in 0.05 N sulfuric acid were added, mixed and after 10 minutes the absorbances were read at 700 nm. Inorganic phosphate standards containing 4-30 μg phosphorous per reaction tube were prepared in 4% TCA. Phosphate was determined in not more than 10 tubes at any one time, due to instability of the color. Enzyme activity was expressed as micrograms inorganic phosphorous per microgram nitrogen.

Glycogen Extraction and Determination

Glycogen was extracted by a slight modification of the method of Good *et al*. (41). After rapid excision from the animal, approximately one gram of tissue was rapidly placed into a previously weighed test tube containing 3.0 ml of 30% potassium hydroxide, reweighed to determine the tissue weight by difference, and heated in a boiling water bath for 20-30 minutes, with shaking every 5 minutes. The tubes were then cooled in an ice bath. Saturated sodium sulfate (0.5 ml) was added followed by 4.2 ml of 95% ethanol. After mixing, the tubes were placed in an ice bath for 1 hour and then heated in a boiling water

bath to the point of boiling (this process helps to flocculate the glycogen). The tubes were cooled and centrifuged for 15 minutes at 3000 RPM. The supernates were discarded, the tubes were inverted and allowed to drain. The precipitate was re-suspended in 2 ml of water and re-precipitated by the addition of 0.5 ml saturated sodium sulfate and 3.0 ml of 95% ethanol as above.

When glycogen was extracted from adipose tissue, the precipitate was washed with 2 ml of diethyl ether. The ether was aspirated and the glycogen precipitate was diluted as above. A few determinations conducted on purified rabbit liver glycogen indicated that the ether extraction did not seriously affect the assay.

The glycogen was determined by the anthrone method of Seifter _et_ _al_. (90). The extracted glycogen was diluted with water to bring the final concentration to 3-30 μg per ml of glycogen. Five ml portions of the diluted hydrolysates were pipeted into 20 x 150 mm pyrex test tubes and submerged in iced water. Ten ml of freshly prepared anthrone reagent (0.2 g anthrone in 100 ml of 95% sulfuric acid) were added to each tube from a fast flowing buret. The tubes were rapidly mixed, covered with marbles, heated for 10 minutes in a boiling water bath, and finally cooled to room temperature. The absorbances were read at 620 nm in 19 x 105 mm cuvettes.

Determinations of unknown samples from adipose tissue extracts were performed in duplicate, those from liver, in quadruplicate. A standard curve of 4-20 μg per ml of D-glucose (freshly prepared from a 2 mg per ml glucose stock standard preserved in saturated benzoic acid) was made with every set of test tubes. Calculations were based on the conversion factor determined by Morris (79).

Protein Determination

Protein concentrations were determined by a slight modification of the method of Lowry *et al*. (70). Both liver and epididymal fat pad homogenates were diluted with water to 0.1% and 2.5%, respectively, so as to adjust the protein concentration to 10-60 μg per 0.2 ml of homogenate. A standard curve of 5-100 μg bovine serum albumin was constructed for every set of 40-60 tubes. Determinations of unknown protein samples were performed in triplicate. Sodium deoxycholate was added to the digestion mixture to a final concentration of 5 mg per ml. When lipid concentrations were high as in the case of non-centrifuged adipose tissue homogenates, 1.0 ml of cold anhydrous diethyl ether was added after the final development of color. The tubes were capped, shaken, centrifuged at 3000 RPM for 15 minutes. The upper ether layer was aspirated and the absorbance of the clear lower aqueous layer was read at 750 nm. In selected instances, readings

of standards before and after ether extraction indicated that this treatment did not interfere with the assay.

Nitrogen Determination

Nitrogen content of tissue homogenates was determined by the micro-Kjeldahl method with direct Nesslerization. 0.1 and 0.2 ml of appropriately diluted tissue homogenate (containing 50-100 μg nitrogen per ml) were added to 25 x 200 mm pyrex test tubes containing 0.2 ml of 18 N sulfuric acid. The tubes were heated on an electric digestion rack until charred and then cooled to room temperature. Two drops of 30% hydrogen peroxide were added to each tube and the tubes were heated until clear. Marbles were placed on top and heating was continued for a 30 minute reflux period. After cooling the tubes to room temperature, 7.0 ml of water and 3.0 ml of Nessler's reagent were added and the absorbances were read at 480 nm. A standard ammonium sulfate solution containing 100 μg of nitrogen per ml was processed at two levels by the same procedure as the tissue samples. The Nessler's reagent was prepared by dissolving 100 g mercuric iodide and 70 g potassium iodide in 400 ml of water, followed by the addition of a 500 ml solution containing 100 g of sodium hydroxide and the addition of sufficient water to a final volume of 1000 ml. The solution was stored in a dark brown bottle at room temperature.

Chemicals

The alloxan was obtained from Eastman Chemicals, Rochester, New York. Enzyme grade sucrose was purchased from Mann Research Laboratories, New York, New York. Bovine serum albumin and adenosine triphosphate were obtained from Nutritional Biochemical Corporation, Cleveland, Ohio. The following were obtained from the Sigma Chemical Company, St. Louis, Missouri: Alpha-glycerophosphate dehydrogenase from rabbit muscle, DL-cysteine-HCl, cyclohexylamine dihydroxyacetone phosphate dimethyl ketal, disodium D-glucose-6-phosphate, rabbit liver glycogen, monosodium L-malate, sodium beta nicotinamide adenine dinucleotide, reduced sodium beta nicotinamide adenine dinucleotide, sodium nicotinamide adenine dinucleotide phosphate, and sodium pyruvate. Anthrone, glycerol and all other chemicals used were from Fisher Scientific Company, Fair Lawn, New Jersey.

RESULTS

Fasting-Refeeding Experiments

Body Weight

Untreated obese mice were approximately twice as heavy as untreated thin littermates (Table 1). After a 48 hr fast the obese groups lost 6-7g of body weight which was significantly more than the 4-5g lost by identically treated thins (Table 2). Upon refeeding with standard chow plus 15% glucose in the drinking water for 72 hr, the obese failed to regain the weight which had been lost on fasting (Table 2). While the thins regained almost all the weight lost by fasting within 24 hr of refeeding. Refeeding the thins with standard chow plus 15% glycerol in the drinking water for 72 hr did not result in as large a weight gain as with 15% glucose. Nowever, no difference in weight regained was observed in obese mice treated with either glucose or glycerol in the drinking water (Table 2). After each period of refeeding the thin littermates invariably gained significantly more weight than identically treated obese mice.

After a 48 hr fast the per cent weight lost represented approximately 11% of the original body weight of the obese, significantly less than the 17-18% lost by the thins (Table 3). On a refeeding, following the fast,

the thins regained 17.2% of their body weight by 24 hr and 19.7% by 72 hr when refed standard chow supplemented with glucose in the drinking water, but had only a 6.7% weight gain by 72 hr with the glycerol supplement. The thins essentially regained on refeeding the weight that had been lost on fasting (Tables 2, 3). The obese mice, in contrast, lost more weight than the thins on fasting, which represented less on a per cent basis, but failed to regain the major portion of this weight loss even by 72 hr of refeeding.

Since the per cent body weight lost by the obese mice after a 48 hr fast was significantly less than that of the thins (Table 3) and lower metabolic rates are reported for the obese (33), it must be assumed that the fixed time periods in these experiments may not be comparable metabolic treatments for obese and thin mice. Therefore, a group of obese mice were fasted for 96 hr and another group fasted 96 hr followed by 144 hr of refeeding. After a 96 hr fast the obese mice lost only 8-10g of body weight which was not significantly more than that lost after a 48 hr fast (Tables 4, 5). On refeeding the body weights did not approach those prior to treatment. Some obese mice died after a 96 hr fast, but none had died after a 48 hr fast, indicating that this longer fasting period may not be tolerated by these mice. The data from those that died

are not included in the tables. However, it is pertinent that the obese mice exhibited a sluggish response on extended refeeding following prolonged fasting.

Liver and Epididymal Fat Pad Weights

Liver weights were significantly higher in non-treated obese than in thin animals (Table 6). This was not merely a larger liver for a larger body mass, but a disproportionate increase as indicated by the higher liver to body weight ratios of the obese groups (Table 7). Following a 48 hr fast livers of both obese and thin mice weighed less than those from the non-treated groups. After refeeding, the liver weights of the thin groups were the same or slightly higher than those of the non-treated thins (Tables 6, 7). The obese, in contrast, tended to exhibit liver weights after refeeding that were less than those of non-treated obese mice. Only after an extended fast of 96 hr followed by 144 hr of refeeding did the liver weights ofthe obese approach those of non-treated obese animals (Table 8).

The combined weights of left and right epididymal fat pads in thin and obese animals in all groups showed no definite trends. Although epididymal fat pads of the obese groups weighed significantly more than those of the thin groups in every situation (Table 9), no disproportionate increase occurred in non-treated obese as compared

with correspondingly thin groups. The fact that the major deposition of adipose tissue in the obese is subcutaneous (46) may explain these data. Fasting and refeeding did not cause any dramatic changes in epididymal fat pad weights or fat pad to body weight ratios (Tables 9, 10). Even an extreme fast of 96 hr in the obese (Table 11) did not result in any dramatic changes in epididymal fat pad weight. However, the fact that different individuals comprised control and all experimental groups precludes a vigorous appraisal.

Glycogen Content of Liver and Epididymal Fat Pad

The specific hepatic glycogen content was not different in untreated obese and thin mice, but the total hepatic glycogen content was significantly higher among the obese (Table 12). After a 48 hr fast the obese retained considerably more hepatic glycogen on an absolute basis than the thins. The apparent retention is due in part to higher liver to body weight ratios in the obese (Table 7), rather than to a failure to mobilize glycogen. After refeeding, the hepatic glycogen content of both groups was restored to approximately the non-treated levels.

Epididymal fat pad glycogen content was significantly higher in the non-treated obese only when expressed as the total glycogen per fat pad pair (Table 13). After 72 hr of refeeding following a 48 hr fast, the fat pad

glycogen content of the thins was increased 20 fold, expressed on a specific basis (mg glycogen per gram of tissue) and 13 fold, expressed on a total basis (mg glycogen per fat pad pair), over non-treated thin values. While this parallels classic findings in normal rodents (91), it is significant that the obese did not demonstrate dramatic changes in epididymal fat pad glycogen content after refeeding.

Hepatic Enzyme Activities

Hepatic activities of malic enzyme, alpha-glycerophosphate dehydrogenase, glycerol kinase, lactic dehydrogenase, and glucose-6-phosphatase were all significantly higher in non-treated obese mice than in their thin littermates (Table 14). Although activities of glucose-6-phosphate dehydrogenase also tended to be higher in the obese, the difference was not significant. Tables 15 through 19 demonstrate that the adaptive responses of these enzymes to nutritional stresses were different in obese and thin mice.

Glucose-6-phosphate Dehydrogenase

Glucose-6-phosphate dehydrogenase activities (units per mg supernatant protein) of the thins decreased to 76% of the non-treated values after a 48 hr fast. After refeeding with laboratory pellets plus 15% glucose in the drinking water, an increase in activity was not observed

before 48 hr (Table 15). By 48 hr of refeeding, activity values were 1.7 times higher than in non-treated thins and 3.2 times higher after 72 hr of refeeding. When 15% glycerol was substituted for glucose in the drinking water a 1.8-fold increase in activity occurred after 72 hr of refeeding.

Corresponding changes in glucose-6-phosphate dehydrogenase activities of comparably treated obese mice were not observed (Table 15) even after 72 hr of refeeding with glucose in the drinking water. However glycerol substitution resulted in a 1.7 fold increase in enzyme activity over non-treated obese values. Interestingly, the magnitude of increase over appropriate non-treated values in both obese and thins refed with glycerol in the drinking water was approximately the same. In the obese, glycerol gave a greater increase in glucose-6-phosphate dehydrogenase activity than glucose. But in the thins, glucose gave a greater increase after 72 hr of refeeding.

When hepatic enzyme activities were expressed on an organ basis the same qualitative responses were observed as for protein-specific activities. However, the overwhelmingly larger liver mass in the obese (Table 8), affords a greater total glucose-6-phosphate dehydrogenase activity in the non-treated obese than in the thins (Table 15).

Malic Enzyme

Malic enzyme activity (units per mg supernatant protein) was almost 3.9 times higher in non-treated obese livers than in those of non-treated thins (Table 16) and higher than glucose-6-phosphate dehydrogenase activity in both the obese and thin groups. Refeeding with standard chow plus 15% glucose in the drinking water resulted in a 4.0-fold increase in activity in the thins. Glycerol substitution resulted in a 2.8-fold increase in activity in the thins (Table 16). Identically treated obese mice failed to demonstrate any increases above controls in malic enzyme activities with either regimen.

Malic enzyme activities expressed on an organ basis demonstrated similar responses. Either glucose or glycerol in the drinking water during refeeding resulted in increased activity in the thins, with glucose again more effective. Activities in the refed obese did not demonstrate an "overshoot" compared to non-treated values.

Alpha-glycerophosphate Dehydrogenase

Alpha-glycerophosphate dehydrogenase activities (units per mg supernatant protein) were 1.6 times higher in non-treated obese than those in non-treated thin mice (Table 17) and were significantly higher in the obese in every experiment. After a 48 hr fast, activities were not significantly different from non-treated values in either

group when expressed on a protein basis. But, when hepatic enzyme activity was expressed per whole organ, a considerable decrease was observed in both the obese and thin groups.

After refeeding with laboratory pellets plus 15% glucose in the drinking water, approximately a 1.5 fold increase in activity (units per mg supernatant protein) over the non-treated value was observed by 24 hr in the thins. By 72 hr of refeeding, alpha-glycerophosphate dehydrogenase activity was nearly identical to the non-treated thin value (Table 17). However, refeeding with laboratory pellets plus 15% glycerol in the drinking water for 72 hr produced a 1.9-fold increase over control values in activity in the thins. Obese mice showed a 1.6-fold increase in activity (units per mg supernatant protein) by 24 hr of refeeding with the glucose supplement. After 72 hr of refeeding, activity was nearly the same as that in the non-treated obese group. When 15% glycerol was substituted in the water bottle for glucose, enzyme activity was 2.2 times higher than the non-treated obese value after 72 hr of refeeding. Expressing enzyme activity as units per liver does not seriously affect the qualitative interpretation of these enzymatic responses.

Obese and thin mice demonstrated similar responses in alpha-glycerophosphate dehydrogenase activities. In

both phenotypes, approximately a 1.5-fold increase in activity was observed by 24 hr of refeeding with glucose in the water bottle. By 72 hr the activity had fallen to that of the appropriate non-treated level. When glycerol was substituted for glucose in the water bottle, the activity after 72 hr of refeeding was approximately 2.0 times the appropriate non-treated value in both obese and thin groups.

Glycerol Kinase

Glycerol kinase activity (units per mg supernatant protein) was 1.4 times higher in livers of non-treated obese than in non-treated thins and was significantly higher than thins in every situation observed (Table 18). No significant changes in glycerol kinase activities were observed in either thin or obese mice after a 48 hr fast. After refeeding with laboratory pellets plus 15% glucose in the drinking water the activities in both thin and obese groups were approximately 1.5 times higher than in their respective controls by 24 hr. At 72 hr of refeeding, glycerol kinase levels in both the thins and obese approximated non-treated values. When 15% glycerol was substituted for glucose in the drinking water, no significant changes from control values were observed in either the thins or obese after 72 hr of refeeding. It is possible, however, that a rise or fall in glycerol kinase

activity could have occurred prior to 72 hr during glycerol refeeding because no observations were made earlier than 72 hr.

When glycerol kinase activities were expressed per liver (Table 18) the qualitative responses in the thins were found to be essentially the same as when activities were expressed on a protein basis. In the obese groups, however, no significant changes in glycerol kinase were noted when activity was expressed in this manner. The large variations in obese liver weights during fasting-refeeding may account for this slight discrepancy.

Lactic Dehydrogenase

Lactic dehydrogenase activity was 1.4 times higher (units per mg supernatant protein) in non-treated obese mice than in their thin littermates (Table 19). No significant changes in activity were observed in either group after a 48 hr fast. In the thin groups refed laboratory pellets plus 15% glucose in the drinking water a 1.4-fold increase in activity was noted only in thins that had been refed for 48 hr. By 72 hr of refeeding activity returned to the non-treated level. Significant changes in lactic dehydrogenase activities among the obese were not observed (Table 19). When 15% glycerol was substituted for glucose in the water bottles the activity among the thins after 72 hr of refeeding was not

significantly different than the non-treated level. In the obese group, 15% glycerol in the water bottle resulted in a slight, but significant, increase in activity. When lactic dehydrogenase activity was expressed as units per liver, dramatic changes in the activity levels were not observed. Moreover, this expression of activity is associated with a high variability.

Glucose-6-phosphatase

Glucose-6-phosphatase activity of the non-treated obese mice was 1.3 times higher than in the non-treated thins (Table 20). No significant changes in activity were observed after fasting or refeeding in both thins and obese. The lack of an increase in hepatic glucose-6-phosphatase in the mouse has been reported by other investigators (110). The adaptive increase of glucose-6-phosphatase activity after fasting is apparently a species specific response, present in the rat, but absent in the mouse.

Hepatic Enzyme Activities After Prolonged Fasting-Refeeding

As previously indicated, the failure to observe increases in the activities of these hepatic enzymes in the obese mice may be related to the lower metabolic rate of these animals (33). Therefore enzyme activities were

assayed after a 96 hr fast followed by 144 hr of refeeding to test the tentative hypothesis that a longer fasting period in the obese may be required to induce an adaptive increase in enzyme activities upon refeeding. Among the enzymes assayed, no significant differences were observed between the obese group refed 144 hr and the non-treated obese (Table 21). However, a 1.7 fold increase in glycerol kinase activity (units per mg supernatant protein) was noted after the 96 hr fast (Table 21), while no change in activity was observed after the 48 hr fast (Table 18).

When enzyme activity was expressed on a total organ basis, all of the enzymes assayed were observed to decrease (Table 21) after a 96 hr fast. This may be concomitant to the markedly decreased liver weight in the obese (Table 8) during this physiological state. After refeeding for 144 hr, when liver weights have returned to control values (Table 8), no significant differences were observed when compared to non-treated values (Table 21).

Epididymal Fat Pad Enzyme Activities

Glucose-6-phosphate Dehydrogenase

Glucose-6-phosphate dehydrogenase activity was generally higher in the obese than in the thins (Table 22). After a 48 hr fast no significant changes in activity were

observed in either the obese or thin mice. Upon refeeding with laboratory pellets plus 15% glucose in the drinking water a 1.7 fold increase in activity (units per mg supernatant protein) over the non-treated value was observed among the thins by 72 hr. In identically treated obese mice no significant changes in activity were observed. When 15% glycerol was present in the drinking water during the refeeding period, no significant changes in activity were observed in the obese or thins after 72 hr.

Expressing enzyme activity as units per fat pad pair (Table 22) did not seriously alter the qualitative interpretation of the data, although an increase in activity among the thins was observed by 48 hr of refeeding. The obese, on the other hand, consistently did not demonstrate any "overshoot" in glucose-6-phosphate dehydrogenase activity. Since the epididymal fat pad weights were very variable among the thins (Tables 9, 10), this expression of activity may reflect changes in organ weight more than changes in enzyme activity.

Malic Enzyme

Malic enzyme activity in non-treated obese mice was half the value (units per mg supernatant protein) of non-treated thins (Table 23). This was the only case where an enzyme involved in the lipogenic process was

lower in the obese than in the thins. Fasting for 48 hr did not result in any significant changes in activity in obese or thins. After refeeding for 72 hr with laboratory pellets plus 15% glucose in the drinking water, activity in the thins was 2.7 times higher than the non-treated value. A 15% glycerol supplement in the water bottles did not result in any significant change in activity. Significant changes in activity among refed obese mice were not observed.

Alpha-glycerophosphate Dehydrogenase

Alpha-glycerophosphate dehydrogenase activity (units per mg supernatant protein) was not significantly different in obese and thin mice (Table 24). Fasting for 48 hr did not result in any significant changes in activity. Upon refeeding with laboratory pellets plus 15% glucose in the drinking water, activity was 3.9 times higher after 72 hr than the non-treated value in the thins. Substitution of 15% glycerol in the water bottles during refeeding did not result in any significant increase in activity among the thins.

A similar pattern of response was not observed in the obese. No significant changes in activity were observed during refeeding when glucose was in the water bottles. But when glycerol was in the drinking water during refeeding a 1.6 fold increase in activity was

observed (Table 24). Expression of activity on an organ basis reflects the variation in epididymal fat pad weights (Tables 9, 10) more than changes in enzyme activity and probably is not the expression of choice.

Lactic Dehydrogenase

Lactic dehydrogenase activities (units per mg protein) were similar in non-treated obese and thin mice (Table 25). A 48 hr fast resulted in a slight decrease in activity among the obese while a significant change was not observed among the thins. Upon refeeding with glucose in the drinking water, a 1.4 fold increase in activity was observed in the thins after 72 hr, while only a slight increase was observed in the obese. When glycerol was in the drinking water, no significant changes were noted in the thins but a slight, highly significant increase in activity was observed among the obese (Table 25). A similar pattern was observed when activity was expressed as units per fat pad pair. But since epididymal fat pad weights are extremely variable (Tables 9, 10) and other enzymes assayed did not demonstrate consistent results using this expression (Tables 22, 23, 24), it is not regarded as a significant trend.

Epididymal Fat Pad Enzyme Activities After Prolonged Fasting-Refeeding

For reasons previously indicated, enzyme activities in the epididymal fat pads of obese mice were assayed after a 96 hr fast followed by 144 hr of refeeding. When activity was expressed as units per mg supernatant protein no significant changes were observed in the enzymes assayed either after the 96 hr fast or after 144 hr of refeeding (Table 26).

When activity was expressed on a per fat pad pair basis, no changes were observed in glucose-6-phosphate dehydrogenase (Table 26). Alpha-glycerophosphate dehydrogenase activity was 1.5 times higher in obese mice refed for 144 hr than in the non-treated obese group. Lactic dehydrogenase was 0.67 times the non-treated value after a 96 hr fast. Since variables results were obtained in general when enzyme activity was expressed on an organ basis, perhaps caution should be observed in interpreting these changes.

Effects of Alloxan

Body Weight

The weights of the animals in these experiments were comparable to those used in the fasting-refeeding studies (Tables 1, 27). Seventy-two hours after administration of alloxan, obese and thin mice had lost

a minimal amount of weight (Tables 27, 28). Weight loss in grams and on a percentage basis was similar in both groups (Table 28). Glucosuria was present by this time in the thin mice as shown by a positive urine "dextrostix."

Liver and Epididymal Fat Pad Weights

The liver weights after alloxan treatment were significantly higher in the obese than in the thin littermates (Table 29). Liver: body weight ratios were comparable to those of the non-treated animals used as controls in the fasting-refeeding studies (Tables 7, 29). Alloxan, at least at the dose used, appears to have no effect on liver weight.

The epididymal fat pad weights were much higher in the obese than in the thins (Table 29). The fat pad weights and the fat pad: body weight ratios of thins (Table 29) were comparable to those of non-treated thins (Tables 9, 10). Those of the alloxan-treated obese (Table 29) were considerably higher than those of non-treated obese (Tables 9, 10). This group of obese animals may have had more adipose tissue than the non-treated obese mice or alternatively alloxan may affect epididymal fat pad density. Further work is needed to choose between these alternative conclusions.

Hepatic Enzyme Activities

Activities of glucose-6-phosphate dehydrogenase and glycerol kinase in livers of thin mice were not significantly different after alloxan treatment (Table 30). Alpha-glycerophosphate dehydrogenase activity was 1.3 times higher than the non-treated value. Lactic dehydrogenase activity (units per mg supernatant protein) was 80% of the non-treated value after alloxan treatment.

In the livers of obese mice treated with alloxan, glycerol kinase activity was not significantly different from the non-treated value (Table 31). Both glucose-6-phosphate dehydrogenase and lactic dehydrogenase activities were 75% of the non-treated values. Alpha-glycerophosphate dehydrogenase activity was 1.3 times higher than the non-treated value in the obese (Table 31), clearly the only enzyme that was significantly increased after alloxan treatment in both phenotypes.

Epididymal Fat Pad Enzyme Activities

In the thin mice, glucose-6-phosphate dehydrogenase and lactic dehydrogenase activities (units per mg protein) were lower in the alloxan treated group than in the non-treated thins. Alpha-glycerophosphate dehydrogenase activity was not significantly different from the non-treated value.

In the alloxan treated obese mice, glucose-6-phosphate dehydrogenase and lactic dehydrogenase activities were not significantly different than the non-treated values when expressed as units per mg supernatant protein (Table 33). Alpha-glycerophosphate dehydrogenase activity (units per mg protein) was 1.8 times higher in the alloxan-treated group than in the non-treated obese animals. Since the epididymal fat pad weights of the two groups were not comparable and show large variations, the expression of activity as units per fat pad pair is of limited usefulness.

DISCUSSION

Responses of experimental animals to fasting-refeeding manipulation have been extensively studied in rats (30, 40, 69, 112, 113, 114, 120), but only minimal work has been reported for similar studies in mice (51, 54, 123). There is no *a priori* reason to assume that mice would respond to such nutritional manipulation in a manner similar to that of rats. Fasting-refeeding responses in genetically obese hyperglycemic mice and other common obese models have been examined to an even lesser extent. Fasting-refeeding has been used as an experimental model for inducing high rates of lipogenesis with concomitant induction of enzyme systems associated with this process. "Adaptive hyperlipogenesis," the term designated for this model by Tepperman (112), has been useful in elucidating mechanisms involved in controlling metabolic flow over not only the fatty acid synthesizing pathway itself (40) but also over associated pathways which provide both the required reducing equivalents (112, 114) in the form of NADPH as well as the alpha-glycerophosphate that is required for the esterification process (55, 113).

In these studies, emphasis has been placed on the expression of enzyme activity as micromoles of substrate

turned over per unit time per milligram of protein. Other expressions of enzyme activity have generally been used. These relate the amount of substrate turned over per unit time to tissue weight, tissue protein or nitrogen content, DNA content, cell number, total organ weight, or unit of body weight. The manner of expressing enzyme activity has received much attention. By general agreement of the International Union of Biochemists (29) the most acceptable mode of expressing enzyme activity is micromoles of substrate turned over per minute per milligram of tissue or cell fraction protein. However, the problem of which expression has the greatest physiological significance has not been completely resolved as evidenced by the variety of expressions used in the metabolic literature.

Recently, it has been re-emphasized that liver wet weight varies considerably due to changes in water and glycogen content and therefore is probably the poorest base for expressing hepatic enzyme activity (28). Alternatively, the expression of hepatic enzyme activity as units per 100 grams of body weight, largely reflects organ wet weight and parallels the expression as units per milligram of protein. The lipid content, which largely determines the wet weight of the epididymal fat pad, varies markedly with nutritional status. Therefore, units on a per protein basis probably carries the most significance for the epididymal fat pad (82).

Some workers (88, 89) have argued for relating enzyme activity to tissue DNA content since this may provide a "per cell" basis for enzyme expression. This is probably no better or worse an expression of activity than using protein content as a basis. But since protein content and not DNA content can effectively be used in evaluating changes in enzyme activity within various cell fractions or compartments, it is probably a more useful base in the expression of enzyme activity. In general, the activities of the hepatic enzymes in the present study have shown the same qualitative response, whether expressed on a protein or total liver basis. In the epididymal fat pad, enzyme activity as units per milligram protein and per fat pad pair did not qualitatively demonstrate the same type of responses.

Obese animals lost more weight on fasting but gained less than thins on refeeding (Table 2), failing to regain by 72 hr the weight lost on fasting (Tables 2, 3). It was observed that the obese mice did not lose much more weight after a 96 hr fast than after a 48 hr fast (Table 4). This failure of the obese animals to rapidly regain body weight after a fast was unexpected, especially in one considers the rapid weight gains of these animals early in life (21, 49). The general inability of obese mice to make homeostatic adaptations to physiological stresses such as lower ambient temperature (74) and decompression

sickness (3) may be associated with the failure to regain body weight after fasting.

The finding of higher liver weights in untreated obese mice both on absolute (Table 6) and relative bases (Table 7) is consistent with the concept that the liver may be the major lipogenic organ in obesity (25, 26, 50, 93, 126). While the thins tended to exhibit higher relative liver weights on refeeding (Table 7) compared to non-treated values, the obese tended to display lower ones. Only after a prolonged refeeding period did the liver weights of the obese approach those of the non-treated obese animals (Table 8). But this may not be simply a matter of an overall sluggish response since obese animals exhibited responses comparable to those of thins in terms of other parameters.

A considerable amount of glycogen was mobilized from the obese liver after a 48 hr fast when glycogen content was expressed on a total organ basis (Table 12). This was replenished on refeeding, the response being comparable in the obese and thins. Hepatic glucose-6-phosphatase levels were higher in the obese (Tables 14, 20), consistent with the findings of Seidman *et al*. (88, 89). In the obese mouse, high levels of blood insulin and blood glucose coexist with higher than normal hepatic activities of glucose-6-phosphatase (Table 20) and

fructose-1,6-diphosphatase (88, 89), the "gluconeogenic" enzymes of Weber (120, 122). However, concomitant activities of glucokinase, phosphofructokinase, and pyruvic kinase (88, 89) are also high which is inconsistent with Weber's hypothesis that activities of the unidirectional enzymes, glucokinase, phosphofructokinase and pyruvic kinase are high when glycolytic activity is high and low when glycolytic activity is low (120, 122). Conversely, activities of unidirectional enzymes, directing metabolic flow in the gluconeogenic direction (pyruvic carboxylase, phosphoenolpyruvate carboxykinase, fructose-1, 6-diphosphatase, and glucose-6-phosphatase) are elevated under conditions promoting gluconeogenesis. Activities of the unidirectional glycolytic enzymes are decreased while those of the gluconeogenic set are increased in rat liver after fasting (120, 122). After refeeding, or when glucose is available, activities of the gluconeogenic set of enzymes are decreased and those of the glycolytic set are increased. Insulin is believed to play a major role in the induction of the glycolytic set and repression of the gluconeogenic set (107, 120, 122) while cortisone is believed to induce only the "gluconeogenic" enzymes (120, 122). Perhaps the high insulin (37, 46, 73, 104, 127) and corticoid levels (16, 46) in the obese mouse induce high activity in both directions simultaneously. Since

metabolism of carbohydrate by extra-hepatic sites may be required for the insulin repression of "gluconeogenic" enzymes (95), the impaired glucose uptake by muscle and adipose tissue of obese mice may also be involved in the high activity of both glycolytic and "gluconeogenic" enzymes. Hepatic glucose-6-phosphatase activity of both thin and obese animals was not increased after fasting nor did it change after refeeding (Table 20). A rise in glucose-6-phosphatase activity after fasting has been reported in rat liver (118). But this has been shown to be a species-specific response (110), indicating that enzymes which are adaptive in some species may not be adaptive in others.

Hepatic activities of glucose-6-phosphate dehydrogenase and malic enzyme were higher in the obese (Tables 14, 15, 16) than in the thins, although the difference in glucose-6-phosphate dehydrogenase was not statistically significant. These may be merely a reflection of the higher insulin levels of the obese mice (37, 46, 73, 104, 127) or may be related to diet and chronic inactivity as in rats (57). Both of these enzymes are believed to be crucial in providing reducing equivalents for fatty acid synthesis and have been observed to decrease after a fast and to increase under conditions of high lipogenesis, e.g., fasting-refeeding (7, 30, 112, 114) and meal-feeding

(66, 67). Fasted thin littermates showed a small decrease in glucose-6-phosphate dehydrogenase activity which was not observed in the obese (Table 15). On refeeding, the activities of both glucose-6-phosphate dehydrogenase (Table 15) and malic enzyme (Table 16) increased dramatically in the thins but not in the obese. Even after prolonged fasting and refeeding (Table 21) glucose-6-phosphate dehydrogenase activity did not substantially change in the obese. One possibility is that insulin release from the obese pancreas is not stimulated by feeding so that insulin levels are constant and enzyme activity merely reflects insulin stasis. However, the obese pancreas is stimulated both by an oral glucose load (38) and by *in vitro* glucose administration (73) to release insulin, which tends to rule out such an explanation.

Further evidence of a differential response to fasting-refeeding is obtained by noting that when glycerol was substituted for glucose in the drinking water during refeeding, a smaller response was obtained in the thins for both glucose-6-phosphate dehydrogenase (Table 15) and malic enzyme (Table 16). In the obese, a slightly larger response was obtained in hepatic glucose-6-phosphate dehydrogenase with glycerol (Table 15). Glycerol has been shown to be a stimulant of lipogenesis and can

increase hepatic activities of glucose-6-phosphate dehydrogenase and malic enzyme in rats (111, 113, 114). Theoretically, one would expect a larger malic enzyme response to glycerol than to glucose refeeding in a normal animal liver because glycerol enters glycolysis below the phosphofructokinase control point (69, 114). Failure to observe this in mice indicates a possibility that different control mechanisms exist for this organism.

The findings of high alpha-glycerophosphate dehydrogenase (Table 17) and glycerol kinase (Table 18) activities in the obese liver are in agreement with reported observations (34, 58). Since alpha-glycerophosphate is obligatory for esterification of fatty acids (55, 117) and also stimulates fatty acid synthesis (48), it is expected that pathways generating alpha-glycerophosphate would be elevated in obesity. Neither alpha-glycerophosphate dehydrogenase nor glycerol kinase activities were decreased by fasting (Tables 17, 18). However, Tepperman and Tepperman (113) reported both decreased glycerol kinase and alpha-glycerophosphate dehydrogenase activities in rat liver after fasting. They observed an increase in alpha-glycerophosphate dehydrogenase and a decrease in glycerol kinase activities on refeeding (113). In the present studies both enzymes were shown (Tables 17, 18) to have increased activities on refeeding high carbohydrate in

thin and obese mice. The Teppermans (113) also observed an increase in alpha-glycerophosphate dehydrogenase activity when 15% glycerol was in the drinking water, which is in complete agreement with results reported here (Table 17). However, glycerol in the drinking water of refed obese mice resulted in very dramatic changes in alpha-glycerophosphate dehydrogenase activity (Table 17), while no significant changes in glycerol kinase activity in either obese or thin mice were observed (Table 18). The Teppermans (113), on the other hand, reported a decrease in glycerol kinase activity with 15% glycerol in the drinking water indicating perhaps that as in the case of glucose-6-phosphatase (110), rat and mouse respond differently.

Current research orientation has placed major emphasis on the role of adipose tissue in rodent lipogenesis (114). The relationship of glycogen synthesis and degradation to fatty acid syntehsis and esterification has recently been reviewed (91, 92). High levels of glycogen in adipose tissue correlate well with increased rates of fatty acid synthesis (91, 92). When the adipocyte is flooded with glucose, such as during realimentation following a fast, glycogen is believed to represent a temporary storage depot until glycolysis can gradually dissipate the carbon flow to fatty acids and

alpha-glycerophosphate (91, 92). While adipose tissue from fasted-refed thin mice had very high glycogen levels, that from identically treated obese animals showed no changes on refeeding (Table 13). The failure of the obese to deposit high adipocyte glycogen is probably related to the insulin insensitivity of this tissue (20, 23, 64, 103, 105) which has led some workers (17, 58, 68, 116) to investigate alternate routes for glycerophosphate generation other than glycolysis. Of these, the adipose tissue glycerol kinase pathway appears to be the most likely candidate because the necessary glycerol can be taken up by the adipocyte and glycerol kinase activity can be increased by insulin (58).

Since glucose is poorly utilized by obese adipose tissue (23, 64) and lipogenesis from acetate is high, one might expect activity of glucose-6-phosphate dehydrogenase to be lower while that of malic enzyme to be higher in the obese. As compared to thins, glucose-6-phosphate dehydrogenase was actually significantly higher (Table 22) while malic enzyme was lower (Table 23) in adipose tissue of non-treated obese animals. This is in sharp contrast to the expectation that one might derive from Flatt's hypothesis (31) that the malate cycle is the sole source of NADPH for fatty acid synthesis when glycogen stores and glucose availability are low. Also, neither activities of

glucose-6-phosphate dehydrogenase nor malic enzyme showed any changes in the obese on refeeding (Tables 22, 23).

The significance of the various parameters measured in the epididymal fat pad may be questioned. All the fat pads, including the epididymal fat pad, show early hypertorphy due to the multiplication of adipocytes and accumulation of glycerides (25). Although the overwhelming adipose deposition is subcutaneous in the obese animals, the epididymal fat pad is hypertrophied during the early stages of obesity through at least five months of age (47). Therefore, for the age span (4-6 months) of animals used in the present studies, the epididymal fat pad is probably representative, in a general way, of the adipose tissue throughout the body. Additionally, the lack of subcutaneous adipose tissue in the thin littermates precludes the use of subcutaneous adipose tissue for metabolic comparisons between obese and thin animals.

Results from the experiments with the alloxan-treated obese and thin mice must be interpreted with reservation. The alloxan dose selected (170 mg per Kg of body weight) was based on the work of Mayer and co-workers (59, 72, 101) who were interested primarily in effects on blood glucose levels and pancreatic islet histology. This dose was higher than that of other workers (72, 82, 128) and the time after injection that was chosen for killing

the animals was comparable with the procedures of these workers (59, 72, 78, 82, 101, 128). Since few obese animals were available, a dose-response curve of alloxan's effects on enzyme activities was not established. Thus, the possibility remains that the alloxan dose used may have been inadequate to produce maximum effects on enzyme activities. As a monitor of the diabetic effect of alloxan in thin mice, only the presence of glucosuria was used.

In rats, decreased hepatic activities of glycolyptic enzymes, including glucose-6-phosphate dehydrogenase (111) and citrate cleavage enzyme (69, 111) are reported after alloxan treatment. Adipose tissue activities of glucose-6-phosphate dehydrogenase and malic enzyme (92) are also lower after alloxan treatment. In adipose tissue of thin mice, activity of glucose-6-phosphate dehydrogenase was significantly lower after alloxan administration (Table 32) while hepatic activity remained unchanged (Table 30). Mice may respond differently than rats in that the adipose tissue may be more responsive to *in vivo* alloxan than liver. Alternatively, the alloxan dose used may not have been adequate, as indicated in the preceding paragraph. The obese mice, on the other hand, showed significantly lower hepatic glucose-6-phosphate dehydrogenase activity after alloxan (Table 31) in accord with the results from other

species. But in obese adipose tissue, no change in glucose-6-phosphate dehydrogenase was observed (Table 33). Alloxan is known to restore insulin sensititivy to at least one insulin-resistant tissue of obese mice, namely muscle (72). The concomitantly lowered blood glucose levels (59, 78, 101) and increased alpha-glycerophosphate dehydrogenase activities in obese adipose tissue observed after alloxan treatment (Tables 31, 33) indicate that there might be a reversal of the insulin resistance in adipose tissue as well. An *in vitro* measurement of glucose transport in tissues of obese mice after alloxan treatment is required to fully answer these questions.

The findings reported here indicate that species differences in glycolytic enzyme response exist in the normal mouse and the normal rat. In the liver of the thin mouse the relative levels of glucose-6-phosphate dehydrogenase are much lower than those of malic enzyme (Table 14) while in the rat the level of glucose-6-phosphate dehydrogenase is approximately the same as that of amlic enzyme (121). Hepatic glucose-6-phosphatase activity did not increase after a fast (Table 20) as has been reported for the rat (118). The fasting-refeeding responses of some of the hepatic enzymes reported here appear to be unique to the mouse. In rats a larger malic enzyme response has been reported with glycerol than with

glucose refeeding (111, 114). Similar experiments in normal mice show larger responses with glucose refeeding (Table 16). Unlike the rat (113), no decreases in alpha-glycerophosphate and glycerol kinase activities were observed in thin mice after fasting (Tables 17, 18). In the literature, metabolic changes in mice and rats are referred to almost interchangeably. The differences between mice and rats indicated here suggest greater caution in transposing metabolic characteristics and point to the necessity of a further cataloguing of specific differences between these species.

Generally, hepatic enzyme activities are higher in obese than in thin mice. These are referred to both in the Introduction and in the Results. Any change in specific activity, which is expressed on a protein or nitrogen basis, must be interpreted in terms of the large hepatosomatic index of obese mice (Table 7). A change in specific activity in the obese animals represents an even greater increase for the total animal. This fits will with the concept of some workers who believe that the liver is the major lipogenic organ in obesity (25, 26, 50, 93, 126) in spite of the larger adipose tissue mass. It is also interesting that alloxan treatment primarily affected hepatic enzymatic activities in the obese while adipose tissue enzymatic activities were affected only

in the thin mice. If the liver is, indeed, the major lipogenic organ in obese animals, with adipose tissue serving mainly as a depot, then one might expect alloxan to affect liver to a greater extent than adipose tissue in obesity. The greater enzyme activity, larger hepato-somatic index, and the greater hepatic effect of alloxan in obese mice supports the view that the liver plays a major role in the etiology of obesity.

Surprisingly, little work has been reported for enzyme activities in adipose tissue of obese mice, perhaps limited only to exploration of glyceroneogenesis (17, 58, 68, 116). Spencer and Lowenstein (102) have observed that hepatic citrate cleavage enzyme is higher in the obese and decreases after fasting in both the thins and obese. But after refeeding, the hepatic activity of the thin litter-mates actually exceeds that of the obese (102). The failure to observe very large increases in body weight, liver weight, and activities of NADPH generating enzymes in liver and adipose tissue of obese mice on fasting-refeeding regimens is strikingly different from the thins. Even after a prolonged fast and a prolonged refeeding period the activities of both hepatic and adipose tissue glucose-6-phosphate dehydrogenase did not increase (Tables 21, 26) indicating that the failure to observe a response with the shorter refeeding periods was not merely

due to a lag or sluggish response. Given the collective role of glucose-6-phosphate dehydrogenase, malic enzyme, and citrate cleavage enzyme in lipogenesis, one can conclude that obese mice lack an "adaptive enzyme" response to fasting-reffeding manipulation and are suggestive of an impaired "adaptive hyperlipogenic" response.

Since the hepatic alpha-glycerophosphate generating enzymes increased in the obese animals on refeeding, an alternate hypothesis is that there is an increase in activity of the glycerophosphate shuttle. The shuttle couples the cytoplasmic and mitochondrial alpha-glycerophosphate dehydrogenases, bringing alpha-glycerophosphate into the mitochondrion. Within the mitochondrion, alpha-glycerophosphate is oxidized, completing the transfer of hydrogen atoms from the cytoplasm into the mitochondrion (15, 85, 87). The importance of this shuttle has been well documented in insect flight muscle (85). However, in rat liver the significance of the glycerophosphate shuttle is implied mainly from data demonstrating alterations in mitochondrial alpha-glycerophosphate dehydrogenase concomitant with fluctuations in mitochondrial respiratory activity in thyroxine-treated rats (87). Recently, however, the importance of the glycerophosphate shuttle in rat liver has been

questioned because the rate of detritiation of 2-H^3-alpha-glycerophosphate is much too high to be accounted for by the activity of the mitochondrial alpha-glycerophosphate dehydrogenase (15). Also, rotenone fails to inhibit the oxidation of alpha-glycerophosphate (15) which would tend to de-emphasize any importance assigned to the mitochondrial enzyme in the rat liver. Therefore, the possibility of an increased glycerophosphate shuttle operating during refeeding, to account for the observed increases in hepatic alpha-glycerophosphate generating enzymes tends to be remote.

Human adipose tissue is believed to function mainly as a depot while the liver serves as the main lipogenic organ (94, 96). In human adipose tissue enzymatic activities of the classic lipogenic enzymes (glucose-6-phosphate dehydrogenase, malic enzyme, citrate cleavage enzyme, and alpha-glycerophosphate dehydrogenase) in obese individuals are generally the same or slightly lower than those of normal weight individuals (12). The findings reported here and in the literature indicate that the obese hyperglycemic syndrome in the mouse is similar in part to human obesity. The total lipogenesis is believed to be mainly a hepatic function and the adipose tissue functions mainly as a depot for esterification of fatty acids that are either synthesized in the liver or ingested (25, 26, 50, 93, 126). Adipose tissue enzymatic

activities are generally the same or lower in the obese compared to those in lean littermates, except for glucose-6-phosphate dehydrogenase and glycerol kinase which are higher in obese mice. Therefore it is not unreasonable to suggest that the lack of an "adaptive enzyme" response in this model system may have implications directly applicable to the human situation.

TABLE 1

INITIAL BODY WEIGHTS OF INDIVIDUAL GROUPS OF THIN AND OBESE MICE

	Non-treated	Fasted 48 hr	Fasted 48 hr +24 hr Refed A	Fasted 48 hr +48 hr Refed A	Fasted 48 hr +72 hr Refed A	Fasted 48 hr +72 hr Refed B
Thin	33.4 ±1.8 (10)	30.7 ±1.0 (10)	29.7 ±1.0 (7)	30.1 ±0.7 (7)	30.7 ±0.9 (10)	30.3 ±0.7 (7)
Obese	67.4 ±1.6 (10)	65.5 ±1.5 (10)	62.7 ±0.7 (7)	64.6 ±1.4 (7)	57.7* ±2.6 (10)	63.7 ±0.8 (7)
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values are the means (grams) ± SEM of the number of observations in parentheses.

Refed A and Refed B refer to refeeding with laboratory pellets plus 15% glucose and 15% glycerol in the drinking water, respectively.

*Significantly different from the non-treated obese at P < 0.02 level.

TABLE 2

BODY WEIGHT CHANGES IN GROUPS OF THIN AND OBESE MICE

	Fasted 48 hr	Fasted 48 hr +24 hr Refed A	Fasted 48 hr +48 hr Refed A	Fasted 48 hr +72 hr Refed A	Fasted 48 hr +72 hr Refed B
			Weight Lost After a 48 Hour Fast		
Thin	4.9±0.2 (10)	4.9±0.1 (7)	4.2±0.3 (7)	4.7±0.2 (10)	5.3±0.3 (7)
Obese	7.1±0.3 (10)	6.8±0.2 (7)	7.3±0.6 (7)	5.9±0.7 (10)	7.0±0.1 (7)
P	<0.001	<0.001	<0.001	N.S.	<0.001
			Weight Gained After Refeeding		
Thin		4.3±0.2 (7)	4.2±0.2 (7)	5.1±0.2 (10)	1.7[a]±0.2 (7)
Obese		1.9±0.2 (7)	1.8±0.4 (7)	3.4±0.4 (10)	2.7 ± 0.2 (7)
P		<0.001	<0.001	<0.001	<0.01

Values are the means (grams) ± SEM of the number of observations in parenthesis. Refed A and Refed B refer to refeeding with laboratory pellets plus 15% glucose and 15% glycerol in the drinking water, respectively.

[a]Significantly different from thin, fasted 48 hr + 72 hr Refed A at P < 0.001 level.

TABLE 3

PERCENT BODY WEIGHT CHANGES IN GROUPS OF THIN AND OBESE MICE

	Fasted 48 hr	Fasted 48 hr +24 hr Refed A	Fasted 48 hr +48 hr Refed A	Fasted 48 hr +72 hr Refed A	Fasted 48 hr +72 hr Refed B
Loss After 48 Hour Fast					
Thin	15.6±0.8 (10)	16.6±0.6 (7)	14.0±0.9 (7)	15.5±0.6 (10)	17.6±1.3 (7)
Obese	10.8±0.4 (10)	10.9±0.2 (7)	11.2±0.8 (7)	10.9±1.0 (10)	11.0±0.2 (7)
P	<0.001	<0.001	<0.05	<0.001	<0.001
Gain After Refeeding					
Thin		17.2±0.7 (7)	16.1±0.7 (7)	19.7±1.1 (10)	6.7[a]±0.7 (7)
Obese		3.4±0.3 (7)	3.1±0.7 (7)	6.5±0.6 (10)	4.7±0.3 (7)
P		<0.001	<0.001	<0.001	<0.05

Values are the means ± SEM of the number of observations in parenthesis. Refed A and Refed B refer to refeeding with laboratory pellets plus 15% glucose and 15% glycerol, respectively, in the drinking water. Percent loss is calculated as (g loss/pretreated wt) x 100. Percent gain is calculated as (g gain/fasted wt) x 100.

[a]Significantly different from thin, fasted 48 hr + 72 hr Refed A at $P < 0.001$ level.

TABLE 4

BODY WEIGHT CHANGES IN OBESE MICE SUBJECTED TO EXTENDED PERIODS OF FASTING AND REFEEDING

Fasted 48 hr	Fasted 48 hr +72 hr Refed	Fasted 96 hr	Fasted 96 hr +144 hr Refed
Loss After Fasting			
7.1 ± 0.3 (10)	5.9 ± 0.7 (10)	8.3 ± 0.9 (5)	10.8 ± 0.6 (5)
Gain After Refeeding			
	3.4 ± 0.4 (10)		6.3 ± 1.1 (5)

Values are the means (grams) ± SEM of the number of observations in parentheses. Animals were either fasted for the periods indicated or fasted and refed with laboratory pellets plus 15% glucose in the drinking water.

TABLE 5

PERCENT BODY WEIGHT CHANGES IN OBESE MICE SUBJECTED TO EXTENDED PERIODS OF FASTING AND REFEEDING

Fasted 48 hr	Fasted 48 hr +72 hr Refed	Fasted 96 hr	Fasted 96 hr +144 hr Refed
Loss After Fasting			
10.8 ± 0.4 (10)	10.9 ± 1.0 (10)	13.6 ± 0.9 (5)	16.2* ± 0.6 (5)
Gain After Refeeding			
	6.5 ± 0.6 (10)		11.3 ± 2.0 (5)

Values are the means ± SEM of the number of observations in parentheses. Animals were either fasted for the periods indicated or fasted and then refed with laboratory pellets plus 15% glucose in the drinking water.

Percent loss is calculated as (g loss/pre-treated wt) x 100.

Percent gain is calculated as (g gained/fasted wt) x 100.

*Not significantly different from the fasted 96 hr value.

TABLE 6

LIVER WEIGHTS IN THIN AND OBESE MICE

	Non-treated	Fasted 48 hr	Fasted 48 hr +24 hr Refed A	Fasted 48 hr +48 hr Refed A	Fasted 48 hr +72 hr Refed A	Fasted 48 hr +72 hr Refed B
Thin	1.55 ±0.10 (10)	1.08[a] ±0.03 (10)	1.66 ±0.05 (7)	1.42 ±0.06 (7)	1.60 ±0.05 (10)	1.34 ±0.05 (7)
Obese	4.94 ±0.24 (10)	3.01[b] ±0.22 (10)	3.64 ±0.40 (7)	3.79 ±0.21 (7)	3.08[b] ±0.36 (10)	3.78 ±0.15 (7)
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values are the means (grams) ± SEM of the number of observations in parentheses

Refed A and Refed B refer to refeeding with laboratory pellets plut 15% glucose and 15% glycerol, respectively, in the drinking water.

[a]Significantly different from the non-treated and refed thin groups at the $P < 0.001$ level.

[b]Significantly different from the non-treated obese at the $P < 0.001$ level.

TABLE 7

PERCENT BODY WEIGHT REPRESENTED BY LIVER IN THIN AND OBESE MICE

	Non-treated	Fasted 48 hr	Fasted 48 hr +24 hr Refed A	Fasted 48 hr +48 hr Refed A	Fasted 48 hr +72 hr Refed A	Fasted 48 hr +72 hr Refed B
Thin	4.6 ±0.1 (10)	4.2[a] ±0.1 (10)	5.7[a] ±0.1 (7)	4.7 ±0.2 (7)	5.2[b] ±0.1 (10)	5.1 ±0.1 (7)
Obese	7.4 ±0.5 (10)	5.2[c] ±0.3 (10)	6.3 ±0.7 (7)	6.4 ±0.3 (7)	5.5[c] ±0.6 (10)	6.4 ±0.3 (7)
P	<0.001	<0.01	N.S.	<0.001	N.S.	<0.001

Values are calculated as (liver wt/body wt) x 100 and are listed as the means ± SEM of the number of observations in parentheses.

Refed A and Refed B refer to refeeding with laboratory pellets plus 15% glucose and 15% glycerol, respectively, in the drinking water.

[a]Significantly different from the non-treated thins at the $P < 0.02$ level

[b]Significantly different from the non-treated thins at the $P < 0.001$ level.

[c]Significantly different from the non-treated obese at the $P < 0.02$ level.

TABLE 8

LIVER WEIGHTS IN OBESE MICE SUBJECTED TO EXTENDED PERIODS OF FASTING AND REFEEDING

Non-Treated	Fasted 48 hr	Fasted 48 hr +72 hr Refed	Fasted 96 hr	Fasted 96 hr +144 hr Refed
		Absolute Weight (grams)		
4.94 ± 0.24 (10)	3.01[a] ± 0.22 (10)	3.08[a] ± 0.36 (10)	2.60[a] ± 0.10 (5)	4.31 ± 0.29 (5)
		Relative Weight (Heptosomatic Index)		
7.4 ± 0.5 (10)	5.2[b] ± 0.3 (10)	5.5[b] ± 0.6 (10)	5.1[b] ± 0.4 (5)	7.0 ± 0.3 (5)

Values are the means ± SEM of the number of observations in parentheses.

The hepatosomatic index is calculated as (liver wt/body wt) x 100.

Animals were either fasted or refed with laboratory pellets plus 15% glucose in the drinking water.

[a]Significantly different from the non-treated and the fasted 96 hr + 144 hr refed obese groups at the $P < 0.001$ level.

[b]Significantly different from the non-treated and the fasted 96 hr + 144 hr refed obese groups at the $P < 0.02$ level.

TABLE 9

EPIDIDYMAL FAT PAD WEIGHTS IN THIN AND OBESE MICE

	Non-treated	Fasted 48 hr	Fasted 48 hr +24 hr Refed A	Fasted 48 hr +48 hr Refed A	Fasted 48 hr +72 hr Refed A	Fasted 48 hr +72 hr Refed B
Thins	0.73 ±0.13 (10)	0.51 ±0.06 (10)	0.49 ±0.08 (7)	0.71 ±0.04 (7)	0.59 ±0.07 (10)	0.46 ±0.07 (7)
Obese	1.50 ±0.13 (10)	1.36 ±0.11 (10)	1.66 ±0.07 (7)	1.74 ±0.11 (7)	1.54 ±0.10 (10)	1.83 ±0.15 (7)
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values are the means (grams) ± SEM of the number of observations in parentheses.

Refed A and Refed B refer to refeeding with laboratory chow plus 15% glucose and 15% glycerol in the drinking water, respectively.

TABLE 10

PERCENT BODY WEIGHT REPRESENTED BY EPIDIDYMAL FAT PAD IN MICE

	Non-treated	Fasted 48 hr	Fasted 48 hr +24 hr Refed A	Fasted 48 hr +48 hr Refed A	Fasted 48 hr +72 hr Refed A	Fasted 48 hr +74 hr Refed B
Thin	2.5 ±0.3 (10	1.9 ±0.2 (10	1.6 ±0.2 (7)	2.4 ±0.1 (7)	1.8 ±0.2 (10)	1.7 ±0.2 (7)
Obese	2.2 ±0.2 (10)	2.3 ±0.2 (10)	2.9 ±0.2 (7)	2.9 ±0.2 (7)	2.8 ±0.2 (10)	3.1 ±0.3 (7)
P	N.S.	N.S.	<0.001	<0.05	<0.001	<0.01

Values are the means ± SEM of the number of observations in parentheses

Values are calculated as (epididymal fat pad wt/body wt) x 100.

Refed A and Refed B refer to refeeding with laboratory pellets plus 15% glucose and 15% glycerol in the drinking water, respectively.

TABLE 11

EPIDIDYMAL FAT PAD WEIGHTS IN OBESE MICE SUBJECTED TO EXTENDED PERIODS OF FASTING AND REFEEDING

Non-Treated	Fasted 48 hr	Fasted 48 hr +72 hr Refed	Fasted 96 hr	Fasted 96 hr +144 hr Refed
Absolute Weight (grams)				
1.50 ± 0.13 (10)	1.36 ± 0.11 (10)	1.54 ± 0.10 (10)	1.59 ± 0.12 (4)	1.47 ± 0.08 (5)
Relative Weight (Epididymal Fat Pad - Somatic Index)				
2.2 ± 0.2 (10)	2.3 ± 0.2 (10)	2.8 ± 0.2 (10)	3.1 ± 0.1 (4)	2.4 ± 0.1 (5)

Values are the means ± SEM of the number of observations in parentheses.

The epididymal fat pad-somatic index is calculated as (epididymal fat pad weight/body wt) x 100. Animals were either fasted or refed with laboratory pellets plus 15% glucose in the drinking water.

TABLE 12

LIVER GLYCOGEN CONTENT IN THIN AND OBESE MICE

	Non-Treated	Fasted 48 hr	Fasted 48 hr +72 hr Refed
	Milligram Glycogen Per Gram Liver		
Thin	43.50 ± 4.24 (15)	6.53[a] ± 2.80 (8)	38.30 ± 3.78 (9)
Obese	38.50 ± 1.93 (15)	16.76[b] ± 2.05 (7)	44.18 ± 7.01 (9)
P	N.S.	<0.02	N.S.
	Milligram Glycogen Per Liver		
Thin	61.40 ± 6.28 (15)	6.67[a] ± 2.65 (8)	55.40 ± 0.75 (9)
Obese	152.00 ±12.13 (15)	48.70[b] ± 8.78 (7)	136.99 ±32.99 (9)
P	<0.001	<0.001	<0.05

Values are the means of the number of observations in the parentheses ± SEM. Animals were refed with laboratory pellets plus 15% glucose in the drinking water.

[a]Significantly different from the non-treated and fasted-refed thins at the <0.001 level.

[b]Significantly different from the non-treated and fasted-refed obese at the 0.05 > P > 0.02 level.

TABLE 13

EPIDIDYMAL FAT PAD GLYCOGEN CONTENT IN THIN AND OBESE MICE

	Non-Treated	Fasted 48 hr	Fasted 48 hr +72 hr Refed
	Milligram Glycogen Per Gram Tissue		
Thin	0.076 ± 0.038 (11)	0.043 ± 0.044 (7)	1.46[a] ± 0.46 (9)
Obese	0.089 ± 0.020 (13)	0.045 ± 0.020 (6)	0.071 ± 0.024 (10)
P	N.S.	N.S.	<0.01
	Milligram Glycogen Per Epididymal Fat Pad Pair		
Thin	0.035 ± 0.017 (11)	0.008 ± 0.008 (7)	0.465[a] ± 0.134 (9)
Obese	0.168 ± 0.037 (13)	0.071 ± 0.037 (6)	0.076 ± 0.024 (10)
P	<0.01	N.S.	<0.02

Values are the means of the number of observations in the parentheses ± SEM. Animals were refed with laboratory pellets plus 15% glucose in the drinking water.

[a]Significantly different from the non-treated thins at the $P < 0.01$ level.

TABLE 14

HEPATIC ENZYME ACTIVITIES IN NON-TREATED OBESE AND THIN MICE

	Thin	Obese	P
G6PDH	0.00669 ± 0.00075 (10)	0.00841 ± 0.00048 (10)	N.S.
Malic Enz.	0.0229 ± 0.0018 (4)	0.0889 ± 0.0074 (5)	<0.001
AGPDH	0.204 ± 0.011 (10)	0.329 ± 0.021 (10)	<0.001
Glycerol Kinase	0.00394 ± 0.00018 (7)	0.00553 ± 0.00062 (9)	<0.05
LDH	1.54 ± 0.05 (10)	2.14 ± 0.10 (10)	<0.001
G-6Pase	0.297 ± 0.018 (8)	0.384 ± 0.016 (8)	<0.01

Values are the means of the number of observations in parentheses ± SEM. One unit of enzyme activity is defined as micromoles product turned over per minute per milligram supernatant protein, except for glucose-6-phosphatase where the unit is defined as micrograms inorganic phosphorous released per microgram nitrogen.

TABLE 15

HEPATIC GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITIES IN THIN AND OBESE MICE

	Non-treated	Fasted 48 hr	Fasted 48 hr +24 hr Refed A	Fasted 48 hr +48 hr Refed A	Fasted 48 hr +72 hr Refed A	Fasted 48 hr +72 hr Refed B
Per Milligram Supernatant Protein						
Thin	0.00669 ±0.00075 (10)	0.00492[a] ±0.00027 (10)	0.00549 ±0.00030 (7)	0.0119[a] ±0.0022 (7)	0.0220[b] ±0.0027 (10)	0.0125[b] ±0.0016 (7)
Obese	0.00841 ±0.00048 (10)	0.0104 ±0.0021 (10)	0.0105 ±0.0008 (7)	0.0115 ±0.0009 (7)	0.0111[c] ±0.0011 (10)	0.0142[d] ±0.0008 (7)
P	N.S.	<0.02	<0.001	N.S.	<0.01	N.S.
Per Liver						
Thin	1.13 ±0.22 (10)	0.609[a] ±0.044 (10)	0.838 ±0.063 (7)	1.72 ±0.36 (7)	3.75[b] ±0.36 (10)	1.83 ±0.36 (7)
Obese	3.91 ±0.32 (10)	3.06 ±0.41 (10)	3.37 ±0.46 (7)	3.79 ±0.33 (7)	3.26 ±0.48 (10)	5.32[d] ±0.26 (7)
P	<0.001	<0.001	<0.001	<0.01	N.S.	<0.001

Values are the means of the number of observations in parentheses ± SEM. Refed A and Refed B refer to refeeding with laboratory pellets plus 15% glucose and 15% glycerol in the drinking water, respectively. One unit of enzyme activity is defined as micromoles NADPH generated per minute.

[a] Significantly different from the non-treated thins at $0.05 > P > 0.02$ level.

[b] Significantly different from the non-treated thins at the $P < 0.01$ level.

[c] Significantly different from the non-treated obese of the $P < 0.05$ level.

[d] Significantly different from the non-treated obese at the $P < 0.01$ level.

TABLE 16

HEPATIC MALIC ENZYME ACTIVITIES IN THIN AND OBESE MICE

	Non-Treated	Fasted 48 hr	Fasted 48 hr +72 hr Refed A	Fasted 48 hr +72 hr Refed B
Per Milligram Supernatant Protein				
Thin	0.0229 ±0.0018 (4)	0.0223 ±0.0031 (6)	0.0918[a] ±0.0092 (5)	0.0636[a] ±0.0073 (7)
Obese	0.0889 ±0.0074 (5)	0.0679[c] ±0.0027 (5)	0.0788 ±0.0154 (7)	0.0915 ±0.0046 (7)
P	<0.001	<0.001	N.S.	<0.01
Per Liver				
Thin	2.98 ±0.31 (4)	2.76 ±0.48 (6)	15.6[a] ±1.1 (5)	10.6[a] ±1.1 (7)
Obese	44.7 ±3.5 (5)	20.8[b] ±1.5 (5)	27.6[b] ±3.1 (7)	34.7 ±2.9 (7)
P	<0.001	<0.001	<0.01	<0.001

Values are the means of the number of observations in parentheses ± SEM. Refed A and Refed B refer to refeeding with laboratory pellets plus 15% glucose and 15% glycerol in the drinking water, respectively. One unit of enzyme activity is defined as micromoles of NADPH generated per minute.

[a]Significantly different from the non-treated thins at the $P < 0.001$ level.

[b/c]Significantly different from the non-treated obese at the $P < 0.01$ and $P < 0.05$ levels respectively.

TABLE 17

HEPATIC ALPHA-GLYCEROPHOSPHATE DEHYDROGENASE ACTIVITIES IN THIN AND OBESE MICE

	Non-treated	Fasted 48 hr	Fasted 48 hr +24 hr Refed A	Fasted 48 hr +48 hr Refed A	Fasted 48 hr +72 hr Refed A	Fasted 48 hr +72 hr Refed B
			Per Milligram Supernatant Protein			
Thin	0.204 ±0.011 (10)	0.199 ±0.011 (10)	0.284[a] ±0.009 (7)	0.305[a] ±0.009 (7)	0.234 ±0.021 (10)	0.389[a] ±0.044 (7)
Obese	0.329 ±0.021 (10)	0.280 ±0.020 (10)	0.521[c] ±0.035 (7)	0.449[d] ±0.024 (7)	0.377 ±0.032 (10)	0.737[c] ±0.024 (7)
P	<0.001	<0.01	<0.001	<0.001	<0.01	<0.001
			Per Liver			
Thin	32.1 ±2.6 (10)	24.4[b] ±1.4 (10)	43.4[b] ±2.6 (7)	43.6[b] ±2.9 (7)	41.1 ±4.1 (10)	62.7[a] ±6.2 (7)
Obese	154 ±13 (10)	90.7[d] ±9.3 (10)	166 ±23 (7)	149 ±13 (7)	117 ±17 (10)	278[c] ±14 (7)
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values are the means of the number of observations in parentheses ± SEM. Refed A and Refed B refer to refeeding with laboratory pellets plus 15% glucose and 15% glycerol in the drinking water, respectively. One unit of enzyme activity is defined as a micromole of NADH oxidized per minute.

[a/b]Significantly different from the non-treated thins at the $P < 0.001$ and $P < 0.02$ levels respectively.

[c/d]Significantly different from the non-treated obese at the $P < 0.001$ and $P < 0.02$ levels respectively.

TABLE 18

HEPATIC GLYCEROL KINASE ACTIVITIES IN THIN AND OBESE MICE

	Non-treated	Fasted 48 hr	Fasted 48 hr +24 hr Refed A	Fasted 48 hr +48 hr Refed A	Fasted 48 hr +72 Refed A	Fasted 48 hr +72 hr Refed B
Per Milligram Supernatant Protein						
Thin	0.00394 ±0.00018 (7)	0.00406 ±0.00043 (9)	0.00574[a] ±0.00037 (7)	0.00628[a] ±0.00021 (7)	0.00479[b] ±0.00032 (10)	0.00472 ±0.00041 (6)
Obese	0.00553 ±0.00062 (9)	0.00609 ±0.00046 (10)	0.00855[c] ±0.00030 (7)	0.00759[d] ±0.00033 (7)	0.00630 ±0.00046 (10)	0.00734 ±0.00075 (7)
P	<0.05	<0.01	<0.001	<0.01	<0.02	<0.02
Per Liver						
Thin	0.642 ±0.089 (7)	0.486 ±0.045 (9)	0.865[b] ±0.045 (7)	0.896[b] ±0.054 (7)	0.839 ±0.044 (10)	0.756 ±0.048 (6)
Obese	2.52 ±0.25 (9)	1.98 ±0.26 (10)	2.70 ±0.31 (7)	2.51 ±0.17 (7)	1.93 ±0.30 (10)	2.73 ±0.24 (7)
P	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001

Values are the means of the number of observations in parentheses ± SEM. Refed A and Refed B refer to refeeding with laboratory pellets plus 15% glucose and 15% glycerol in the drinking water, respectively. One unit of enzyme activity is defined as micromoles of NADH generated per minute.

[a/b]Significantly different from the non-treated thins at the $P < 0.001$ and $P < 0.05$ levels respectively.

[c/d]Significantly different from the non-treated obese at the $P < 0.001$ and $P < 0.01$ levels respectively.

TABLE 19

HEPATIC LACTIC DEHYDROGENASE ACTIVITIES IN THIN AND OBESE MICE

	Non-treated	Fasted 48 hr	Fasted 48 hr +24 hr Refed A	Fasted 48 hr +48 hr Refed A	Fasted 48 hr +72 hr Refed A	Fasted 48 hr +72 hr Refed B
Per Milligram Supernatant Protein						
Thin	1.54 ±0.05 (10)	1.39 ±0.07 (10)	1.60 ±0.07 (7)	2.13[a] ±0.20 (7)	1.50 ±0.04 (10)	1.73 ±0.14 (7)
Obese	2.14 ±0.10 (10)	2.09 ±0.10 (10)	2.17 ±0.13 (7)	2.45 ±0.17 (7)	2.15 ±0.15 (9)	2.57[b] ±0.08 (7)
P	<0.001	<0.001	<0.01	<N.S.	<0.001	<0.001
Per Liver						
Thin	248 ±23 (10)	171[a] ± 8 (10)	241 ±10 (7)	302 +29 (7)	262 + 8 (10)	279 +17 (7)
Obese	989 ±49 (10)	671[b] ±70 (10)	678[b] ±74 (7)	818 ±76 (7)	630 ±92 (9)	963 ±23 (7)
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values are the means of the number of observations in parentheses ± SEM. Refed A and Refed B refer to refeeding with laboratory pellets plus 15% glucose and 15% glycerol in the drinking water, respectively. One unit of enzyme activity is defined as micromoles of NADH oxidized per minute.

[a]Significantly different from the non-treated thins at the $P < 0.02$ level.

[b]Significantly different from the non-treated obese at the $P < 0.01$ level.

TABLE 20

HEPATIC GLUCOSE-6-PHOSPHATASE ACTIVITIES IN THIN AND OBESE MICE

	Thin	Obese	P
Non-Treated	0.297 ± 0.018 (8)	0.384 ± 0.016 (8)	0.01
Fasted 48 hrs	0.308 ± 0.027 (5)	0.354 ± 0.036 (5)	N.S.
Fasted 48 hrs +72 hrs Refed	0.313 ± 0.009 (7)	0.350 ± 0.27 (7)	N.S.

Values are the means of the number of observations in parentheses ± SEM. The animals were either fasted or refed with laboratory pellets plus 15% glucose in the drinking water. One unit of enzyme activity is defined as micrograms inorganic phosphorous released per 15 minutes per microgram of nitrogen.

TABLE 21

HEPATIC ENZYME ACTIVITIES IN OBESE MICE SUBJECTED TO EXTENDED PERIODS OF FASTING AND REFEEDING

	Non-Treated	Fasted 96 hr	Fasted 96 hr + 144 hr Refed
	Per Milligram Supernatant Protein		
G6PDH	0.00841 ± 0.00048 (10)	0.0111 ± 0.0024 (5)	0.0111 ± 0.0010 (5)
AGPDH	0.329 ± 0.021 (10)	0.333 ± 0.011 (5)	0.363 ± 0.017 (5)
Glycerol Kinase	0.00553 ± 0.00062 (9)	0.00929[a] ±0.0014 (5)	0.00552 ± 0.00022 (5)
LDH	2.14 ± 0.10 (10)	2.23 ± 0.20 (5)	2.05 ± 0.12 (4)
	Per Liver		
G6PDH	3.91 ± 0.22 (10)	2.04[b] ± 0.29 (5)	4.95 ± 0.78 (5)
AGPDH	154 ±13 (10)	67.2[b] ± 8.2 (5)	157 ±10 (5)
Glycerol Kinase	2.52 ± 0.25 (9)	1.76[a] ± 0.15 (5)	2.39 ± 0.13 (5)
LDH	989 ±49 (10)	441[a] ±52 (5)	1360 ±495 (5)

Values are the means of the number of observations in parentheses ± SEM. Animals were fasted and refed with laboratory pellets plus 15% glucose in the drinking water.

One unit of enzyme activity is defined as micromoles of substrate either oxidized or reduced per minute.

[a/b]Significantly different from non-treated obese at the $P < 0.05$ and $P < 0.001$ levels respectively.

TABLE 22

EPIDIDYMAL FAT PAD GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITIES IN THIN AND OBESE MICE

	Non-treated	Fasted 48 hr	Fasted 48 hr +24 hr Refed A	Faster 48 hr +48 hr Refed A	Fasted 48 hr +72 hr Refed A	Fasted 48 hr +72 hr Refed B
			Per Milligram Supernatant Protein			
Thin	0.210 ±0.010 (10)	0.237 ±0.031 (10)	0.226 ±0.008 (7)	0.227 ±0.010 (7)	0.357[a] ±0.038 (10)	0.176 ±0.032 (7)
Obese	0.311 ±0.021 (10)	0.327 ±0.020 (10)	0.277 ±0.011 (7)	0.327 ±0.022 (7)	0.356 ±0.018 (10)	0.284 ±0.009 (7)
P	<0.001	<0.05	<0.01	<0.01	N.S.	<0.01
			Per Fat Pad Pair			
Thin	0.755 ±0.086 (10)	0.681 ±0.061 (10)	0.840 ±0.117 (7)	1.16[a] ±0.07 (7)	1.30[a] ±0.09 (10)	0.482[b] ±0.080 (7)
Obese	3.45 ±0.34 (10)	2.99 ±0.15 (10)	3.37 ±0.18 (7)	4.54 ±0.53 (7)	3.30 ±0.34 (10)	3.79 ±0.37 (7)
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values are the means ± SEM. Refed A and Refed B refer to refeeding with laboratory pellets plus 15% glucose and 15% glycerol in the drinking water respectively. One unit of enzyme activity is defined as micromoles of NADPH generated per minute.

[a/b] Significantly different from the non-treated thins at the $P < 0.01$ and $P < 0.05$ levels, respectively.

TABLE 23

EPIDIDYMAL FAT PAD MALIC ENZYME ACTIVITIES IN THIN AND OBESE MICE

	Non-treated	Fasted 48 hr	Fasted 48 hr +72 hr Refed A	Fasted 48 hr +72 hr Refed B
		Per Milligram Supernatant Protein		
Thin	0.0471 ±0.0029 (5)	0.0358 ±0.0045 (3)	0.127[a] ±0.011 (5)	0.0376 ±0.0041 (5)
Obese	0.0216 ±0.0012 (5)	0.0173 ±0.0021 (5)	0.0192 ±0.0010 (5)	0.0236 ±0.0011 (6)
P	<0.001	<0.01	<0.001	<0.01
		Per Fat Pad Pair		
Thin	0.239 ±0.035 (5)	0.134[c] ±0.010 (3)	0.636[a] ±0.047 (5)	0.121[b] ±0.011 (5)
Obese	0.239 ±0.030 (5)	0.167 ±0.026 (5)	0.150[c] ±0.014 (5)	0.278 ±0.029 (6)
P	N.S.	N.S.	<0.001	<0.001

Values are the means ± SEM. Refed A and Refed B refer to refeeding with laboratory pellets plus 15% glucose and 15% glycerol in the drinking water, respectively. One unit of enzyme activity is defined as micromoles of NADPH generated per minute.

[a/b]Significantly different from the non-treated thins at the $P < 0.001$ and $P < 0.01$ levels, respectively.

[c]Significantly different from the non-treated obese at the $P < 0.05$ level.

TABLE 24

EPIDIDYMAL FAT PAD ALPHA-GLYCEROPHOSPHATE DEHYDROGENASE ACTIVITIES IN THIN AND OBESE MICE

	Non-treated	Fasted 48 hr	Fasted 48 hr +24 hr Refed A	Fasted 48 hr +48 hr Refed A	Fasted 48 hr +72 hr Refed A	Fasted 48 hr +72 hr Refed B
Per Milligram Supernatant Protein						
Thin	0.122 ±0.110 (10)	0.164 ±0.016 (10)	0.184 ±0.021 (7)	0.242 ±0.030 (7)	0.481[a] ±0.061 (10)	0.245 ±0.039 (7)
Obese	0.141 ±0.033 (10)	0.110 ±0.011 (10)	0.150 ±0.011 (7)	0.212 ±0.014 (7)	0.145 ±0.009 (10)	0.229[a] ±0.008 (7)
P	N.S.	<0.02	N.S.	N.S.	<0.001	N.S.
Per Fat Pad Pair						
Thin	0.477 ±0.084 (10)	0.583 ±0.081 (10)	0.696 ±0.141 (7)	1.23[b] ±0.14 (7)	1.73[b] ±0.10 (10)	0.738 ±0.165 (7)
Obese	1.57 ±0.18 (10)	0.967[a] ±0.110 (10)	1.82 ±0.18 (7)	3.07[a] ±0.48 (7)	1.36 ±0.17 (10)	3.04[b] ±0.26 (7)
P	<0.001	<0.02	<0.001	<0.01	N.S.	<0.001

Values are the means of the number of observations in parentheses ± SEM. Refed A and Refed B refer to refeeding with laboratory pellets plus 15% glucose and 15% glycerol in the drinking water, respectively.

One unit of enzyme activity is defined as micromoles of NADH oxidized per minute.

[a]Significantly different from the appropriate non-treated value at $P < 0.02$ level.

[b]Significantly different from the appropriate non-treated value at the $P < 0.001$ level.

TABLE 25

EPIDIDYMAL FAT PAD LACTIC DEHYDROGENASE ACTIVITIES IN THIN AND OBESE MICE

	Non-treated	Fasted 48 hr	Fasted 48 hr +24 hr Refed A	Fasted 48 hr +48 hr Refed A	Fasted 48 hr +72 hr Refed A	Fasted 48 hr +72 hr Refed B
			Per Milligram Supernatant Protein			
Thin	1.42 ±0.09 (10)	1.39 ±0.18 (10)	1.42 ±0.04 (7)	1.31 ±0.04 (7)	2.02[a] ±0.15 (10)	1.40 ±0.07 (5)
Obese	1.34 ±0.02 (10)	1.19[c] ±0.06 (10)	1.43 ±0.12 (7)	1.26 ±0.07 (7)	1.41[c] ±0.02 (10)	1.52[d] ±0.03 (7)
P	N.S.	N.S.	N.S.	N.S.	<0.001	N.S.
			Per Fat Pad Pair			
Thin	5.34 ±0.68 (10)	3.97 ±0.30 (10)	5.17 ±0.54 (7)	6.66 ±0.36 (7)	7.36[b] ±0.56 (10)	4.56 ±0.37 (5)
Obese	14.8 ±1.0 (10)	10.5 ±0.9 (10)	17.1 ±1.1 (7)	15.9 ±2.1 (7)	12.8 ±1.0 (10)	20.3[c] ±1.8 (7)
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values are the means of the number of observations in parenthesis ± SEM. Refed A and Refed B refer to refeeding with laboratory pellets plus 15% glucose and 15% glycerol in the drinking water respectively. One unit of enzyme activity is defined as micromoles as NADH oxidized per minute.

[a/b] Significantly different from the non-treated thins at the $P < 0.01$ and $P < 0.05$ levels, respectively.

[c/d] Significantly different from the non-treated obese at the $P < 0.05$ and $P < 0.001$ levels, respectively.

TABLE 26

EPIDIDYMAL FAT PAD ENZYME ACTIVITIES IN OBESE MICE SUBJECTED TO EXTENDED PERIODS OF FASTING AND REFEEDING

	Non-Treated	Fasted 96 hr	Fasted 96 hr + 144 hr Refed
	Per Milligram Supernatant Protein		
G6PDH	0.311 ± 0.021 (10)	0.358 ± 0.038 (5)	0.246 ± 0.031 (5)
AGPDH	0.141 ± 0.033 (10)	0.156 ± 0.032 (5)	0.177 ± 0.011 (5)
LDH	1.34 ± 0.02 (10)	1.31 ± 0.20 (5)	1.34 ± 0.14 (5)
	Per Fat Pad Pair		
G6PDH	3.45 ± 0.34 (10)	2.70 ± 0.24 (5)	3.17 ± 0.19 (5)
AGPDH	1.57 ± 0.18 (10)	1.18 ± 0.24 (5)	2.38 ± 0.21 (5)[a]
LDH	14.8 ± 1.0 (10)	9.89 ± 1.36 (5)[a]	17.5 ± 0.9 (5)

Values are the means of the number of observations in parentheses ± SEM.

Animals were fasted and refed with laboratory pellets plus 15% glucose in the drinking water. One unit of enzyme activity is defined as micromoles of substrate oxidized or reduced per minute.

[a]Significantly different from the non-treated value at the $P < 0.02$ level.

TABLE 27

BODY WEIGHT OF ALLOXAN-TREATED THIN AND OBESE MICE

	Before Treatment	After Treatment	P
Thin	30.4 ± 0.9 (9)	29.1 ± 0.8 (9)	N.S.
Obese	57.3 ± 1.4 (10)	55.8 ± 1.5 (10)	N.S.
P	<0.001	<0.001	

Values are the means (grams) of the number of observations in parentheses ± SEM.

All the animals were injected intraperitoneally with 5 mg of alloxan in 0.5 ml of 0.9% sodium chloride and killed 72 hr later as described in Materials and Methods.

TABLE 28

WEIGHT LOST AFTER ALLOXAN TREATMENT IN THIN AND OBESE MICE

	Grams Lost	Percent Lost
Thin	1.3 ± 0.2 (9)	4.1 ± 0.9 (9)
Obese	2.0 ± 0.3 (8)	3.6 ± 0.5 (8)
P	N.S.	N.S.

Values are the means of the number of observations in parentheses ± SEM. Animals were weighed prior to alloxan treatment and 72 hr after treatment. All the animals were injected intraperitoneally with 5 mg of alloxan.

TABLE 29

LIVER AND EPIDIDYMAL FAT PAD WEIGHTS IN THIN AND OBESE MICE AFTER ALLOXAN TREATMENT

	Liver Weight (grams)	Liver/B.W. X 100
Thin	1.33 ± 0.03 (9)	4.6 ± 0.1 (9)
Obese	3.91 ± 0.38 (10)	7.0 ± 0.4 (10)
P	<0.001	<0.001
	Epididymal Fat Pad Weight (grams)	**Fat Pad/B.W. X 100**
Thin	0.57 ± 0.04 (9)	2.0 ± 0.1 (9)
Obese	2.30 ± 0.07 (10)	4.2 ± 0.2 (10)
P	<0.001	<0.001

Values are the means of the number of observations in parentheses ± SEM. Animals were injected intraperitoneally with 5 mg of alloxan and killed 72 hr later.

TABLE 30

HEPATIC ENZYME ACTIVITIES IN ALLOXAN-TREATED THIN MICE

	Non-Treated	Alloxan-Treated	P
	Per Milligram Supernatant Protein		
G6PDH	0.00669 ± 0.00075 (10)	0.00606 ± 0.00061 (9)	N.S.
AGPDH	0.204 ± 0.011 (10)	0.268 ± 0.011 (9)	<0.001
Glycerol Kinase	0.00394 ± 0.00018 (7)	0.00370 ± 0.00027 (9)	N.S.
LDH	1.54 ± 0.05 (10)	1.27 ± 0.05 (9)	<0.01
	Per Liver		
G6PDH	1.13 ± 0.22 (10)	0.966 ± 0.085 (9)	N.S.
AGPDH	32.1 ± 2.6 (10)	43.2 ± 2.0 (9)	<0.01
Glycerol Kinase	0.642 ± 0.089 (7)	0.589 ± 0.043 (9)	N.S.
LDH	248 ± 23 (10)	204 ± 7 (9)	N.S.

Values are the means of the number of observations in parentheses ± SEM. Animals were injected intraperitoneally with 5 mg of alloxan and killed, 72 hr later. One unit of enzyme activity is defined as micromoles of substrate either oxidized or reduced per minute.

TABLE 31

HEPATIC ENZYME ACTIVITIES IN ALLOXAN-TREATED OBESE MICE

	Non-Treated	Alloxan-Treated	P
	Per Milligram Supernatant Protein		
G6PDH	0.00841 ± 0.00048 (10)	0.00629 ± 0.00042 (10)	<0.01
AGPDH	0.329 ± 0.021 (10)	0.428 ± 0.022 (10)	<0.01
Glycerol Kinase	0.00553 ± 0.00062 (9)	0.00527 ± 0.00032 (10)	N.S.
LDH	2.14 ± 0.10 (10)	1.63 ± 0.10 (10)	<0.01
	Per Liver		
G6PDH	3.91 ± 0.31 (10)	2.59 ± 0.21 (10)	<0.01
AGPDH	154 ± 13 (10)	175 ± 10 (10)	N.S.
Glycerol Kinase	2.52 ± 0.25 (9)	2.15 ± 0.15 (10)	N.S.
LDH	989 ± 49 (10)	667 ± 48 (10)	<0.001

Values are the means of the number of observations in parentheses ± SEM. Animals were injected intraperitoneally with 5 mg of alloxan and killed 72 hr later. One unit of enzyme activity is defined as micromoles of substrate either oxidized or reduced per minute.

TABLE 32

EPIDIDYMAL FAT PAD ENZYME ACTIVITIES IN ALLOXAN-TREATED THIN MICE

	Non-Treated	Alloxan-Treated	P
	Per Milligram Supernatant Protein		
G6PDH	0.210 ± 0.010 (10)	0.182 ± 0.005 (9)	0.05
AGPDH	0.122 ± 0.110 (10)	0.186 ± 0.010 (9)	N.S.
LDH	1.42 ± 0.09 (10)	1.02 ± 0.05 (9)	0.01
	Per Fat Pad Pair		
G6PDH	0.755 ± 0.086 (10)	0.723 ± 0.068 (9)	N.S.
AGPDH	0.477 ± 0.084 (10)	0.752 ± 0.089 (9)	0.05
LDH	5.34 ± 0.68 (10)	3.98 ± 0.31 (9)	N.S.

Values are the means of the number of observations in parentheses ± SEM. Animals were injected intraperitoneally with 5 mg of alloxan and killed 72 hr later. One unit of enzyme activity is defined as micromoles of substrate either oxidized or reduced per minute.

TABLE 33

EPIDIDYMAL FAT PAD ENZYME ACTIVITIES IN ALLOXAN-TREATED OBESE MICE

	Non-Treated	Alloxan-Treated	P
	Per Milligram Supernatant Protein		
G6PDH	0.311 ± 0.021 (10)	0.305 ± 0.009 (10)	N.S.
AGPDH	0.141 ± 0.033 (10)	0.252 ± 0.023 (10)	<0.02
LDH	1.34 ± 0.02 (10)	1.35 ± 0.04 (10)	N.S.
	Per Fat Pad Pair		
G6PDH	3.45 ± 0.34 (10)	5.93 ± 0.19 (10)	<0.001
AGPDH	1.57 ± 0.18 (10)	4.84 ± 0.44 (10)	<0.001
LDH	14.8 ± 1.0 (10)	26.3 ± 0.9 (10)	<0.001

Values are the means of the number of observations in parentheses ± SEM. Animals were injected intraperitoneally with 5 mg of alloxan and killed 72 hr later. One unit of enzyme activity is defined as micromoles of substrate either oxidized or reduced per minute.

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