Plasma Lipoproteins and the Synthesis and Turnover of Plasma Triglyceride in Normal and Genetically Obese Mice

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1. Lipoproteins in the plasma of mice were characterized by agarose-gel chromatography and polyacrylamide-gel electrophoresis: genetically obese (*ob/ob*) mice exhibited hyperlipoproteinaemia (compared with lean mice), largely owing to an increase in the concentration of cholesterol in high-density lipoprotein. Plasma concentrations of triglyceride and phospholipid were not markedly increased in genetically obese mice. 2. The formation of glycerolipids in liver and plasma was investigated with ^{14}C -labelled precursors. The synthesis of hepatic triglyceride and phospholipid from glucose or palmitate was enhanced in *ob/ob* mice, compared with lean mice. The rate of entry of triglyceride into plasma, calculated from the time-course of incorporation of ^{14}C from [^{14}C]palmitate into plasma triglyceride, was increased in *ob/ob* mice ($0.5\ \mu\text{mol}$ of fatty acid/min, compared with 0.2 in lean mice). 3. The removal from plasma of murine lipoprotein triglyceride-[^{14}C]fatty acid was increased in *ob/ob* mice (half-time 2.2 min , compared with 7.2 min in lean mice). Similar results were obtained with an injected lipid emulsion (Intralipid). 4. From these measurements, estimates of the rates of turnover of plasma triglyceride in mice (fed on a mixed diet, female, 3 months old) are about $1.0\ \mu\text{mol}$ of fatty acid/min in *ob/ob* mice, and 0.25 in lean mice. 5. The major precursor of hepatic and plasma triglyceride in lean and *ob/ob* mice was calculated to be plasma free fatty acid. 6. These results are discussed, in connexion with the role of the liver in triglyceride metabolism in mice, especially in relation to genetic obesity.

One approach to the understanding of abnormal metabolism in obesity is the study of genetically obese rodents (for reviews see Mayer, 1960; Renold, 1968; Stauffacher *et al.*, 1971; Bray & York, 1971). In genetically obese mice, an aspect which has not been systematically explored is the metabolism of plasma triglyceride. The incorporation of ^{14}C from ^{14}C -labelled precursors into hepatic fatty acids is more extensive in obese mice (Zomzely & Mayer, 1959; Shigeta & Shreeve, 1964; Shreeve *et al.*, 1967; Christophe *et al.*, 1970; Jansen *et al.*, 1967). In addition, enzymes, such as acetyl-CoA carboxylase (EC 6.4.1.2) and citrate cleavage enzyme (EC 4.1.3.8), which may be regulatory in fatty acid synthesis, exhibit enhanced activities in the liver of genetically obese mice (Spencer & Lowenstein, 1966; Chang *et al.*, 1967; Nakanishi & Numa, 1971). These observations imply that the secretion of newly synthesized fatty acids by the liver (in plasma lipids) may be accelerated in genetically obese mice. As obese mice have elevated concentrations of insulin and glucose in blood [see Abraham *et al.* (1971) for references] it might be expected that they would show an increased flux of plasma triglyceride (Nikkila, 1969). Such an increased turnover has been reported in obese humans (Birkenhager & Tjabbes, 1969; Nestel & Whyte, 1968). Finally, as plasma triglyceride can be

taken up by adipose tissue and muscle (Robinson, 1970), its metabolism in obese mice is of interest in relation to the deposition of adipose-tissue triglyceride, and to the impairment in obesity of insulin-dependent glucose uptake from blood (Stauffacher & Renold, 1969), through the possibility of competition between glucose and triglyceride in tissues.

In the present paper, an investigation of the turnover of circulating triglyceride in normal and genetically obese mice is reported. This problem has been tackled in regard to both the secretion and assimilation of circulating triglyceride. In addition, the plasma lipoproteins, in which triglyceride is carried, have been characterized. The data suggest that the flux of plasma triglyceride is enhanced in genetically obese mice, and that the extra triglyceride is formed in the liver of obese mice, both from plasma free fatty acids and by synthesis *de novo* of fatty acids from carbohydrate.

Materials and Methods

Animals

Mice were from a mixed strain into which the *ob* gene had been incorporated (see Abraham & Beloff-Chain, 1971). Obese mice were homozygotes (*ob/ob*),

and lean mice were mixed hetero- and homo-zygotes. All mice used were female; fed mice had access to a standard supplemented high-carbohydrate diet of rat cubes (Pilsburys Ltd., Birmingham, U.K.) and to water. Obese mice on a restricted diet were selected at age 6–8 weeks and allowed 5 g of food each morning; from 3–8 months old, they weighed 30–45 g.

Chemicals

Solvents and chemicals were the highest grade available commercially from Fisons Ltd. (Loughborough, Leics., U.K.) or Hopkin and Williams (Romford, Essex, U.K.) unless indicated. Alcohols were from James Burrough Ltd. (London S.E.11, U.K.), and glycerol from BDH Chemicals Ltd. (Poole, Dorset, U.K.). Standard lipids were from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. [U - ^{14}C]Palmitic acid and [U - ^{14}C]glucose were from The Radiochemical Centre, Amersham, Bucks., U.K.

Enzymes and substrates for glycerol determination were from Boehringer Corp. (London) Ltd., London W.5, U.K. Agarose beads (Bio-Gel A; 50m; 50–100 mesh) were from Bio-Rad Laboratories, Richmond, Calif., U.S.A. Intralipid, a stable soya-bean oil emulsion (10% triglyceride, w/v), was from Vitrum, Stockholm, Sweden. One litre of this emulsion contains fractionated soya-bean oil (100 g), egg lecithin (12 g) and glycerol (25 g). Silica gel for t.l.c. (Merck Ltd.) was from Anderman Ltd., London S.E.1., U.K. The anaesthetic Avertin was obtained from Winthrop Laboratories Ltd., Surbiton, Surrey, U.K.

Preparation and treatment of lipid extracts

Extraction of lipids from serum, lipoprotein fractions, or tissues was done by the method of Folch *et al.* (1957). Before extraction, liver samples were frozen in liquid N_2 . Blood was obtained by cardiac puncture from mice anaesthetized with diethyl ether + air. Serum was obtained by centrifugation, after clotting at room temperature.

Lipid classes were separated by t.l.c. (Freeman & West, 1966) on silica gel, made up in 0.01% (w/v) rhodamine 6G (Christophe & Mathijus, 1967). Bands were identified under u.v. light and eluted with diethyl ether–light petroleum (b.p. 40–60°C)–formic acid (50:50:1, by vol.), as described by Bickerstaffe & Annison (1970).

Analytical methods

Glycerides were determined in serum or in lipid extracts as glycerol after saponification (Albrink, 1959). Glycerol was determined as described by Pinter

et al. (1967); for measurements in serum, the values for initial glycerol were subtracted from those for total glycerol, to give the content of glyceride-glycerol. Glycerides in fractions obtained after agarose-gel chromatography were measured colorimetrically (Snyder & Stephens, 1959) after t.l.c. Free and esterified cholesterol were determined after separation by t.l.c. with a $FeCl_3$ method (Rosenthal *et al.*, 1957). Phospholipids were measured as P_1 (Martin & Doty, 1949) after degradation in perchloric acid (60%, w/v). Glucose was determined by a glucose oxidase method. Protein was determined by the method of Lowry *et al.* (1951), with recrystallized bovine serum albumin (Sigma Chemical Co. Ltd.) as reference protein.

Characterization of plasma lipoproteins in mice

In an initial attempt to separate plasma lipoprotein classes in mice, electrophoresis on cellulose acetate strips was employed. The resolution of fractions was not satisfactory, despite the adequate separation of human plasma lipoproteins in parallel experiments. However, acceptable resolution of murine plasma lipoprotein classes was achieved with other methods. (1) Ultracentrifugation. Plasma was obtained by centrifugation after the addition of 20 μ l of trisodium citrate (16%, w/v) to each ml of whole blood. The density of the plasma was adjusted by the addition of calculated volumes of a mixture of concentrated salt solutions (Havel *et al.*, 1955). Plasma was centrifuged at 105000g for 22h in a 3 $\times$ 5ml swing-out rotor (59589) of the MSE 65 ultracentrifuge at 15–20°C, in four spins at successive densities: 1.006 (Gustafson *et al.*, 1965), 1.050, 1.075 (Faloona *et al.*, 1969) and 1.21. The upper fractions were collected from the centrifuge tubes. (2) Agarose-gel chromatography at room temperature. Agarose beads (2%, w/v) in a 45cm $\times$ 2.5cm column were equilibrated with a buffer containing 0.4M-NaCl, 0.1M-potassium phosphate, pH 7.0, and 0.2mm-EDTA (Bowden & Fried, 1970; Margolis, 1967). Total lipoprotein was obtained from serum after ultracentrifugation at density 1.21 (as described above) and layered on to the column (in a volume of about 2.5ml). Elution was carried out with the above buffer at a flow rate of 9 ml/h, and fractions (5–9ml) were collected in a fraction collector. The performance of the column was checked by confirming that human serum lipoproteins behaved as described by Sata *et al.* (1970) and with marker particles (Bowden & Fried, 1970). The recovery of serum lipids in combined fractions was 60–90% of the quantity in serum. (3) Polyacrylamide-gel electrophoresis. Serum lipids were stained before electrophoresis with Sudan Black B (BDH Chemicals Ltd.), as described by McDonald & Ribeiro (1959). Electrophoresis was carried out on 30 μ l of serum by the method of Narayan *et al.* (1966); gels (3.75%) were fixed in 7.5% (w/v) acetic acid at 2°C.

Experiments with [U-¹⁴C]glucose or [U-¹⁴C]palmitate

Fed mice, aged 3–4 months, received [U-¹⁴C]-glucose intraperitoneally (1 mmol; 20–30 μ Ci) between 14:00h and 15:00h. After a time-interval, mice were lightly anaesthetized with diethyl ether+air. Lipid extracts of liver and plasma were prepared (see above), and ¹⁴C in lipids was determined as described below. In experiments with [U-¹⁴C]-palmitate, a solution was prepared (Friedberg *et al.*, 1960) containing [¹⁴C]palmitic acid (200 mCi/mmol, 10–20 μ Ci/ml) bound to bovine serum albumin (Fraction V: Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K.). Albumin was dialysed before use against a bicarbonate-buffered saline (Krebs & Henseleit, 1932). Mice, aged about 3 months, were anaesthetized with 2.5% (v/v) Avertin (0.5–1 ml) and 15 min later 100 μ l of the above solution was injected into a tail vein. In one group of experiments a series of blood samples was taken from the tip of the tail, into heparinized capillary tubes, removing 50–100 μ l at each time. The tubes were sealed and centrifuged at about 1000 rev./min and 4°C for 30 min. Plasma (50 μ l) was removed with a syringe and needle. In a second group of experiments, mice were killed 5 min after injection of [¹⁴C]palmitate, and liver and plasma samples were obtained. Lipid extracts were prepared and treated as described above, for the determination of ¹⁴C in lipids; carrier lipids (including triglyceride) were added to extracts to facilitate separation. The plasma volume was presumed to be 1.5 ml in lean mice and 2.0 ml in *ob/ob* mice (see Abraham *et al.*, 1971).

Experiments with murine lipoprotein labelled with [U-¹⁴C]palmitate

Groups of four to five mice (aged 6–10 months) were injected intravenously with 5–10 μ Ci of [U-¹⁴C]-palmitate (see Fig. 5). After 30 min, they were anaesthetized with diethyl ether; blood was removed by cardiac puncture, and serum was prepared. Samples of serum were pooled, and lipoprotein (VLD lipoprotein*; density less than 1.006) was prepared by centrifugation as described above. The major lipid classes in a small sample of the preparation of lipoprotein were separated by t.l.c., and their content of ¹⁴C determined. In the preparations from both lean and *ob/ob* mice, 70–80% of the total ¹⁴C was contained in triglyceride; most of the remainder was in free fatty acids. Freshly prepared lipoprotein (0.2 ml containing about 10³ d.p.m. in 0.2 μ mol of triglyceride) was injected into the tail vein of mice. After various times, blood was obtained by cardiac puncture and the ¹⁴C in serum triglyceride was determined after

t.l.c. of lipid extracts, as described elsewhere. When the interval between injection of VLD lipoproteins and sampling was less than 4 min, mice were anaesthetized with Avertin (see previous section) before injection, to facilitate accurate timing. In the remaining experiments, VLD lipoprotein was injected into restrained conscious mice, which were killed after a suitable time with diethyl ether.

Measurement of fat-tolerance with Intralipid

Mice were anaesthetized with Avertin (2.5%, w/v). After about 15 min Intralipid was injected into the tail vein (Boberg *et al.*, 1969). Over the next 30 min, serial blood samples (50–75 μ l) were withdrawn from the tip of the tail into heparinized capillary tubes and cooled in ice. Intralipid in plasma was measured within a few hours by nephelometry, after suitable dilution with 0.9% NaCl (Lewis *et al.*, 1972).

Determination of ¹⁴C in lipids and glucose

Lipid classes were separated by t.l.c. and the bands scraped into 20 ml glass counting vials. Glucose, in perchloric acid extracts of blood, was isolated by t.l.c. (Jacin & Mishkin, 1965). Standard glucose spots were detected with a diphenylamine–aniline spray, plus heating to 60°C for 5 min. Unstained [¹⁴C]-glucose bands were eluted into vials with 0.5 ml of water, followed by 5 ml of 2-methoxyethanol and 10 ml of liquid-scintillation fluid [6 g of 5-(4-biphenyl)-2-(4-*t*-butylphenyl)-1-oxa-3,4-diazole (CIBA Ltd., Basle, Switzerland) and 60 g of naphthalene/l of toluene]. Radioactivity was measured in a Packard Tri-Carb liquid-scintillation spectrometer. The d.p.m. present were computed from recorded c.p.m. by a channels-ratio method (Baillie, 1960) and checked with [¹⁴C]hexadecane as an internal standard. The efficiency of counting of ¹⁴C was about 70% for lipids and 40% for aqueous samples. Recovery of ¹⁴C-labelled lipids after t.l.c., which was assessed by comparing the d.p.m. recovered (in all bands) from the t.l.c. plate and d.p.m. in the total lipid extract before separation, was about 90%; thus elution of ¹⁴C-labelled lipids from silica gel was not essential for the recovery of ¹⁴C.

Results*Concentrations of plasma lipids in mice*

In serum from *ob/ob* mice, the concentration of cholesterol was higher than in lean mice; this increase was partly accounted for by cholesterol esters (Table 1). Most of the cholesterol in serum (of lean or *ob/ob* mice) was in the last major lipoprotein fraction to be

* Abbreviations: VLD lipoproteins, very-low-density lipoproteins; LD lipoproteins, low-density lipoproteins; HD lipoproteins, high-density lipoproteins.

Table 1. Concentrations of lipids in serum of fed and starved mice

Blood was obtained from male mice, aged 2-4 months, under light ether anaesthesia. The methods of lipid analysis and other experimental details are given in the text. The concentrations of free fatty acid in plasma, which have been reported previously*, are included for comparison. Results are expressed as averages, or as means $\pm$ S.E.M., of the numbers of observations indicated in parentheses. †, ‡; $P < 0.02$, calculated by Student's t test.

Nutritional state of mice	Average weight of mice (g)	Lipid class	Concentration of lipid (μ mol/ml of serum)				
			Free fatty acid*	Glyceride-glycerol	Cholesterol, esterified	Cholesterol, total	Lipid phosphorus
Lean, fed	27.9		0.26 $\pm$ 0.03 (12)	0.68 $\pm$ 0.08 (13)	1.61 (2)	2.02 $\pm$ 0.23 (10)†	1.89 $\pm$ 0.41 (3)
Lean, starved (24h)	26.3		1.02 $\pm$ 0.10 (12)	0.43 $\pm$ 0.10 (6)	1.71 (2)	2.72 $\pm$ 0.24 (6)‡	1.06 (2)
<i>ob/ob</i> , fed	64.1		0.29 $\pm$ 0.06 (6)	0.82 $\pm$ 0.09 (13)	2.06 (2)	2.64 $\pm$ 0.25 (10)†	2.58 $\pm$ 0.50 (3)
<i>ob/ob</i> , starved (24h)	59.6		0.89 $\pm$ 0.11 (6)	0.47 $\pm$ 0.08 (5)	2.25 (2)	3.96 $\pm$ 0.45 (6)‡	1.14 (2)

* From Elliott *et al.* (1971).

obtained on agarose-gel chromatography (Fig. 1), which corresponds to the HD lipoprotein (Bowden & Fried, 1970). Hyperlipoproteinaemia in *ob/ob* mice was also demonstrated by polyacrylamide-gel electrophoresis; the excess of lipid in one fraction of mouse serum (Fig. 2) was probably due to cholesterol in a HD-lipoprotein fraction (Narayan, 1967).

The concentrations of other lipids in the plasma of *ob/ob* mice were not markedly increased (Table 1). The plasma glyceride in both lean and *ob/ob* mice was located mainly in the initial (largest-particle-size) fraction on agarose-gel chromatography (Fig. 1a). In this initial fraction, the quantity of triglyceride was larger in *ob/ob* mice than in lean mice (Fig. 1a); this may have reflected a difference in recovery of serum triglyceride after lipoprotein preparation and chromatography, rather than a true hyperglyceridaemia in *ob/ob* mice of this older age group. The plasma glyceride in mice comprised largely triglyceride, as separate experiments employing t.l.c. did not reveal partial glycerides in serum. Separation of lipoprotein classes by ultracentrifugation showed that most of the triglyceride was in a fraction of density less than 1.006, which consisted of VLD lipoprotein rather than chylomicrons (see the Discussion section): in three experiments with pooled sera from fed mice aged 3 months (separated by the method of Havel *et al.*, 1955) the total glyceride in this fraction was 0.75 ± 0.05 in lean mice and 0.86 ± 0.04 in *ob/ob* mice (expressed as μ mol/ml of original serum, $\pm$ S.E.M.), whereas more than two-thirds of the plasma cholesterol and phospholipid (in lean or *ob/ob* mouse serum) was recovered in a fraction of density greater than 1.075.

Formation of hepatic triglyceride and phospholipid from 14 C-labelled precursors

The sources of hepatic triglyceride were investigated with 14 C-labelled precursors. Since the mice were ingesting a starch-based mixed diet, [U- 14 C]glucose (1 mmol) was used in one group of experiments. The amount of [14 C]glucose was 20-30 μ Ci; to correct for variation in the injected dose, the results in Fig. 3 (and Fig. 4 and Table 3) have been adjusted as if the injected 14 C was 10^8 d.p.m. in all animals. The specific radioactivity of blood [14 C]glucose (adjusted in the same way) declined gradually between 0 and 90 min from about 8×10^4 d.p.m./ μ mol to about 2×10^4 d.p.m./ μ mol in lean and *ob/ob* mice. During the period 40-90 min, the average blood glucose concentration was about 20 mM in lean mice, and 27 mM in *ob/ob* mice. The incorporation of 14 C into liver triglyceride, after an initial lag period, was more rapid and more extensive in *ob/ob* mice than in lean mice (Fig. 3). About 60% of the 14 C in *ob/ob* mice was present in the fatty acid part of the total lipid fraction (Table 2) compared with about 40% in lean mice. There were no differences between lean and *ob/ob* mice

Fig. 1. Separation of plasma lipoproteins by agarose-gel chromatography

Agarose-gel chromatography was carried out with lipoprotein from fed mice aged 6 months [void vol. (V_0), 64 ml; included vol., 205 ml; elution vol. (V_e) for bovine serum albumin, 185 ml]. Fractions were analysed (a) for protein: —, lean mice; ----, *ob/ob* mice; or pooled for lipid determination: (a), glycerides (●, ○); (b), cholesterol, esterified (●, ○), or non-esterified (■, □). Other details are in the text. Results are from single analyses of pooled serum (about 4 ml) from more than 12 lean (○, □) or *ob/ob* mice (●, ■). No fractions were omitted from analysis, although they were pooled.

in the incorporation of ^{14}C into liver phospholipids (expressed per g of fresh liver); the amount of ^{14}C in phospholipid after 90 min was up to one-half of that in triglyceride (Fig. 3).

The formation of hepatic triglyceride from circulating free fatty acid was investigated with [$\text{U-}^{14}\text{C}$]-palmitate, which was presumed to be representative of plasma free fatty acids (Hagenfeldt *et al.*, 1972). At 5 min after a trace dose of [^{14}C]palmitate, the incorporation of ^{14}C into liver lipids was about twice as extensive in *ob/ob* mice as in lean mice (Table 3).

Over 70% of the injected ^{14}C was recovered in hepatic glycerolipids of *ob/ob* mice (Table 3).

Calculation of rates of fatty acid synthesis from glucose in the liver of *ob/ob* mice

Since the mice in the present study were ingesting a diet that was predominantly carbohydrate, it is pertinent to calculate the rate of hepatic synthesis of fatty acids from glucose in *ob/ob* mice. This is possible from the present data, partly because the injected

Fig. 2. Separation of plasma lipoproteins by polyacrylamide-gel electrophoresis

Lipoproteins were pre-stained, and separated by polyacrylamide-gel electrophoresis as described in the text. The diagram is representative of results that were obtained three times each in serum from fed lean mice (a) or *ob/ob* mice (b). The designation HD₁ (a HD-lipoprotein fraction) is derived from Narayan (1967).

Table 2. Distribution of ¹⁴C from injected [U-¹⁴C]-glucose in hepatic lipids

Fed mice received [U-¹⁴C]glucose, as described in Fig. 3. Extracts of total liver lipids were washed and saponified (Albrink, 1959); after acidification free fatty acids were extracted twice with light petroleum (b.p. 40–60°C) and their ¹⁴C content determined. Other details are given in the text. Results are the averages of two, or means ± S.E.M. of three, measurements.

Time of sampling (min after injection)	% of lipid ¹⁴ C in fatty acids	
	Lean mice	<i>ob/ob</i> mice
60	39 ± 6 (3)	51 ± 6 (3)
90	35 ± 7 (3)	63 ± 13 (3)
120	39 (2)	70 (2)

glucose (1 mmol) approximately corresponded to the dietary glucose that would have been ingested during the period of the experiments. Since the decline in specific radioactivity of the blood [¹⁴C]glucose was slow (owing to the excess of injected glucose) and since the most-rapid rising phases in the time-courses of incorporation of ¹⁴C into lipids were established (Fig. 3), the rates of fatty acid synthesis in the liver may reasonably be calculated from the rate of accumulation of ¹⁴C in products and the average specific radioactivity of the circulating [¹⁴C]glucose (about 5×10^4 d.p.m./μmol or 8×10^3 d.p.m./μg-atom of

Fig. 3. Time-course of incorporation of ¹⁴C from [U-¹⁴C]glucose into liver lipids

Fed mice received 1 mmol of [¹⁴C]glucose intraperitoneally at about 11:00h. After various times, ¹⁴C was determined in triglyceride (a) or phospholipid (b). The injected ¹⁴C was 20–30 μCi. Other details are in the text. Results are adjusted as if the injected dose was 10^8 d.p.m., and are means of three to five measurements; bars represent the S.E.M. Lean mice (○); *ob/ob* mice (●).

Table 3. Incorporation of ¹⁴C from [U-¹⁴C]palmitate into liver and plasma lipids of mice

Fed mice, aged 6–8 weeks, received [U-¹⁴C]palmitate intravenously, as an average trace dose of 11.3×10^6 d.p.m. After 5 min, the content of ¹⁴C in lipids was determined. The mean liver weights were 1.08 g in lean mice and 1.70 g in *ob/ob* mice. Other details are given in the text. Results are means ± S.E.M. of three observations, unless indicated. *, $P < 0.05$; †, $P < 0.02$, calculated by Student's *t* test.

Type of mouse	Tissue	$10^{-4} \times ^{14}\text{C}$ in lipids (d.p.m./g of fresh liver or ml of plasma)	
		Triglyceride	Phospholipid
Lean	Liver	167 ± 10*	58 ± 17†
Lean	Plasma	0.06 ± 0.02	0.11 (1)
<i>ob/ob</i>	Liver	303 ± 62*	188 ± 36†
<i>ob/ob</i>	Plasma	0.15 ± 0.01	0.14 (1)

C: see previous section). For a C₁₆ fatty acid, taking the results in Fig. 3, the calculated rate of formation of triglyceride-fatty acid from circulating

glucose in the liver of *ob/ob* mice (since about 60% of the ^{14}C was in the fatty acid moiety) was about $0.03\ \mu\text{mol}$ of fatty acid/min per g fresh liver (assessed between 40 and 60 min), compared with about 0.01 in lean mice. The rate of synthesis of phospholipid fatty acid, similarly calculated, was less than $0.01\ \mu\text{mol}$ of fatty acid/min per g in lean or *ob/ob* mice. Since each glucose molecule may be presumed to generate two C_2 (acetyl) fragments, the total rate of synthesis of fatty acid in *ob/ob* mouse liver represents the consumption of about $0.20\ \mu\text{mol}$ of blood glucose/min per g.

Formation of plasma triglyceride from glucose

In an attempt to measure the rate of formation of plasma triglyceride from glucose the incorporation of ^{14}C into plasma triglyceride from $[\text{U-}^{14}\text{C}]\text{glucose}$ was followed. There was a delay in the appearance of ^{14}C in plasma triglyceride (Fig. 4), especially in *ob/ob* mice. The fastest rate of appearance of ^{14}C in plasma triglyceride in *ob/ob* mice was about 600 d.p.m./min, estimated from the period 40–90 min in Fig. 4. This corresponds to about $0.01\ \mu\text{mol}$ of glucose/min per mouse converted into plasma triglyceride (calculated as in the previous section). However, this value probably underestimates the rate of this process, which cannot be satisfactorily calculated because of the rapid removal of plasma triglyceride ^{14}C , even during the rising phase of entry of ^{14}C into plasma triglyceride.

Fig. 4. Time-course of incorporation of ^{14}C from $[\text{U-}^{14}\text{C}]\text{glucose}$ into plasma triglyceride

Fed mice received ^{14}C glucose, as described for Fig. 3, and ^{14}C in plasma triglyceride was determined after various times. Other details are in the text. Results are means of three measurements (bars indicate S.E.M.), except for the 120 min point in lean mice, and the 40 and 90 min points in *ob/ob* mice, which are averages of two measurements (bars indicate half the range): lean mice (○); *ob/ob* mice (●).

Measurement of the total rate of entry of triglyceride into plasma with $[\text{U-}^{14}\text{C}]\text{palmitic acid}$

A major proportion of plasma triglyceride is derived from circulating free fatty acids (Havel *et al.*, 1970). Hence the appearance of ^{14}C from $[\text{U-}^{14}\text{C}]\text{palmitate}$ in plasma triglyceride of mice was studied. After a trace dose of $[\text{U-}^{14}\text{C}]\text{palmitic acid}$, there was a delay of a few minutes before ^{14}C was detected in plasma triglyceride (Fig. 5). The amount of ^{14}C in triglyceride rose to a maximum after 20–40 min; that in plasma phospholipid was less than one-third of that in triglyceride between 20 and 60 min after injection (results not shown). In these experiments the decline in specific radioactivity of circulating $[\text{U-}^{14}\text{C}]\text{palmitate}$ was presumably rapid, in view of the known rapid turnover of plasma free fatty acids in mice (Dade & Hems, 1972). Hence no satisfactory calculation of the rates of its conversion into plasma triglyceride is possible. However, these experiments permit calculation of the total rate of release of triglyceride-fatty acid into blood, as follows. The amount of ^{14}C in serum triglyceride became approximately constant (Fig. 5). The most likely explanation for this is that it reflected continuous release into plasma from an extravascular pool (or pools) of ^{14}C -labelled triglyceride, which behaved as if it retained fairly constant specific radioactivity during the latter phase of the experiment (see the Discussion section). This could be partly due to recycling between ^{14}C -labelled fatty acids in non-triglyceride pools and triglyceride in

Fig. 5. Time-course of incorporation of ^{14}C from $[\text{U-}^{14}\text{C}]\text{palmitate}$ into plasma triglyceride

Fed mice received ^{14}C palmitate intravenously, as an average trace dose of 2.8×10^6 d.p.m. The content of ^{14}C in plasma triglyceride was determined after various times. Except for the 5 min point, the time-course was followed in serial samples from single mice. Other details are in the text. The values after 5 min are in Table 3. Results are means of three to five measurements, in five lean (○) or *ob/ob* (●) mice, and bars indicate S.E.M.

Table 4. *Calculated rates of entry of triglyceride into plasma of mice*

The results of Fig. 5 have been used to calculate rates of triglyceride secretion into plasma, as described in the text. The designation '%D' refers to the percentage of the injected trace dose of [U-¹⁴C]palmitate.

	Lean mice	<i>ob/ob</i> mice
Plasma triglyceride pool (μ mol of triglyceride)*	1.0	1.6
Approximate specific radioactivity of plasma [¹⁴ C]triglyceride during steady phase in Fig. 5: <i>s</i> (%D/ μ mol of triglyceride)	2.1	0.45
Approximate initial rate of entry of [¹⁴ C]triglyceride into plasma during rising phase in Fig. 5 <i>r</i> : (%D/min)	0.13 (between 10 and 20 min)	0.08 (between 5 and 10 min)
Calculated rate of triglyceride secretion: $3 \times r/s$ (μ mol of triglyceride-fatty acid/min)	0.18	0.54

* From Table 1 and measurements of plasma volume (Abraham *et al.*, 1971).

tissues. Such recycling could occur within the liver lipid pools (Farquhar *et al.*, 1965; Gross *et al.*, 1967, 1968; Havel *et al.*, 1962) or via adipose tissue and blood (Laurell, 1959). Such a postulated pool of mobilizable tissue [¹⁴C]triglyceride, of relatively constant specific radioactivity, is likely to be located mainly in the liver (Havel *et al.*, 1962, 1970; Baker & Schotz, 1964).

If one presumes that triglyceride from such a tissue pool of [¹⁴C]triglyceride was being released at a relatively constant rate into the initially unlabelled pool of plasma triglyceride during the period between 5 and 40 min (Fig. 5), then a rate of release of triglyceride may be calculated from the initial rising phase of incorporation of ¹⁴C in triglyceride (Fig. 5) and the specific radioactivity of the serum triglyceride, estimated from the steady phase of the time-course (Fig. 5). Such calculations of rates of secretion of triglyceride are shown in Table 4. From the nature of the calculation, these probably represent minimum values for this process in the present conditions.

Removal of circulating triglyceride in mice

The assimilation of plasma triglyceride was investigated by injecting lipoprotein (VLD lipoprotein) in which the triglyceride was labelled by prior administration of [U-¹⁴C]palmitate to separate donor mice. Blood was obtained about 30 min after injection of [¹⁴C]palmitate (as in Fig. 5); lipoprotein was prepared as described in the Materials and Methods section, and the fresh preparation was injected into mice. The disappearance of injected ¹⁴C in triglyceride from blood was more rapid in *ob/ob* mice than in lean mice (Fig. 6). Although the present results were not adequate to discern the kinetic order of this process, the initial phase of triglyceride clearance from blood exhibited approximately first-order kinetics in other similar experiments (Havel, 1965; Gross *et al.*, 1968; Farquhar *et al.*, 1965; see Nikkila, 1969, for dis-

Fig. 6. *Time-course of disappearance from plasma of ¹⁴C in lipoprotein triglyceride-[¹⁴C]fatty acid*

Fed mice, aged 2 months, were injected intravenously with VLD lipoprotein containing [¹⁴C]triglyceride. VLD lipoprotein from lean mice was injected into other lean mice (○) and that from *ob/ob* mice into other *ob/ob* mice (●). After various times, ¹⁴C in plasma triglyceride was determined. Other details are in the text. Each result represents a single measurement, and lines were fitted by regression analysis, taking 100% (at time zero) as one value; *r* was -0.88 for lean mice, and -0.77 for *ob/ob* mice.

cussion). Presuming that such kinetics pertained in the present instance, half-times of clearance from blood of injected VLD lipoprotein triglyceride-fatty acid may be estimated from Fig. 6. These half-times are 7.2 min in lean mice and 2.2 min in *ob/ob* mice.

If it is presumed that the clearance from blood of endogenous triglyceride-fatty acid also follows first-order kinetics, and that the injected VLD lipoprotein triglyceride-[¹⁴C]fatty acid mixed with and resembled that in plasma, then the rate constant, *k*, for the uptake of endogenous triglyceride-fatty acid may be obtained from the estimated half-time, *t*, of clear-

ance of injected VLD lipoprotein, since (from the properties of first-order processes) $k \times t = \ln 2 = 0.69$. Thus, from the estimated values of t , the calculated rate constants (k) are 0.10min^{-1} in lean mice and 0.31min^{-1} in *ob/ob* mice. The turnover rate (corresponding to the uptake rate at a steady reactant concentration, since the triglyceride is replenished as it is consumed) equals $k \times (\text{pool size in plasma})$, as elabo-

rated by Farquhar *et al.* (1965). The plasma triglyceride pool consists of $1.0\mu\text{mol}$ in lean mice, and $1.6\mu\text{mol}$ in *ob/ob* mice (from Table 1 and measurements of plasma volume: Abraham *et al.*, 1971). Expressed as μmol of triglyceride-fatty acid, these pool sizes are $3.0\mu\text{mol}$ and $4.8\mu\text{mol}$ respectively. Hence the calculated rates of turnover of plasma triglyceride (in the present experiments) are about $0.30\mu\text{mol}$ of fatty acid/min in lean mice, and 1.50 in *ob/ob* mice. These rates pertain to conscious unstressed mice, since they were calculated from parameters which were measured in such animals (although mice were briefly anaesthetized before being killed).

The capacity for uptake of lipids from blood by mouse tissues was also investigated with Intralipid, which is a stable emulsion of soya-bean oil; the particles in such emulsions resemble chylomicrons (Gries *et al.*, 1967). The time-course of removal of injected 'Intralipid' from blood, shown in representative experiments in Fig. 7, followed first-order kinetics, so that results may be expressed as half-times of disappearance of lipid from blood (Table 5). The rates of fat clearance, which were about twice as rapid in *ob/ob* mice as in lean mice, resembled those obtained after injection of VLD lipoprotein. The rapid fat clearance in *ob/ob* mice was not reversed by diet restriction or starvation, and was present in young animals (Table 5).

Composition of liver lipids in mice

In situations where the secretion of hepatic triglyceride is enhanced, there may be increased deposition of liver fat (for discussion see Nikkila, 1969). This was observed in *ob/ob* mice (Table 6). The excess of hepatic lipid consisted of triglyceride.

Fig. 7. Time-course of removal of injected lipid (Intralipid) from blood

Fed mice, anaesthetized lightly with ether, received intravenous Intralipid (10%, w/v; 0.1 ml in lean mice, 0.13 ml in *ob/ob* mice). Serial samples (50 μ l) were subsequently removed from the tail vein, and Intralipid was measured by nephelometry. Other details are in the text. Representative single time-courses are shown from lean (●, ▲) and *ob/ob* (○, □) mice.

Table 5. Half-times of removal of injected lipid (Intralipid) from the blood of mice

A small dose of Intralipid was injected intravenously into mice that were anaesthetized with Avertin. The half-time of its disappearance from plasma was calculated from the time-course of removal in each mouse, which was followed in sequential blood samples, and which exhibited first-order kinetics (Fig. 7). Other details are given in the text. Results are averages, or means $\pm$ S.E.M., of the numbers of observations in parentheses.

State of mice	Age (months)	Half-time of disappearance of Intralipid (min)	
		Lean mice	ob/ob mice
Fed	1	6.2 (3)	3.2 (2)
Fed	3	5.1 $\pm$ 0.7 (5)	2.5 $\pm$ 0.2 (6)
Fed	4	7.0 (2)	3.5 (2)
Starved (24h)	3	7.0 $\pm$ 1.5 (3)	3.3 $\pm$ 0.3 (3)
Starved (24h)	5	8.3 (2)	4.0 $\pm$ 0.3 (3)
Diet-restricted starved (24h)	6	—	2.7 $\pm$ 0.2 (3)
Diet-restricted starved (24h)	14	—	3.3 (2)

Table 6. *Lipid composition of livers of mice*

The lipids in livers of mice aged about 3 months were extracted and analysed as described in the text. Results are means $\pm$ S.E.M. of the numbers of observations in parentheses.

Mice	Lipid content (μ mol/g of fresh liver)		
	Total glycerides	Lipid phosphorus	Total cholesterol
Lean, fed	31 $\pm$ 3 (9)	37 $\pm$ 3 (3)	11 $\pm$ 1 (3)
Lean, starved (24h)	72 $\pm$ 5 (6)	—	—
<i>ob/ob</i> , fed	72 $\pm$ 7 (8)	39 $\pm$ 5 (4)	11 $\pm$ 1 (3)
<i>ob/ob</i> , starved (24h)	39 $\pm$ 4 (6)	—	—

Starvation brought about a decrease in the content of triglyceride in the liver of *ob/ob* mice, but an increase in that of lean mice. The larger pool of hepatic triglyceride in *ob/ob* mice presumably contributed to the delay in the appearance of 14 C in plasma triglyceride from 14 C-labelled precursors (Figs. 4 and 5), which does not reflect an impairment in the release process, as shown by the present measurements of triglyceride secretion into plasma.

Discussion

Hyperlipoproteinaemia in genetically obese mice

The present experiments confirm the occurrence of hyperlipoproteinaemia in genetically obese mice (Tuller & Mayer, 1959). The excess of lipid in plasma of *ob/ob* mice comprised mainly cholesterol (Mayer & Jones, 1953), in both free and esterified forms, as reported by Enser (1972). These animals thus do not resemble genetically obese ('fatty') rats, in which there is an excess of plasma triglyceride and free fatty acids (Zucker, 1965; Barry & Bray, 1969; Schonfeld & Pfleger, 1971).

There was an increase in the rate of turnover of plasma triglyceride in *ob/ob* mice, compared with lean mice, which was not associated with significant hyperglyceridaemia, except perhaps in older mice (see Fig. 1). The triglyceride in the plasma of *ob/ob* mice was predominantly in a very-low-density fraction, as would be expected, since the major part of circulating triglyceride occurs in this fraction in other species (Havel *et al.*, 1955; Nichols, 1969). The question arises whether the triglyceride-laden particles in the plasma of *ob/ob* mice on a mixed diet are chylomicrons or VLD lipoproteins. The identity of triglyceride-rich particles in plasma of *ob/ob* mice as VLD lipoprotein is suggested by the following evidence: (i) on electrophoresis on cellulose acetate strips, no lipid from plasma of lean or *ob/ob* mice remained near the origin (D. M. W. Salmon, unpublished work), which is where chylomicrons remain in this procedure; (ii) chylomicrons cause turbidity (e.g. on nephelometry) in plasma, and on centrifugation (e.g. 10000g for 1 h)

rise to the top of serum samples; these characteristics were not observed in plasma from *ob/ob* mice.

The cholesterol in the plasma of genetically obese mice was largely in a fraction of density 1.075–1.21, i.e. HD lipoprotein. A significant proportion of the excess of serum lipid (probably cholesterol) in *ob/ob* mice was in a fraction ('HDL₁': identified on polyacrylamide-gel electrophoresis), which has been reported in rats, but appears to be absent from man (Narayan, 1967; Narayan *et al.*, 1966). In man, blood cholesterol is located largely in LD lipoprotein (Havel *et al.*, 1955). The three techniques for characterization of lipoproteins which were employed in the present experiments showed that mouse serum, like rat serum (Havel *et al.*, 1955), contains relatively less LD lipoprotein (density 1.006–1.075) than plasma from humans, and relatively more HD lipoprotein (density greater than 1.075). The significance of this observation, and of the excess of cholesterol in the plasma of *ob/ob* mice, which is not associated with atheromatous arterial deposits, remain to be elucidated.

Turnover of plasma triglyceride in mice

The present experiments show that the rate of turnover of circulating triglyceride is faster in genetically obese mice than in lean mice. This follows from the calculations of both the rate of secretion of triglyceride, and of the clearance from blood of endogenous triglyceride. These calculations are considered in detail, in the following discussion.

The rate of secretion of plasma triglyceride was calculated from the time-course of incorporation of 14 C from a trace intravenous dose of [U- 14 C]palmitate into serum triglyceride. The assumption was made that pools of extravascular triglyceride (likely to be located mainly in the liver) became labelled with 14 C over an initial period during which release of 14 C into serum triglyceride was negligible, and that the time-course of appearance of 14 C in serum triglyceride depended on these extravascular pools subsequently behaving as if there were one pool which retained fairly constant specific radioactivity (for 5–60 min).

This analysis of the events after a trace intravenous dose of ^{14}C -labelled fatty acid agrees in general with the results and conclusions of Baker & Schotz (1964), Havel *et al.* (1962) and Gross *et al.* (1967, 1968), which concerned the behaviour of liver triglyceride pools in particular. As the calculation concerned the rate of triglyceride secretion from a hypothetical triglyceride pool (pre-labelled with [^{14}C]palmitate), it yielded values for the total rate of entry of triglyceride into plasma, but gave no measure of the contribution of individual precursors to this process.

Turning to the calculation of triglyceride turnover in plasma from the measurements of clearance of injected VLD lipoprotein, this depended on the assumptions that the assimilation of the injected VLD lipoprotein exhibited first-order kinetics, and that the VLD lipoprotein mixed with and resembled that in the plasma of mice. The first assumption is supported by studies of the assimilation of plasma triglyceride in other species (Havel, 1965; Nikkila, 1969). The second assumption was reasonable, because the VLD lipoprotein was prepared from donor mice that resembled the recipient mice, including the presence (or not) of genetic obesity. The delay of 30 min before collecting the VLD lipoprotein from donors presumably allowed formation from [^{14}C]palmitate of a variety of ^{14}C -labelled triglycerides, so that the preparations may be regarded as resembling the VLD lipoprotein pool of triglyceride in the recipients.

The calculated rates of secretion and assimilation of endogenous triglyceride-fatty acid in lean mice were of the same order; hence the estimated rate of turnover of plasma triglyceride in lean mice is about $0.25\text{ }\mu\text{mol}$ of fatty acid/min. In *ob/ob* mice, considering that the calculated secretion rate was a minimum value, a reasonable estimate of the rate of triglyceride turnover in the plasma of *ob/ob* mice (female, aged 2–3 months, conscious and fed) is about $1.0\text{ }\mu\text{mol}$ of triglyceride fatty acid/min. This process is likely to play a major part in the production of 'positive' triglyceride balance in adipose tissue (during weight gain in *ob/ob* mice), since plasma triglyceride can be rapidly assimilated by adipose tissue (Bezman *et al.*, 1962) and since the above rate is faster than the rate of gain of fat-weight in mice aged 2–3 months (up to 0.5 g/day).

The occurrence of enhanced turnover of plasma triglyceride in obese mice is in agreement with observations in obese humans (Nestel & Whyte, 1968; Birkenhager & Tjabbes, 1969).

Precursors of plasma triglyceride in genetically obese mice

The triglyceride that enters the plasma in *ob/ob* mice is likely to be mainly formed in the liver, which is the major source of VLD lipoprotein (Havel *et al.*, 1962, 1970). Hence the nature of the circulating pre-

cursors of plasma triglyceride may reasonably be inferred from measurements of the incorporation of ^{14}C from ^{14}C -labelled precursors into hepatic lipids. The present results suggest that both free fatty acids, and fatty acid synthesized *de novo* in the liver, contribute to the extra triglyceride secretion in *ob/ob* mice. This follows from the more rapid formation in *ob/ob* mice of hepatic triglyceride and phospholipid from [^{14}C]palmitate and [^{14}C]glucose.

The rate of formation from circulating glucose of fatty acid (in triglyceride plus phospholipid) in the liver of *ob/ob* mice, which in general confirmed the results of Shreeve *et al.* (1967) and Jansen *et al.* (1967), was calculated to be about $0.20\text{ }\mu\text{mol}$ of glucose converted into fatty acid/min per g of liver (see the Results section). This corresponds to about $0.15\text{ }\mu\text{mol}$ of fatty acid/min per mouse, and may represent a maximum potential rate of hepatic secretion of synthesized fatty acid (in plasma lipids), since there is a likelihood that some ^{14}C -labelled fatty acid reached the liver via blood, and that some of the lipid produced is not released from the liver (Farquhar *et al.*, 1965). This calculated rate of synthesis of fatty acid from glucose is low when compared with the estimated rate of turnover of plasma triglyceride in *ob/ob* mice ($1.0\text{ }\mu\text{mol}$ of triglyceride-fatty acid/min). Further, the total rate of fatty acid synthesis *de novo* (in all tissues) in *ob/ob* mice on a high-carbohydrate diet is about $1.0\text{ }\mu\text{mol/min}$ (N. L. Bowen & D. A. Hems, unpublished work) in accord with the rate of fat deposition (up to 0.5 g/day), and the fact that some fatty acid is degraded by tissues. Hence it appears that hepatic lipogenesis from circulating glucose, although faster in *ob/ob* mice than in lean mice, makes only a minor contribution (one-third or less) to either the total rate of triglyceride secretion by the liver, or to the total rate of fatty acid synthesis in *ob/ob* mice on a starch-based diet. Although the possibility remains open that the synthesis *de novo* of fatty acids from glucose in the liver of *ob/ob* mice was not investigated in optimal conditions, this conclusion is in accord with the generally low capacity for fatty acid synthesis in the liver, relative to adipose tissue.

A major portion of the triglyceride that is secreted in *ob/ob* mice appears to be formed from circulating free fatty acid. This is reflected by the ease with which ^{14}C from [^{14}C]palmitate was detected in plasma triglyceride, and by the faster rate of esterification of palmitate to form liver triglyceride or phospholipid in *ob/ob* mice. The turnover rate of long-chain free fatty acids in plasma of conscious unstressed mice aged 3 months has been estimated to be about $1.8\text{ }\mu\text{mol/min}$ in *ob/ob* mice, and $0.9\text{ }\mu\text{mol/min}$ in lean mice (measured with [^{14}C]palmitate, and calculated for the combined pool of all acids; E. Dade & D. A. Hems, unpublished work). Minimum rates of esterification of plasma free fatty acids in the liver in the present experiments may be calculated as the product of these rates and the

percentage of the intravenous trace dose of ^{14}C in [^{14}C]palmitate that was recovered in hepatic glycerolipids, since the latter values, i.e. 74% and 22% in *ob/ob* and lean mice respectively, were obtained when the incorporation of ^{14}C from [^{14}C]palmitate into liver triglyceride is maximal (Baker & Schotz, 1964). Estimated rates of esterification of plasma free fatty acid in the liver are thus about $1.3\ \mu\text{mol}$ of fatty acid/min in *ob/ob* mice and $0.2\ \mu\text{mol}$ /min in lean mice. Hence this process can account for most of the triglyceride that enters plasma in lean or *ob/ob* mice. The increase in esterification of plasma free fatty acids in *ob/ob* mice (compared with lean mice) is not due to a raised concentration of plasma free fatty acid (Abraham *et al.*, 1971). It is partly due to heptomegaly, and also may be interpreted as a direction of free fatty acid into esterification reactions, rather than into degradative pathways; thus the formation of ketone bodies from free fatty acids is slower in the liver of obese mice (Stein *et al.*, 1970; Winand *et al.*, 1968).

Assimilation of plasma triglyceride in genetically obese mice

The experiments in which triglyceride was injected into mice provided a measure of the capacity for removal by tissue of plasma triglyceride, i.e. of 'fat tolerance'. This process is enhanced in *ob/ob* mice, which explains why their increased triglyceride turnover is not associated with marked hypertriglyceridaemia. Blood triglyceride is taken up by tissues via the lipoprotein lipase reaction (Robinson, 1970). The activity of lipoprotein lipase is enhanced in the heart and adipose tissue of *ob/ob* mice (de Gasquet & Pequignot, 1972; Enser, 1972), especially when calculated per mouse, and also in skeletal muscle (E. Rath, D. A. Hems & A. Beloff-Chain, unpublished work). Hence these tissues are likely to be implicated in the assimilation of plasma triglyceride in *ob/ob* mice. Accelerated activity of lipoprotein lipase in *ob/ob* mice would produce glycerol that cannot be rapidly consumed by adipose tissue or muscle, and could provide part of the extra flux of blood glycerol in *ob/ob* mice (Dade & Hems, 1972).

Increased assimilation of plasma triglyceride in *ob/ob* mice may contribute to their high blood glucose concentration and 'insulin resistance' (Stauffer & Renold, 1969; Abraham & Beloff-Chain, 1971). Increased metabolism of free fatty acid by tissues can cause impairment of glucose uptake (as if there is competition between substrates) at least in muscle; this process is a part of the 'glucose-fatty acid cycle' [see Ruderman *et al.* (1969) and Randle *et al.* (1966) for reviews]. Since triglyceride is metabolized after hydrolysis to free fatty acid and glycerol by the enzyme lipoprotein lipase, similar relationships may obtain between triglyceride and glucose metabolism

in muscle or adipose tissue. The complex links between hypertriglyceridaemia and hyperglycaemia are reviewed by Ruderman *et al.* (1969).

Role of glucose and insulin in the triglyceride metabolism of genetically obese mice

Genetically obese mice have high circulating concentrations of glucose and insulin [see Renold (1968) and Abraham *et al.* (1971) for references], which could be implicated in the enhancement of hepatic glyceride synthesis (Christophe *et al.*, 1970; Winand *et al.*, 1968, 1969). In genetically obese mice, the sluggish response to starvation of hepatic lipogenesis (Jansen *et al.*, 1967) and of blood insulin concentrations (Abraham *et al.*, 1971), despite the rapid decline of the blood glucose concentration (Abraham *et al.*, 1971), suggests that insulin could be controlling the synthesis *de novo* of liver fatty acid. Indeed it has been speculated that genetically obese mice, as a result of their hyperinsulinaemia, might exhibit enhanced turnover of plasma triglyceride (Nikkila, 1969). The present results vindicate this prediction.

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