Lipogenesis from glucose and pyruvate in fat cells from genetically obese rats

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ABSTRACT Subcutaneous fat cells were isolated from genetically obese rats and from rats with obesity produced by hypothalamic lesions. Insulin did not augment the oxidation of fatty acids or their synthesis from glucose-1-^{14}C or glucose-1-^{3}H by fat cells from either group. Radioactivity from pyruvate-3-^{14}C was incorporated into fatty acids to the same degree by fat cells from these two groups.

The presence of 5 mM glucose in the incubation medium containing fat cells and pyruvate-3-^{14}C or aspartate-3-^{14}C stimulated the synthesis of fatty acids to a greater extent in cells of genetically obese rats. Fasting, in contrast, reduced the incorporation of radioactivity from pyruvate and glucose into fatty acids by fat cells from the genetically obese animals. In all experiments the fat cells from genetically obese rats converted more radioactivity into glyceride-glycerol relative to CO_2 than did fat cells from hypothalamic obese rats.

Parabiosis between one thin and one genetically obese litter mate was performed in three pairs of rats without influencing growth of either rat.

Thus in the present studies fat cells from genetically obese rats showed two differences from normal fat cells: they channeled more radioactivity from pyruvate into fatty acids in the presence of glucose, and they uniformly converted more radioactivity into glyceride-glycerol.

SUPPLEMENTARY KEY WORDS parabiosis · oxidation · glycerol synthesis · hypothalamic obese controls

THE INVESTIGATION of human disease has often benefited from the development of animal models and in the area of research on obesity, several such models are available. Obesity as an inherited trait has been described in several strains of mice (1–3) and in one strain of rats (4). It can also be produced by destruction of the ventromedical nuclei in the hypothalamus either with an electrical current (5) or by using gold thioglucose (6). A third experimental model for the study of obesity is available in animals with transplanted ACTH-producing tumors (7). A review of some of the differences between these forms of experimentally induced obesity has been published recently (8).

The present studies on experimental obesity have concentrated on a strain of rats in which obesity is inherited as an autosomal Mendelian recessive trait (4). Previous studies on these rats by Zucker and Zucker (9) have shown an unusual degree of hyperlipemia but normal levels of plasma glucose. Mobilization of the excessive quantities of adipose tissue in these animals occurs normally with fasting (10), and the release of free fatty acids from adipose cells in vitro, in response to epinephrine, is the same in genetically obese rats and in rats with obesity induced by destruction of the ventromedial hypothalamic nuclei (11).

This report extends our observations on this group of rats to some aspects of lipogenesis in the subcutaneous cells of the genetically obese rats and suitably obese control rats with hypothalamic lesions. We have found that the fat cells from the genetically obese rats show two differences from fat cells of hypothalamic obese rats. A preliminary publication of some of these data has already appeared (11).

METHODS AND MATERIALS

Animals

The genetically obese rats used in these experiments were bred in our laboratory from heterozygous parents, obtained through the kindness of Dr. Lois Zucker of the Harriet G. Bird Memorial Laboratory, Stow, Mass. They were fed Purina laboratory chow (Ralston Purina Co., St. Louis, Mo.) or a high- carbohydrate diet (Nutritional Biochemicals Corporation, Cleveland, Ohio). Most

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studies were conducted on female rats between 3 and 6 months of age weighing 400–600 g. Control obese rats with hypothalamic lesions were prepared from thin rats of the same strain as the genetic obese rats, or from female albino rats that were not gaining weight, purchased from Charles River Breeding Laboratories (Wilmington, Mass.). Bilateral lesions in the ventromedial nuclei were produced by applying a 2 ma current for 15–30 sec through a platinum needle electrically insulated, except for its tip, which had been placed in the hypothalamus according to the coordinates of DeGroot (12). Obesity developed in 10–20% of the strain with genetic obesity and 50–60% of the albino rats with hypothalamic lesions. Three pairs of rats were parabiosed by suturing litter mates of the same sex together along a lateral incision that included the peritoneum.

Incubation Procedures

Subcutaneous adipose tissue was removed under light ether anesthesia from four genetic and four hypothalamic obese rats. For each experiment adipose tissue was removed from corresponding sites on each animal. Adipose tissue was obtained from no more than one genetically obese albino rat in any given experiment, the other three being hypothalamic obese rats of the Zucker strain. Isolated fat cells were prepared separately from each biopsy by the procedure of Rodbell (13), except that albumin was omitted from the incubation to permit determination of nitrogen on aliquots of cells, since omitting albumin does not affect the metabolism of fat cells (14).

After the biopsy specimens had been washed four or five times with buffer to remove the excess collagenase (Worthington Biochemicals, Freehold, N.J.), 0.1–0.3 ml portions of fat cells from each specimen were placed in siliconized vessels containing 1 ml of Krebs–Ringer bicarbonate buffer with 40 mg/ml of albumin (Fraction V, Armour Pharmaceuticals, Chicago, Ill.) and either 2 mM glucose (uniformly labeled or labeled in the 1-position with tritium or carbon), 40 mM pyruvate-3-^{14}C (Table 3, below), 5 mM pyruvate-3-^{14}C (Tables 4 and 6, below), or 5 mM aspartate-3-^{14}C. Radioactive substrates were used as supplied (New England Nuclear Corp., Boston, Mass.); they were stated by the suppliers to be chromatographically pure. After the cells had been incubated for 1 hr at 37°C under 95% oxygen–5% CO_2, the radioactive carbon dioxide was collected by the addition of 0.5 ml of 0.5 N sulfuric acid to the incubation medium and 0.5 ml of 1.0 M Hyamine [*p*-(diisobutylcresoxy-ethoxyethyl) dimethyl benzylamine, Packard Instrument Co., LaGrange, Ill.] to a polyethylene cup suspended from the rubber cap. After shaking had continued for an additional hour the polyethylene cup was removed, carefully rinsed, and transferred to counting solution for radioactive assay (15). The total lipid in the incubation medium and cells was extracted by the method of Dole (16), and the heptane phase was washed three times with 3 ml of 0.1 N NaOH. 1 ml of the washed heptane layer was counted to determine the radioactivity in the triglyceride fraction, and the remainder was hydrolyzed for 1 hr at 60°C in 10% alcoholic KOH. The hydrolysate was acidified and the fatty acids were extracted three times with equal volumes of heptane. These heptane washes were pooled and evaporated, and the residue was counted for assay of the radioactivity in the fatty acids. Quenching was corrected for by addition of internal standards. The data were expressed in terms of the quantity of triglyceride in the aliquots of fat cells, which was determined by drying further aliquots to constant weight at 100°C. Triglyceride contents for aliquots of fat cells from genetic and hypothalamic obese rats were the same and averaged 64 mg. The nitrogen contents of subcutaneous fat from genetic and hypothalamic obese rats were identical (1.4 ± 0.19 vs. 1.5 ± 0.15 mg/g). The nitrogen content of fat cells, however, was about 30% less in the genetic obese rats (0.9 vs. 1.2 mg/ml of cells for hypothalamic obese rats).

RESULTS

Parabiosis

Three pairs of litter mates, each containing one genetically obese and one nonobese rat of the same sex, were parabiosed at 5–6 wk of age (80–90 g), which was the earliest age at which the genetically obese rats could first be unequivocally identified. Two pairs lived 2–3 months, and one pair survived 10 months (Fig. 1). In each case, the obese rat became fat and the thin rat remained thin.

Effect of Insulin

Insulin, 0.1 mU/ml, did not stimulate the production of CO_2 from glucose-1-^{14}C or the incorporation of radioactivity into fatty acids by fat cells of genetic or hypothalamic obese rats fed a chow diet (Table 1). Rats fed a high-carbohydrate diet for 4 wk prior to biopsy also did not show any stimulatory effect of insulin on the conversion of glucose-1-^{3}H into fatty acids. In contrast, however, the incorporation of radioactivity from glucose-1-^{3}H into fatty acids by adipose cells was reduced 90% by fasting the rats for 10 days (Table 2).

Metabolism of Pyruvate and Glucose

Conversion of radioactivity from uniformly labeled glucose-^{14}C and pyruvate-3-^{14}C into fatty acids, CO_2, and glyceride-glycerol was observed in fat cells from the two

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TABLE 1 EFFECT OF INSULIN ON THE METABOLISM OF GLUCOSE-1-^{14}C BY FAT CELLS*

	Additions to Medium	Radioactivity Incorporated into CO_2	Glyceride-Glycerol	Fatty Acids	Glyceride-Glycerol/CO_2
		cpm/mg triglyceride/hr			
Hypothalamic	None	57.4 ± 3.1†	47.4 ± 10.2	0.150 ± 0.046	0.83
	Insulin‡	59.6 ± 11.8	41.3 ± 8.0	0.22 ± 0.079	0.69
Genetic	None	38.6 ± 7.2	73.3 ± 11.0	0.148 ± 0.011	1.90
	Insulin‡	37.0 ± 4.9	84.4 ± 6.0	0.162 ± 0.031	2.39

* Adipose cells equivalent to 150–225 mg of triglyceride incubated for 1 hr at 37°C in 1 ml of Krebs–Ringer bicarbonate containing 40 mg of albumin and 2 mM glucose-1-^{14}C.
† Mean ± SEM for four biopsies.
‡ Insulin = 0.1 mU/ml.

groups of rats (Tables 3–5). Glucose enhanced the conversion of radioactivity from pyruvate-3-^{14}C into fatty acids by fat cells of genetic and hypothalamic obese rats (Tables 3 and 4), but the magnitude of the effect was greater in the fat cells from the rats with genetic obesity. Glucose also stimulated a 10-fold increase in the incorporation of radioactivity from aspartate-3-^{14}C into fatty acids by cells from genetically obese rats ($P < 0.05$) and a 4-fold increase in fat cells from hypothalamic obese rats (NS) (Table 4).

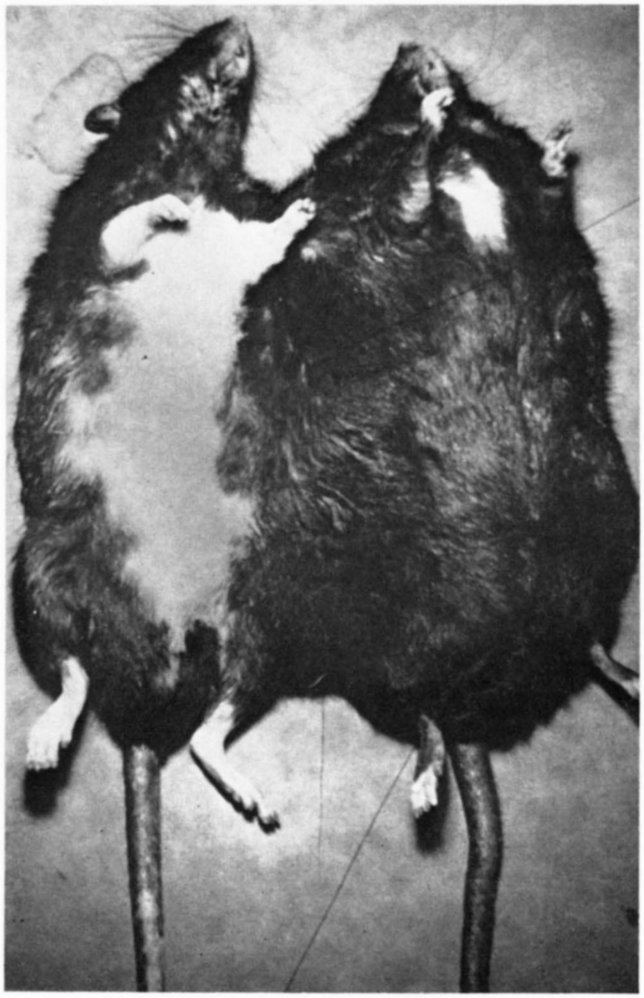

FIG. 1. Parabiosed rats. Male rats from the same litter were parabiosed at 6 wk of age. This picture was taken at 10 months of age.

TABLE 2 EFFECT OF INSULIN AND FASTING ON THE INCORPORATION OF TRITIUM FROM GLUCOSE-1-^{3}H INTO FATTY ACID BY ADIPOSE CELLS FROM GENETICALLY OBESE RATS*

	Additions to Medium	Radioactivity Incorporated into Fatty Acids
		cpm/mg triglyceride/hr
Fed	None	3.12 ± 0.32†
	Insulin‡	3.45 ± 0.28
Fasted	None	0.14 ± 0.04
	Insulin‡	0.13 ± 0.03

* Adipose cells equivalent to 150–225 mg of triglyceride were obtained from rats either fed or fasted for 10 days and incubated for 1 hr at 37°C in 1 ml of Krebs–Ringer bicarbonate containing 40 mg of albumin and 2 mM glucose-1-^{3}H.
† Mean ± SEM.
‡ Insulin = 0.1 mU/ml.

The stimulated incorporation of radioactivity from pyruvate-3-^{14}C into fatty acids occurred with fructose as well as glucose, but citrate, aspartate, and glutamate had no effects (Table 4). In contrast, the metabolism of uniformly labeled glucose-^{14}C (Table 5) was not affected by fructose, aspartate, or glutamate. Pyruvate, however, reduced the incorporation of radioactivity from glucose-^{14}C into glyceride-glycerol (Table 5). In all experiments the ratio of radioactivity incorporated into glyceride-glycerol relative to that incorporated into CO_2 was higher in the fat cells from genetic obese rats (2.2 vs. 1.1).

Effects of Fasting

The effect of fasting on the incorporation of radioactivity was examined in two experiments (Tables 2 and 6). A reduction of more than 90% in the incorporation of radioactivity from glucose-1-^{3}H and pyruvate-3-^{14}C into fatty acids was found in each experiment.

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TABLE 3 METABOLISM OF PYRUVATE-3-^{14}C BY ADIPOSE CELLS*

	Additions to Medium	Incorporation of Radioactivity into CO_2	Glyceride-Glycerol	Fatty Acids	Glyceride-Glycerol/CO_2
		cpm/mg triglyceride/hr			
Hypothalamic	None	1.39 ± 0.49†	1.06 ± 0.29	0.45 ± 0.13	0.76
	Glucose‡	1.65 ± 0.27	1.74 ± 0.31	0.68 ± 0.17	1.05
Genetic	None	1.07 ± 0.08	1.20 ± 0.16	0.33 ± 0.10	1.12
	Glucose‡	1.46 ± 0.54	1.65 ± 0.25	0.74 ± 0.17	1.13

* Adipose cells equivalent to 150–225 mg of triglyceride were incubated for 1 hr at 37°C in 1 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 40 mg of albumin and 40 mM pyruvate-3-^{14}C.
† Mean ± SEM of four biopsies.
‡ Glucose 5.6 mM.

TABLE 4 EFFECT OF SEVERAL NONRADIOACTIVE SUBSTRATES ON THE METABOLISM OF PYRUVATE-3-^{14}C AND ASPARTATE-3-^{14}C BY ADIPOSE CELLS*

	Additions to Medium	Incorporation of Radioactivity into CO_2	Glyceride-Glycerol	Fatty Acids	Glyceride-Glycerol/CO_2
		cpm/mg triglyceride/hr			
Hypothalamic					
Pyruvate-3-^{14}C	None	1.76 ± 0.15†	2.16 ± 0.13	0.056 ± 0.031	1.23
	Glucose‡	2.18 ± 0.52	1.26 ± 0.14	0.155 ± 0.018	0.58
	Fructose‡	1.64 ± 0.15	1.50 ± 0.43	0.200 ± 0.113	0.91
	Citrate‡	1.55 ± 0.22	1.99 ± 0.18	0.079 ± 0.035	1.28
	Aspartate‡	1.78 ± 0.23	1.54 ± 0.08	0.054 ± 0.011	0.87
	Glutamate‡	1.81 ± 0.17	1.47 ± 0.56	0.051 ± 0.034	0.81
Aspartate-3-^{14}C	None	0.42 ± 0.066	0.19 ± 0.036	0.021 ± 0.011	0.45
	Glucose‡	0.47 ± 0.054	0.32 ± 0.093	0.086 ± 0.051	0.68
Genetic					
Pyruvate-3-^{14}C	None	1.27 ± 0.15	2.08 ± 0.42	0.078 ± 0.043	1.64
	Glucose	1.71 ± 0.23	1.67 ± 0.37	0.320 ± 0.091	0.98
	Fructose	1.53 ± 0.11	2.18 ± 0.08	0.160 ± 0.033	1.42
	Citrate	1.46 ± 0.14	3.04 ± 0.35	0.109 ± 0.034	2.08
	Aspartate	1.46 ± 0.18	2.43 ± 0.30	0.082 ± 0.031	1.67
	Glutamate	1.45 ± 0.19	2.43 ± 0.27	0.144 ± 0.066	1.68
Aspartate-3-^{14}C	None	0.408 ± 0.050	0.216 ± 0.011	0.017 ± 0.006	0.53
	Glucose	0.647 ± 0.067	0.570 ± 0.109	0.202 ± 0.060	0.88

* Adipose cells equivalent to 150–225 mg of triglyceride were incubated for 1 hr at 37°C in 1 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 40 mg albumin and either 5 mM pyruvate-3-^{14}C or 5 mM aspartate-3-^{14}C.
† Mean ± SEM for four biopsies.
‡ Glucose, fructose, aspartate, citrate, glutamate 5 mM.

DISCUSSION

In the present experiments we examined two questions about the genesis of obesity in the strain of obese rats called "fatty" (4). In the first experiment we were asking whether the obesity in these rats might result from an excess or deficiency of some circulating substance which could be transmitted between animals. If so, the thin litter mates with peritoneal surfaces contiguous with their obese sibling might remove or supply this missing metabolite. Hervey (17) has observed that induction of obesity with hypothalamic lesions in one parabiont reduces the body fat in the other. Han, Mu, and Lepkovsky (18), who have also examined this question, observed an increased food intake in the animal with the hypothalamic lesion and a small reduction of food intake in the parabiotic partner. The failure of parabiosis, in our experiments, to induce obesity in the thin rat, or to prevent the development of obesity in the "fatty," supports the concept that the genetic expression of obesity in these animals does not involve a readily diffusible substance. However, our experiments do not rule out the possibility that a circulating substance was transiently present at some critical time in the initiation of obesity, prior to parabiosis.

In the second group of experiments we explored the question of whether lipogenesis was abnormal in the fat cells of the genetically obese rats. All experiments were performed on adipose cells from genetically obese rats and compared with results on adipose cells from rats with obesity induced by hypothalamic lesions, the biopsies

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TABLE 5 EFFECT OF SEVERAL NONRADIOACTIVE SUBSTRATES ON THE METABOLISM OF UNIFORMLY LABELED GLUCOSE-^{14}C BY FAT CELLS*

	Additions to Medium	Incorporation of Radioactivity into			Glyceride-Glycerol/ CO_2
		CO_2	Glyceride-Glycerol	Fatty Acids	
		cpm/mg triglyceride/hr			
Hypothalamic	None	22.4 ± 2.3†	59.9 ± 15.3	0.208 ± 0.048	2.67
	Fructose‡	23.8 ± 5.9	43.2 ± 4.1	0.175 ± 0.006	1.82
	Pyruvate‡	20.6 ± 4.7	24.9 ± 5.3	0.230 ± 0.070	1.21
	Aspartate‡	27.1 ± 6.5	53.3 ± 13.1	0.209 ± 0.050	1.97
	Glutamate‡	31.6 ± 5.5	50.9 ± 9.4	0.159 ± 0.046	1.61
Genetic	None	18.6 ± 5.8	56.4 ± 11.1	0.115 ± 0.013	3.03
	Fructose	13.8 ± 1.9	61.8 ± 3.5	0.120 ± 0.024	4.47
	Pyruvate	12.4 ± 0.7	34.7 ± 4.2	0.131 ± 0.012	2.80
	Aspartate	12.5 ± 0.9	64.2 ± 3.1	0.134 ± 0.007	5.13
	Glutamate	11.5 ± 1.4	61.8 ± 11.7	0.108 ± 0.019	5.38

* Adipose cells equivalent to 150–225 mg of triglyceride were incubated for 1 hr at 37°C in 1 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 40 mg of albumin and 5 mM uniformly labeled glucose-^{14}C.
† Mean ± SEM of four observations.
‡ Fructose, pyruvate, aspartate, and glutamate 5 mM.

TABLE 6 EFFECT OF FASTING ON THE METABOLISM OF PYRUVATE-3-^{14}C BY FAT CELLS FROM GENETICALLY OBESE RATS*

	Incorporation of Radioactivity into		
	CO_2	Glyceride-Glycerol	Fatty Acids
	cpm/mg triglyceride/hr		
Fed	10.4 ± 1.2†	23.2 ± 3.2	1.62 ± 0.92
Fasted 8 days	1.3 ± 0.3	4.1 ± 1.0	0.018 ± .004

* Adipose cells equivalent to 225 mg of triglyceride were incubated for 1 hr at 37°C in 1 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 40 mg of albumin and 5 mM pyruvate-3-^{14}C.
† Mean ± SEM for four observations.

for each experiment taken from the same anatomical location. With this latter group as a control we hoped to avoid the pitfalls that might have arisen from comparisons of animals having grossly different quantities of body fat (19). We also hoped to avoid marked discrepancies in the size of individual fat cells (20), since the metabolism of the fat cell is probably related to its cell size (21), and the cell size increases with body fat content. The nitrogen content of subcutaneous fat tissue from the genetic and hypothalamic obese rats was the same, and the triglyceride content of fat cells was similar. Moreover, histologic examination of pieces of subcutaneous fat showed mature fat cells. Thus the use of triglyceride for comparison of these two types of obesity, although not ideal, appears to be adequate.

Insulin did not stimulate the oxidation of glucose or its synthesis to fatty acids by fat cells of either type of obese rat. Resistance to insulin has been reported among the strains of genetically obese mice (22–24) and resistance of the fat cells from the genetically obese rats to the effect of insulin may represent a generalized insulin resistance in these animals. However, the increase in body weight with a corresponding increase in the size of the fat cells seems to be a more important factor (21) since insulin had no effect on the fat cells from either type of obese rat. A third factor which may be partly responsible for the ineffectiveness of insulin is the site from which the biopsies were obtained, for we have found that fat cells from subcutaneous depots of normal rats have a much smaller response to insulin than epididymal fat cells from the same animals (11).

Pyruvate and glucose are both good sources of carbon for the synthesis of fatty acids by adipose tissue of the rat (25), but pyruvate proved to be a better source of radioactivity for fatty acids in fat cells from obese rats. Only small quantities of radioactivity from glucose were incorporated into fatty acids by the fat cells of either type of obese rats, and various additions to the incubation had no stimulatory effect. The low rate at which fatty acids are synthesized may be a reflection of excess fat in the obese animals (26). In the experiments with radioactive glucose (Tables 1 and 5), as well as those with pyruvate (Tables 3 and 4), the quantity of radioactivity incorporated into glyceride-glycerol relative to that oxidized to CO_2 was uniformly higher in the fat cells from the genetically obese rats. This implies that the cells from genetically obese rats are channeling relatively more radioactive substrate into glycerol than into oxidative pathways. It is interesting to note that an abnormality in mitochondrial oxidation of glycerol 3-phosphate has been observed in adipose tissue from obese human beings (27), and that high levels of glycerokinase have been found in the fat of obese hyperglycemic mice (28). The possibility that an error in glycerol me-

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tabolism may exist in the genetically obese rats is being investigated.

Studies of the metabolism of radioactive pyruvate and aspartate showed a second difference between fat cells of genetic and hypothalamic obese rats. The stimulation by glucose and fructose of the incorporation of radioactivity from pyruvate into fatty acids occurred in fat cells from the obese rats, which confirmed an observation made by others using adipose tissue from normal rats (24). The magnitude of this effect was greater in the cells from genetically obese rats. The stimulation by glucose of the incorporation of radioactivity from aspartate into fatty acids has not been previously observed, and, as with pyruvate, the fat cells from genetically obese rats showed a greater effect. These experiments with radioactive glucose, pyruvate, and aspartate demonstrate that the Krebs cycle glycolytic pathways and the pathways for conversion of pyruvate and aspartate to fatty acids and glyceride-glycerol are intact. They also show that quantitative differences exist in the channeling of these substrates into glycerol and fatty acids by fat cells from genetic and hypothalamic obese rats.

Studies by Mayer and his collaborators have shown that in the obese-hyperglycemic mouse (29), lipogenesis is not inhibited during fasting; this has been supported by several groups of workers (30–32). Their studies suggested that a failure to inhibit lipogenesis in these mice might be the cause of the obesity. This concept, however, does not seem to apply to the New Zealand (NZO) or yellow obese mice (33), and one group of investigators (34) have reported that lipogenesis in the obese-hyperglycemic mouse is reduced during fasting. In the Zucker (fatty) rats used in our studies, lipogenesis was clearly inhibited by a fast of 8 or 10 days, showing that one cannot account for their obesity by this mechanism. This difference between the obese-hyperglycemic mouse on the one hand, and the other strains of mice and the Zucker rats on the other, serves to emphasize that there are probably several biochemical lesions that will induce obesity.

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