Studies on Lipogenesis *in vivo*

FATTY ACID AND CHOLESTEROL SYNTHESIS IN HYPERGLYCAEMIC-OBESSE MICE

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1. Lipogenesis has been studied in intact genetically obese mice by measuring the incorporation of a single oral dose of 250 mg. of [U- 14 C]glucose into fatty acid and cholesterol in the liver and extrahepatic tissues. Studies were also carried out with [U- 14 C]glucose added to the diet and fed for 24 hr. With either method of isotope administration, the conversion of [U- 14 C]glucose into fatty acid was greatly elevated in the livers of the obese mice. In contrast, conversion of the single dose of [14 C]glucose into fatty acid in extrahepatic tissues of obese mice was only half that occurring in the non-obese litter mates. When [14 C]glucose was given in the diet for 24 hr. the total accumulation of labelled fatty acid in extrahepatic tissues of obese mice was slightly less than in the non-obese. Uptake of labelled glucose and conversion into fatty acid in adipose tissue of the obese mice decreased with age. 2. Conversion of the single dose of [14 C]glucose into liver cholesterol was comparable in obese and non-obese mice fed on a purified low-fat diet. However, obese mice given this diet for 12 weeks accumulated 1.54% of cholesterol in the liver compared with 0.29% in the non-obese litter mates. This accumulation apparently resulted from a decrease in removal of cholesterol from the liver, rather than an increased synthesis. 3. Conversion of the single dose of [14 C]glucose into extrahepatic fatty acid was decreased by 18 hr. starvation proportionally as much in obese as in non-obese mice. The decrease in liver fatty acid synthesis caused by starvation also was considerable in obese mice, although somewhat less marked than in the non-obese. 4. The metabolic derangements in the liver could be more fundamental to the development of the obesity than the changes seen in extrahepatic tissues.

One of the most widely studied experimental obesities has been the genetic obesity accompanied by hyperglycaemia that was discovered in mice at the Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A. (Ingalls, Dickie & Snell, 1950). The mutant gene has been designated *ob* and is a Mendelian recessive. Although much is known about the metabolic differences between these mice and their non-obese litter mates, the basic cause of the obesity remains unexplained. Mayer, Russell, Bates & Dickie (1953) postulated that the genetic lesion is a partial block in the oxidation of acetate, causing more pyruvate to be converted into fatty acid. Hughes & Tolbert (1958) also found a decrease in the conversion of injected [14 C]acetate into $^{14}\text{CO}_2$ in the obese mice. However, Parson & Crispell (1955) found that obese and non-obese mice produced equal amounts of $^{14}\text{CO}_2$ after injection of [14 C]acetate.

An increased incorporation of [14 C]acetate into both liver and extrahepatic fatty acid has been found *in vivo* (Bates, Mayer & Nauss, 1955b) and

in vitro (Mayer, Hagman, Marshall & Stoops, 1955; Cristophe, Jeanrenaud, Mayer & Renold, 1961b). However, Hellman, Larsson & Westman (1962) have reported lowered lipogenesis from [14 C]acetate in adipose tissue from the obese mice as compared with lean litter mates when assayed *in vitro* in the presence of glucose.

Shigeta & Shreeve (1964) reported that intact obese mice converted 2–4 times as much [^3H]glucose and [14 C]glucose into extrahepatic fatty acids and 5–10 times as much into fatty acid in the liver as did the controls. In contrast, previous work *in vitro* had indicated that glucose uptake, including conversion into fat, was depressed in adipose tissue taken from obese mice (Cristophe, Jeanrenaud, Mayer & Renold, 1961a). Defects in fat mobilization also have been reported (Bates, Mayer & Nauss, 1955c), and adipose tissue from obese mice contains less lipase and more glycerokinase activity than tissue from non-obese litter mates (Lochaya, Hamilton & Mayer, 1963; Treble & Mayer, 1963).

The present consensus appears to be that both

increased lipogenesis and decreased lipolysis are involved in the aetiology of this genetic obesity of mice (Maier, 1963; Mayer, 1964; Hellman, 1965). However, the primary cause of the obesity is not known. The results of studies of lipogenesis from $[U-^{14}C]$ glucose *in vivo* in both liver and extrahepatic tissues are presented in this paper.

MATERIALS AND METHODS

Male hyperglycaemic-obese mice and their male non-obese litter mates were purchased from the Jackson Memorial Laboratory. Details of age, weight range, dietary treatment and experimental procedures used are given in the legends to each of the Tables. The techniques used in dosing and bleeding mice, preparing and analysing tissues and assaying for radioactivity have been described previously (Jansen, Hutchison & Zanetti, 1966a; Jansen, Zanetti & Hutchison, 1966b). Pairs of carcasses were saponified and the digests made up to 250 ml. and 500 ml. for non-obese mice and obese mice respectively. The procedure for saponification of carcasses has been given in detail elsewhere (Jansen, Zanetti & Hutchison, 1966c). In this paper carcass refers to the body with the liver and epididymal fat pads removed. The $[U-^{14}C]$ glucose was obtained from New England Nuclear Corp., Boston, Mass., U.S.A., and had a specific activity of 10–15 mc/m-mole. In most experiments the $[U-^{14}C]$ glucose was given diluted with unlabelled glucose as a 50% (w/v) soln. by stomach tube. In one experiment the $[U-^{14}C]$ glucose was added to the diet and given for 24 hr.

RESULTS

Effect of age and diet. Obese and non-obese mice were given either Purina Chow (5% of fat) or a

low-fat high-glucose diet (diet 2; Jansen *et al.* 1966a) for 1 week or 12 weeks. At these times the average ages of the mice were 8 and 19 weeks respectively. Incorporation of a single dose of 250 mg. of $[U-^{14}C]$ glucose into liver and extrahepatic cholesterol and fatty acids and uptake by epididymal adipose tissue were measured.

As shown in Table 1 the weight gains and percentages of carcass fatty acid (after saponification) of the obese mice were closely similar on these two diets. As has been reported previously (Bates, Nauss, Hagman & Mayer, 1955a) the difference in weight between obese and non-obese was essentially fat and the fat-free body weights were virtually identical for mice given either diet irrespective of age.

Incorporation of a single dose of 250 mg. of $[U-^{14}C]$ glucose into carcass fatty acids and cholesterol is also shown in Table 1. Incorporation into these fractions in the obese mice was only half that in the non-obese litter mates, irrespective of diet. Incorporation of isotope into fatty acid was closely similar at the two time-periods studied in obese mice and also in non-obese mice when both were given the purified low-fat high-glucose diet. However, in the obese mice given Purina Chow the total incorporation into fatty acid was decreased by almost 50% after the 11 additional weeks on this diet. The concentration of cholesterol in the carcasses of the obese mice was only one-half that found in the non-obese litter mates. This was a consequence of the large amount of extrahepatic fat but the total amounts of cholesterol present in

Table 1. *Effect of age and diet on lipogenesis in the carcass*

Experimental details are given in the text. The mice (eight/group) ranged from 6 to 8 weeks of age at the start of the experiment and were all males. The fat-free body weight equals body weight $- 1.1 \times$ (total weight fatty acid after saponification). After being given Purina Chow or diet 2 (Jansen *et al.* 1966a) for 1 or 11 weeks, the mice were each given 250 mg. of $[U-^{14}C]$ glucose ($2.5 \mu\text{C}$) by stomach tube and killed 60 min. later. Food was removed from the cages at the time of dosing. Where appropriate results are given as means $\pm$ S.E.M.

Diet	Average age (weeks)	Time on diet (weeks)	Body wt. (g.)	Fat-free body wt. (g.)	Fatty acid		Cholesterol	
					%*	10 ⁻³ ×	%*	Counts/ min./ mouse
						Counts/ min./mouse		
Obese								
Low-fat high-glucose	8	1	39.3 ± 1.0	21.4	40.0 ± 1.6	148 ± 6	0.13 ± 0.01	363 ± 48
Low-fat high-glucose	19	12	52.1 ± 1.1	26.5	44.3 ± 0.4	145 ± 5	0.12 ± 0.01	325 ± 35
Purina Chow	8	1	40.3 ± 0.8	21.3	41.2 ± 0.4	86 ± 7	0.13 ± 0.01	344 ± 6
Purina Chow	19	12	52.3 ± 1.8	25.4	46.2 ± 0.7	51 ± 6	0.10 ± 0.01	256 ± 19
Non-obese								
Low-fat high-glucose	8	1	24.4 ± 0.7	21.3	10.6 ± 1.1	265 ± 17	0.22 ± 0.01	613 ± 97
Low-fat high-glucose	19	12	31.1 ± 1.4	25.3	14.0 ± 0.7	313 ± 13	0.19 ± 0.01	650 ± 47
Purina Chow	8	1	23.8 ± 0.5	21.4	8.3 ± 1.2	134 ± 11	0.23 ± 0.02	566 ± 53
Purina Chow	19	12	28.6 ± 0.7	25.3	9.5 ± 1.1	150 ± 11	0.21 ± 0.01	440 ± 45

* Wt. (g.) of total fatty acid or cholesterol (after saponification)/100 g. (body wt. — liver wt. — wt. of epididymal fat pads).

Table 2. *Effect of age and diet on lipogenesis in epididymal fat pads*Experimental details are given in the text and Table 1. Where appropriate results are given as means $\pm$ S.E.M.

Diet	Average age (weeks)	Time on diet (weeks)	Wt. of 2 pads (mg.)	100 $\times$ Pad wt./body wt.	Counts/min./pad	% of pad ^{14}C as fat	% of fat ^{14}C as fatty acid
Obese							
Low-fat high-glucose	8	1	2520 $\pm$ 140	6.4	11810 $\pm$ 1050	59 $\pm$ 5	85 $\pm$ 1
Low-fat high-glucose	19	12	2250 $\pm$ 140	4.3	2340 $\pm$ 120	66 $\pm$ 2	31 $\pm$ 2
Purina Chow	8	1	2700 $\pm$ 120	6.7	8750 $\pm$ 1190	54 $\pm$ 3	65 $\pm$ 5
Purina Chow	19	12	2720 $\pm$ 150	5.2	2760 $\pm$ 160	58 $\pm$ 1	18 $\pm$ 1
Non-obese							
Low-fat high-glucose	8	1	393 $\pm$ 33	1.6	7610 $\pm$ 390	88 $\pm$ 2	85 $\pm$ 1
Low-fat high-glucose	19	12	750 $\pm$ 115	2.4	4610 $\pm$ 600	74 $\pm$ 4	74 $\pm$ 3
Purina Chow	8	1	323 $\pm$ 42	1.4	2110 $\pm$ 310	78 $\pm$ 2	63 $\pm$ 3
Purina Chow	19	12	487 $\pm$ 67	1.7	1920 $\pm$ 200	76 $\pm$ 2	65 $\pm$ 3

Table 3. *Effect of age and diet on lipogenesis in liver*Experimental details are given in the text and Table 1. Where appropriate results are given as means $\pm$ S.E.M.

Diet	Average age (weeks)	Time on diet (weeks)	Liver wt. (g.)	Liver/body wt.*	Liver fatty acid		Liver cholesterol	
					%†	10 ⁻³ $\times$ Counts/min./liver	%†	Counts/min./liver
Obese								
Low-fat high-glucose	8	1	3.90 $\pm$ 0.13	14.0	21.5 $\pm$ 0.07	53.5 $\pm$ 6.0	0.41 $\pm$ 0.02	63 $\pm$ 6
Low-fat high-glucose	19	12	5.58 $\pm$ 0.27	15.8	22.7 $\pm$ 1.5	79.9 $\pm$ 7.2	1.54 $\pm$ 0.10	57 $\pm$ 9
Purina Chow	8	1	3.02 $\pm$ 0.13	11.8	15.2 $\pm$ 1.4	47.0 $\pm$ 4.5	0.31 $\pm$ 0.01	147 $\pm$ 23
Purina Chow	19	12	4.46 $\pm$ 0.27	13.4	21.8 $\pm$ 1.1	41.5 $\pm$ 2.4	0.40 $\pm$ 0.03	67 $\pm$ 28
Non-obese								
Low-fat high-glucose	8	1	1.53 $\pm$ 0.05	6.9	3.58 $\pm$ 0.36	33.5 $\pm$ 3.0	0.31 $\pm$ 0.02	53 $\pm$ 5
Low-fat high-glucose	19	12	1.68 $\pm$ 0.12	6.3	4.81 $\pm$ 0.71	39.0 $\pm$ 3.8	0.29 $\pm$ 0.01	106 $\pm$ 22
Purina Chow	8	1	1.51 $\pm$ 0.05	6.8	2.90 $\pm$ 0.11	8.4 $\pm$ 0.7	0.20 $\pm$ 0.01	167 $\pm$ 23
Purina Chow	19	12	1.60 $\pm$ 0.07	6.1	3.09 $\pm$ 0.10	4.9 $\pm$ 0.5	0.22 $\pm$ 0.01	171 $\pm$ 31

* 100 $\times$ g. fat-free liver wt./g. fat-free body wt.

† Wt. (g.) of total fatty acid or cholesterol (after saponification)/100 g. liver wt.

the extrahepatic tissues were similar in obese and non-obese mice.

Data showing the incorporation of [U- ^{14}C]glucose into the epididymal fat pads are in Table 2. In spite of the fact that there was an increase in extrahepatic fat, the weight of these tissues in the obese mice did not increase with age. Hellman (1965) has reported a decline in the weight of epididymal fat pads with age in this strain of obese mice. Uptake of [^{14}C]glucose by the epididymal pads declined by about 40% with age, when non-obese mice were given the purified low-fat diet, but did not decline when these mice were given Purina Chow. In contrast, the uptake of [^{14}C]glucose by pads of obese mice declined by 70–80% irrespective of diet.

Also, in the pads of obese mice this decline was associated with a large increase in the proportion of label present as glyceride glycerol. These changes, which we have previously observed in the pads of mice given a moderate amount of fat or starved overnight (Jansen *et al.* 1966a), appear to be associated with a considerable decrease in the rate of fatty acid synthesis. When the mice reached the age of 19 weeks the total uptake of [^{14}C]glucose by the pads of obese mice given the low-fat diet was only half that of the pads from non-obese mice. This occurred in spite of the fact that the pads from the obese mice weighed 3 times as much as did the control pads.

The incorporation rates of [U- ^{14}C]glucose into

fatty acids and cholesterol in the liver are shown in Table 3. Considerable liver hypertrophy in the obese mice was apparent at 8 weeks of age and at this early age liver fat was fivefold greater than in livers of the controls. Total incorporation of [^{14}C]glucose into liver fatty acid in the obese mice was greater in all instances than in the non-obese litter mates ranging from 60% greater in 8-week-old mice given the low-fat diet to 800% greater in 19-week-old mice given Purina Chow. Total incorporation into liver cholesterol, in spite of the increased liver size, was less in obese than non-obese mice at 19 weeks of age when they were given either diet. However, even though there was a decrease in synthesis, giving the low-fat diet to the obese mice led to an accumulation of 1.54% of cholesterol in the liver compared with 0.29% in the livers of the non-obese mice.

Effect of starvation. The effects of 18 hr. of food deprivation on lipogenesis were evaluated and the results are shown in Table 4. Large decreases in

fatty acid and cholesterol synthesis in both liver and extrahepatic tissues occurred in obese and non-obese mice alike. These results are comparable with those we have previously reported for another strain of mice (Jansen *et al.* 1966a,b). The 70% decrease in liver fatty acid synthesis in obese mice was slightly less than the 90% decrease seen in the non-obese. The effect of starvation on extrahepatic fatty acid synthesis was comparable in obese and non-obese mice, as was the effect on incorporation into liver and extrahepatic cholesterol.

The decrease in [^{14}C]glucose uptake by epididymal fat pads after starvation was also comparable in obese and non-obese mice. As we have reported previously for another mouse strain, a greater percentage of the triglyceride radioactivity was present as glyceride glycerol after starvation (Jansen *et al.* 1966a,b). Incorporation of [^{14}C]glucose into epididymal triglyceride/mg. of N in fed mice was lower in obese than in non-obese mice. However, after 18 hr. starvation the converse was

Table 4. *Effect of starvation on lipogenesis*

Experimental details are given in the text. The mice (eight/group) were 6-7 weeks old, were all males and were maintained on diet 2 (Jansen *et al.* 1966a). Half of the mice were starved overnight (18 hr.). Each mouse was given 250 mg. of [$\text{U-}^{14}\text{C}$]glucose (2.5 μC) by stomach tube and killed 60 min. later. Food was removed from the cages of the fed animals at the time of dosing. Nitrogen was determined on one of the epididymal fat pads of each mouse by the Kjeldahl method (digestion in H_2SO_4 and distillation followed by titration). Where appropriate results are given as means $\pm$ S.E.M.

	Obese		Non-obese	
	Fed	Starved	Fed	Starved
Body wt. (g.)	35.1 $\pm$ 1.0	32.2 $\pm$ 1.1	24.9 $\pm$ 0.5	21.4 $\pm$ 0.4
Liver wt. (g.)	3.74 $\pm$ 0.26	2.61 $\pm$ 0.14	1.61 $\pm$ 0.06	1.23 $\pm$ 0.03
Liver fatty acid				
%* of liver	23.8 $\pm$ 1.1	22.6 $\pm$ 0.9	4.22 $\pm$ 0.38	6.47 $\pm$ 0.54
Counts/min./liver ($\times 10^{-3}$)	51.4 $\pm$ 13.7	15.7 $\pm$ 5.2	29.2 $\pm$ 2.9	2.8 $\pm$ 0.4
Liver cholesterol				
%* of liver	0.68 $\pm$ 0.03	0.90 $\pm$ 0.07	0.24 $\pm$ 0.01	0.30 $\pm$ 0.01
Counts/min./liver	120 $\pm$ 42	4 $\pm$ 2	140 $\pm$ 12	3 $\pm$ 1
Carcass fatty acid				
%† of carcass	41.3 $\pm$ 2.0	44.7 $\pm$ 2.7	12.7 $\pm$ 1.3	8.1 $\pm$ 0.7
Counts/min./mouse ($\times 10^{-3}$)	112 $\pm$ 10	43.7 $\pm$ 9.3	289 $\pm$ 8	89.6 $\pm$ 3.4
Carcass cholesterol				
%† of carcass	0.16 $\pm$ 0.01	0.15 $\pm$ 0.01	0.22 $\pm$ 0.01	0.26 $\pm$ 0.01
Counts/min./mouse	506 $\pm$ 86	238 $\pm$ 12	757 $\pm$ 41	294 $\pm$ 33
Epididymal fat pads				
Wt. of 2 pads (mg.)	1840 $\pm$ 110	1990 $\pm$ 100	500 $\pm$ 30	340 $\pm$ 40
Counts/min./pad	9090 $\pm$ 970	3650 $\pm$ 470	5020 $\pm$ 500	1050 $\pm$ 130
% of pad ^{14}C as fat	74 $\pm$ 3	70 $\pm$ 3	86 $\pm$ 1	68 $\pm$ 3
% of fat ^{14}C as fatty acid	84 $\pm$ 1	53 $\pm$ 5	84 $\pm$ 1	35 $\pm$ 3
% of nitrogen	0.18 $\pm$ 0.01	0.14 $\pm$ 0.01	0.25 $\pm$ 0.01	0.33 $\pm$ 0.02
Triglyceride counts/min./mg. of N	4040	1840	6910	1290

* Wt. (g.) of total fatty acid or cholesterol (after saponification)/100 g. liver wt.

† Wt. (g.) of total fatty acid or cholesterol (after saponification)/100 g. (body wt. — liver wt. — wt. of epididymal fat pads).

true. It has previously been reported that the obese mice are characterized by a high rate of lipogenesis during starvation (Bates, Zomzely & Mayer, 1955d). In their study [^{14}C]acetate was injected and, although the total incorporation of ^{14}C into fatty acid was approximately as high in the starved obese mice as in the fed non-obese mice, the decrease in incorporation caused by starvation was similar in obese and non-obese mice.

Effect of dietary fat. The foregoing experiments were carried out with low-fat diets since it has been reported that greatest weight gains are achieved with high-carbohydrate diets (Mayer, 1960). However, during some nutritional experiments we observed that obese mice given a diet

containing 20% of corn oil gained weight at approximately twice the rate of those given a diet containing 1% of corn oil or Purina Chow. We therefore investigated the effect of dietary fat on lipogenesis. Although we have shown for another strain of mice that the fate of a single dose of [^{14}C]glucose reflected to a considerable degree the fate of dietary glucose (Jansen, Zanetti & Hutchison, 1967), it was considered desirable to carry out this experiment with [^{14}C]glucose added directly to the diet and given for a 24 hr. period. Glucose-based diets, containing either 1% of corn oil, 20% of corn oil or 1% of corn oil plus 19% of lard, were given for 30 days. For the last 24 hr. all diets contained $0.4\text{ }\mu\text{C}$ of [^{14}C]glucose/g. We

Table 5. *Effect of dietary fat on conversion of dietary [^{14}C]glucose into fatty acid and cholesterol*

Experimental details are given in the text. The mice, all males (eight/group) and 3–6 weeks old at the start of the experiment, were given the experimental diets for 31 days. For the last 24 hr. of this period [^{14}C]glucose was added to the diets to give $0.4\text{ }\mu\text{C/g}$. of diet. The diets used were: (a) diet 2 (Jansen *et al.* 1966a); (b) diet 5 (Jansen *et al.* 1966a); (c) diet 5 with 1% of corn oil + 19% of lard instead of 20% of corn oil. Fat-free body weight equals body weight $- 1.1 \times$ (total weight fatty acid after saponification). Where appropriate results are given as means $\pm$ S.E.M.

Dietary fat	Obese			Non-obese		
	1% of corn oil	20% of corn oil	1% of corn oil + 19% of lard	1% of corn oil	20% of corn oil	1% of corn oil + 19% of lard
Wt. gain (g.)	7.4 ± 0.8	12.5 ± 0.6	16.5 ± 0.5	3.1 ± 0.3	2.7 ± 0.6	3.6 ± 0.5
Final body wt. (g.)	40.5 ± 0.8	48.1 ± 1.2	50.3 ± 1.5	24.1 ± 1.0	24.0 ± 0.9	25.2 ± 0.8
Fat-free body wt. (g.)	20.5	21.1	21.7	20.7	21.0	21.6
[^{14}C]Glucose consumed (counts/min./24 hr.) ($\times 10^{-6}$)	2.5 ± 0.2	2.1 ± 0.2	2.4 ± 0.2	2.1 ± 0.2	1.7 ± 0.2	2.0 ± 0.2
Liver wt. (g.)	3.81 ± 0.28	3.37 ± 0.19	3.31 ± 0.15	1.32 ± 0.04	1.36 ± 0.05	1.37 ± 0.09
Liver fatty acid						
% of liver*	23.9 ± 1.4	17.0 ± 1.0	18.7 ± 0.4	3.41 ± 0.19	3.25 ± 0.11	3.48 ± 0.13
Counts/min./liver ($\times 10^{-3}$)	89.1 ± 17.9	28.4 ± 1.3	26.2 ± 3.1	9.84 ± 0.83	2.78 ± 0.29	3.58 ± 0.68
Counts/min./liver (% of dose)	3.6	1.4	1.1	0.47	0.16	0.18
Liver cholesterol						
% of liver*	1.09 ± 0.08	0.34 ± 0.01	0.45 ± 0.04	0.27 ± 0.01	0.23 ± 0.01	0.24 ± 0.01
Counts/min./liver	468 ± 123	99 ± 8	73 ± 11	40 ± 5	99 ± 10	172 ± 37
Carcass fatty acid						
% of carcass†	43.1 ± 2.0	50.1 ± 1.7	50.2 ± 1.7	11.50 ± 0.73	9.65 ± 0.93	11.62 ± 0.61
Counts/min./mouse ($\times 10^{-3}$)	202 ± 6	75.7 ± 2.5	70.8 ± 4.8	201 ± 11	66.0 ± 9.2	92.2 ± 5.6
Counts/min./mouse (% of dose)	8.1	3.7	3.0	9.6	3.9	4.6
Carcass cholesterol						
% of carcass†	0.12 ± 0.1	0.13 ± 0.02	0.11 ± 0.01	0.23 ± 0.01	0.26 ± 0.01	0.22 ± 0.01
Counts/min./mouse	833 ± 106	1070 ± 160	844 ± 76	991 ± 51	960 ± 41	1400 ± 210
Epididymal fat pads						
Wt. of 2 pads (mg.)	2680 ± 100	3620 ± 130	3830 ± 200	521 ± 50	385 ± 56	497 ± 47
Counts/min./2 pads ($\times 10^{-3}$)	19.2 ± 2.3	14.4 ± 0.6	13.7 ± 1.0	6.98 ± 0.88	3.24 ± 0.40	5.43 ± 1.0
% of pad ^{14}C as fat	85 ± 3	77 ± 3	91 ± 1	88 ± 2	78 ± 3	91 ± 1
% of fat ^{14}C as fatty acid	75 ± 3	26 ± 1	27 ± 4	76 ± 1	40 ± 4	37 ± 3

* Wt. (g.) of total fatty acid or cholesterol (after saponification)/100 g. liver wt.

† Wt. (g.) of total fatty acid or cholesterol (after saponification)/100 g. (body wt. — liver wt. — wt. of epididymal fat pads).

previously compared the plasma [^{14}C]glucose concentrations 60 min. after 250 mg. [^{14}C]glucose ($2.5\mu\text{C}$) doses given by stomach tube to eight obese mice (average wt. 67 g.) and eight non-obese litter mates (average wt. 32 g.) maintained on Purina Chow. The glucose intolerance in the obese mice was shown by a plasma glucose concentration of 370 ± 32 mg./100 ml. compared with 172 ± 7 mg./100 ml. in controls. However, the specific activity of the circulating glucose in the obese mice (10080 ± 680 counts/min./mg.) did not differ significantly from that found for controls (11320 ± 840 counts/min./mg.).

As shown in Table 5 there were no significant differences in weight gain of the non-obese mice given the three diets. However, the obese mice given the diets containing 20% of corn oil or 19% of lard plus 1% of corn oil gained 70% and 120% more respectively than those given the diet containing 1% of corn oil. As shown in Table 1 the growth rate of the obese mice given the 1%-corn oil diet was virtually identical with the growth rate when the animals were given Purina Chow. All fat-free body weights for obese and non-obese mice were closely similar (Table 5).

The high amount of dietary fat, whether lard or corn oil, depressed the incorporation of [^{14}C]glucose into both hepatic and extrahepatic fatty acids in both obese and non-obese mice (Table 5). The results with the non-obese litter mates are similar to those obtained previously with another mouse strain (Jansen *et al.* 1967). However, the decrease in incorporation into fatty acid in adipose and other extrahepatic tissues of the obese mice, given moderate amounts of dietary fat, is in contrast with previous work *in vitro* (Lochaya, Leboeuf, Mayer & Leboeuf, 1961).

Incorporation of ^{14}C into liver cholesterol in the non-obese mice was highest on the diet containing lard (Table 5). In the obese mice, giving the 1%-corn oil diet again led to an accumulation of cholesterol in the liver (1.09% compared with 0.29% in the controls). However, with the [^{14}C]glucose given over a 24 hr. period, there was a tenfold increase in ^{14}C content of the liver cholesterol fraction of obese mice given the low-fat diet. Since the incorporation of ^{14}C from the 250 mg. [^{14}C]glucose meal was not at all elevated (Table 3), these results suggest that the accumulation of liver cholesterol in the obese mice given the low-fat diet was secondary to a decreased turnover rate, rather than caused by an increased synthesis rate.

As shown in Table 5 the ^{14}C content of the liver fatty acid fraction of the obese mice given either the low-fat or the high-fat diet also was increased almost tenfold as compared with non-obese mice. Incorporation of dietary [^{14}C]glucose into extrahepatic fatty acids (exclusive of epididymal fat)

was nearly as high in obese as in non-obese mice. In both obese and non-obese mice dietary fat depressed incorporation into carcass fatty acid to a similar extent. There was no appreciable effect of dietary fat on incorporation into carcass cholesterol and the rates in obese mice were similar to the rates in their non-obese litter mates.

Total uptake and retention of [^{14}C]glucose by epididymal fat pads was considerably greater in obese than non-obese mice (Table 5). As in the previous experiments, uptake by the pads of obese mice was considerably less than by the pads of non-obese mice when expressed/unit of weight. Uptake of [^{14}C]glucose by the pads was depressed by dietary fat to a similar extent in obese and non-obese mice. Also, similar to results obtained with another strain of mice (Jansen *et al.* 1966a), dietary fat increased the percentage of triglyceride radioactivity present as glyceride glycerol in the pads of both obese mice and their non-obese litter mates.

DISCUSSION

The primary metabolic alteration in these genetically obese mice would appear to be fully developed at 8 weeks of age as shown by the liver hypertrophy and increased liver fat, each of which has reached a maximum by this time, and most obviously by the increased body weight and fat content. Although these latter parameters continue to increase with age, the capacity of adipose tissue to take up glucose and synthesize fatty acid declines. The decreased glucose uptake by adipose tissue may be an important reason for the hyperglycaemia and insulin resistance that is reported to develop in these animals by the tenth week of age (Mayer *et al.* 1953). The excess of extrahepatic fat also may contribute to the decrease of glucose uptake by muscle via the glucose-fatty acid cycle as postulated to occur in diabetes (Randle, Garland, Newsholme & Hales, 1963).

Our results suggest that adipose tissue and the other extrahepatic tissues respond to their extra fat content by decreasing the rate of fatty acid synthesis. In contrast, the liver continues to synthesize fatty acid at an elevated rate in spite of the considerable accumulation of liver fat. The greater weight gain on the 20%-fat diet is noteworthy in view of reports that greatest weight gains are obtained on high-carbohydrate diets and an actual weight loss occurs on a high-fat diet (Mayer, 1960). In our experiment when a 20%-fat diet was given fatty acid synthesis was greatly diminished and most of the fat deposited in adipose tissue undoubtedly was of dietary origin. Thus the development of the obesity occurs readily with virtually no fat in the diet when essentially all of the body fat has to be synthesized from glucose, and also

under conditions where 40% of the calories are supplied as fat. Therefore, since a high rate of fatty acid synthesis is apparently not required for development of the obesity, it appears likely that the genetic defect is more fundamental than the control of fatty acid synthesis or the supply of precursors for fatty acid synthesis by the citrate-cleavage enzyme (Kornacker & Lowenstein, 1964).

The large accumulation of cholesterol that occurs in the livers of obese mice given the low-fat diet suggests a marked metabolic abnormality in cholesterol metabolism. It has been reported that giving a fat-free diet to rats causes an accumulation of cholesterol and fatty acid in the liver (Norby, 1965). It may be that the obese mice have a higher requirement for essential fatty acids than do their non-obese litter mates.

There is evidence that defective fat mobilization is important in the aetiology of this genetic obesity (Maier, 1963; Mayer, 1964). Our experiments, however, have been concerned primarily with lipogenesis and we have not directly investigated lipolysis. It has been reported that obese mice mobilize less fatty acid/day than controls, although this lowered mobilization also has been reported for hypothalamic obesity (Bates *et al.* 1955c). However, even if the obese mice mobilized as much fat as the non-obese, the fractional turnover rate would be much lower in the obese mice because of the greater total amount of fatty acid. This decreased fractional turnover rate along with elevated synthesis in the liver is probably why labelled fatty acids accumulated in the livers and carcasses of obese mice given [U-¹⁴C]glucose in the diet for 24 hr.

Mayer (1966) has proposed that the primary inborn error in these genetically obese mice is the lack of the normally present repressor to glycerokinase in white adipose tissue. It is difficult to separate the cause of the obesity from the effects and we consider that it will be desirable to compare the obese and the non-obese mice during the first month of life. Our results do not appear to be completely consistent with Mayer's (1966) theory. We have found that, as the obesity develops, conversion of [U-¹⁴C]glucose into fatty acid is inhibited more than conversion into glyceride glycerol. If the rephosphorylation of glycerol in adipose tissue were the primary lesion, one might expect a lowered incorporation of [U-¹⁴C]glucose into glyceride glycerol because of reutilization of unlabelled glycerol coming from triglyceride hydrolysis. Another observation apparently inconsistent with Mayer's (1966) theory is that adipose tissue transplanted from obese mice to their non-obese litter mates and vice versa takes on the appearance of host tissue and loses the appearance of donor tissue (Hausberger, 1959). This suggests that the

obesity does not result from a defect in genetic expression within the adipose tissue itself. Also, the accumulation of liver cholesterol we have observed in obese mice given a low-fat diet would not appear likely to be secondary to increased adipose-tissue glycerokinase. This is not to imply, however, that the glycerokinase may not be involved in the defective fat mobilization reported for the animals.

It is not possible, on the basis of our experiments, to evaluate the comparative importance of increased lipogenesis versus decreased lipolysis in the aetiology of the obesity. Nevertheless, our results appear more consistent with the possibility that the fundamental metabolic abnormality is expressed more prominently in the liver than in adipose tissue. The liver appears to synthesize excessive quantities of fat that presumably are deposited in adipose tissue. Defective fat mobilization could exacerbate this accumulation as could defective fat oxidation. Also, since food intake continues high and is greatly in excess of the diminished caloric needs of the obese mice, because of their inactivity, it is possible that the appetite regulatory system is faulty. Our experiments suggest that the accumulation of extrahepatic fat diminishes peripheral uptake of glucose and this may be an important cause of the hyperglycaemia. The diabetes could well be secondary to the obesity rather than vice versa. The possibility appears worthy of consideration that a similar mechanism could operate in some cases of maturity-onset diabetes in humans.

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