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Formation and fate of endogenous triglycerides in blood plasma of rabbits*

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SUMMARY

The formation of hepatic triglyceride fatty acids from palmitate-1-C^{14} and their transport from the liver to the blood and from the blood to peripheral tissues were studied in rabbits. Isotopic equilibration of triglyceride fatty acids (TGFA) in subcellular compartments of the liver required up to 2 hr. Hepatic TGFA appear to be the immediate precursor of TGFA contained in very low-density lipoproteins of plasma. Isotopic transfer between hepatic TGFA and TGFA of very low-density lipoproteins occurred rapidly in relation to the turnover rate of TGFA in liver and plasma. Thus, after a few hours, the turnover rate of TGFA in these compartments can be considered as a unit. Part of the turnover of this pool is the result of extrahepatic transport of TGFA in very low-density lipoproteins, but in fasted animals most of it occurred within the liver itself. Experiments in which labeled palmitate or labeled TGFA in very low-density lipoproteins were injected intravenously showed that the distribution in tissues of FFA is not affected by the nutritional state, but that of TGFA is markedly altered. About 20 times as much TGFA radioactivity was deposited in the adipose tissue of re-fed rabbits as in that of fasted animals, but oxidation was considerably less. The rate of esterification of FFA, however, depended greatly on nutritional state in adipose tissue and, to a lesser degree, in skeletal muscle and lung. These findings are discussed in relation to the roles of lipoprotein lipase activity and esterifying capacity of tissue in the fate of circulating FFA and TGFA.

There is abundant evidence that the liver is the chief source of the circulating triglycerides derived from endogenous sources (1–6). The fatty acids from which these triglycerides are formed are derived from *de novo* synthesis in the liver or from the blood. The rate of hepatic lipogenesis in fasting animals is very low (7), whereas influx of free fatty acids (FFA)[1] into the liver is great (8). It therefore appears that the chief source of circulating triglyceride fatty acids (TGFA) in the postabsorptive state is the circulating FFA. Isotopic studies in human subjects have provided quantitative confirmation of this proposition (9). On the other hand, after a meal, hepatic triglycerides derived from chylomicrons or from lipogenesis undoubtedly contribute appreciably to the circulating triglycerides of endogenous origin.

In this report, we present data concerning: 1) the influence of the nutritional state on the immediate fate of circulating FFA, 2) the extent of triglyceride formation in the liver from circulating FFA, and 3) the turnover of TGFA in liver and blood plasma. Hepatic TGFA appear to be the immediate precursors of TGFA contained in very low-density lipoproteins ($d < 1.006$) of plasma. The disposition of TGFA of $d < 1.006$ lipoproteins, as influenced by nutritional state, was also studied. The fate of these circulating TGFA was very similar to that of newly absorbed TGFA carried in chylomicrons and differed markedly from that of FFA.

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[1] The abbreviations used are: FFA = free fatty acids; TGFA = fatty acids in triglycerides.

METHODS

Experimental Animals and Procedures. New Zealand white male rabbits weighing 2,200–3,000 g were used. They were kept in individual cages and fed Nunes rabbit pellets. Before use, they were fasted for 3 days (fasted) or fasted for 3 days and then fed for 1 day (re-fed). They were given a 1% aqueous solution of chloralose (H. P. Rossiger Co.) intravenously in a dose of 55 mg per kg. Lidocaine, 1% (Astra Pharmaceutical Products, Inc.), was injected locally to facilitate operative procedures. Polyethylene catheters were placed in femoral vessels, and tracheotomy was performed before experiments lasting more than a few minutes. Pulse and respiration were recorded at intervals during all studies, and blood pressure was measured with a Statham strain gauge and recorded continuously. Supplemental chloralose was administered at intervals to keep the heart rate below 220. When serial biopsies of liver and adipose tissue were to be taken, a mid-line laparotomy was performed. Samples of liver weighing 300–600 mg were taken from the leading edges of various lobes, with care to take each biopsy from a separate lobe. Bleeding was readily controlled with Gelfoam impregnated with thrombin solution (Upjohn Co.). Arterial blood samples were taken from the femoral artery, mixed with approximately 0.1 mg heparin per ml and chilled in ice immediately. Samples of expired air were collected from the tracheotomy tube in polyvinyl beach balloons through a low-resistance valve with small dead space. At the end of experiments, the liver was removed and weighed and samples of other tissues were taken for analysis.

Preparation of Material for Injection. Palmitic acid-1-C^{14}, over 95% pure on the basis of gas-chromatographic collection of material with appropriate retention time (Research Specialties Co.), was converted to the sodium salt and complexed with crystalline bovine serum albumin (Armour Pharmaceutical Co., Chicago, Ill.) as described previously (10). Very low-density lipoproteins ($d < 1.006$) in which the TGFA were labeled with palmitate-1-C^{14} were obtained in the following manner: 100–500 μc palmitate-1-C^{14} was injected intravenously into fasted rabbits. After about 40 min, the animals were exsanguinated through a catheter placed through the femoral artery into the aorta, and the blood was allowed to clot. To remove bicarbonate and other labeled metabolites, the serum was dialyzed through a cellophane membrane for 6–8 hr at 3° against Krebs' bicarbonate buffer containing 0.1% ascorbic acid. The serum was then centrifuged for 15 hr at 115,000 × g in the 40 rotor of a Spinco Model L ultracentrifuge at about 10°. The $d < 1.006$ lipoproteins contained in the top 2 cm of the tube were harvested with the aid of a tube slicer and equilibrated briefly in a tonometer against 95% O_2–5% CO_2 to remove residual labeled bicarbonate before use. The lipoproteins were used within 3 hr of preparation. Analysis as described below showed that 98% or more of the label was contained in TGFA. I^{131}-labeled albumin was obtained from E. R. Squibb & Sons (New York, N. Y.).

Analytical Methods. Blood samples were centrifuged promptly at 3°, and the plasma was processed within a few hours of collection. The $d < 1.006$ lipoproteins were harvested after centrifugation of 3–4 ml plasma in the 40.3 rotor of the ultracentrifuge for 15 hr at 10° and extracted in chloroform–methanol 2:1 (v/v). The extract was washed with one-fifth volume of approximately 0.03 N HCl. The infranatant plasma was extracted according to the method of Davis (11). Tissue samples were extracted in ethanol–acetone 1:1 (v/v) in a blendor for one minute, and the mixture was heated at 45° overnight. The extracts were then filtered and made to suitable volume.

Lipid classes were separated on small silicic acid columns, 11 mm internal diameter and 130 mm long. Six hundred mg of silicic acid (J. T. Baker Chemical Co., Phillipsburg, N. J.) activated at 110° overnight, and 300 mg Super Cel (Johns Manville Co.) were added to the column in a slurry of heptane-diethyl ether 98:2 (v/v). Five mg or less of lipid was added to the column in a volume of 2 ml. Fractions were eluted with 8 ml heptane–ether (cholesterol esters), 50 ml chloroform (glycerides, FFA, and cholesterol), and 20 ml methanol (phospholipids). The FFA were isolated in the second fraction by the solvent partition method of Borgström (12). The acid phase was washed twice with 0.001 N HCl before analysis. All solvents were evaporated at room temperature under nitrogen.

Homogenates of liver were made with a glass homogenizer fitted with a Teflon pestle in ice-cold 0.15 M saline. The mixture was centrifuged at 400 × g^2 for 5 min at 3° to remove unbroken liver cells, red blood cells, and nuclei. The homogenate was then layered under ice-cold 0.15 M saline in a 40.3 rotor tube and centrifuged at 14,000 × g^2 for 30 min at 10°. This procedure separated the homogenate into three fractions. The fatty layer at the top of the tube ("floating fat")[3] was removed with the aid of a tube slicer. The

[2] Measured at center of tube.

[3] This material seems not to consist of simple globules of fat, since it can be re-emulsified readily, even after recentrifuging in 0.15 M saline. In four samples, such recentrifuged material had the following per cent composition (excluding water): protein, 1.1–3.1; cholesterol, 1.05–1.5; phospholipids, 1.2–1.9; triglycerides, 93.8–95.8. This composition is similar to that of chylomicrons, except for a somewhat lower phospholipid and cholesterol content (13).

clear fluid ("microsomes plus supernatant"), which occupied most of the tube, was removed with a syringe through a long needle. The precipitate ("mitochondria" plus "fluffy layer") was taken up in a small quantity of cold saline. In some cases, "microsomes" were separated from "supernatant" by centrifugation at 114,000 $\times g^2$ for 1 hr. All fractions were extracted in ethanol–acetone 1:1 (v/v) overnight at 45°.

Glyceride glycerol was determined by the method of Carlson (14). FFA were titrated according to Dole (15). Lipid phosphorus was measured by the method of Stewart and Hendry (16) and total and free cholesterol by the method of Sperry and Webb (17). Plasma glucose concentration was determined with Glucostat reagent (18) and total protein concentration by the method of Gornall et al. (19). For determination of the fatty acid composition of lipid classes, the fatty acids were transesterified in the following manner: An aliquot of the lipid extract was taken to dryness under N_2. The residue was dissolved in 5 ml dry methanol containing 1% H_2SO_4 and heated at 70° for 1 hr. An equal volume of water was added, and the methyl esters were extracted in 2 ml heptane. The methyl esters were separated in a gas-liquid chromatography apparatus (Research Specialties Co.) with a 6-ft column containing ethylene–glycol succinate coated on Gas Chrom P (Applied Sciences Laboratory, Inc., State College, Pa.) at 180°, with argon as the gas. The effluent was assayed with a strontium ionization detector, which gave a linear response with mass. The concentration of the methyl esters was determined by the method of Carroll (20). The values were corrected for variable sensitivity of the detector to different methyl esters on the basis of analyses of known mixtures of methyl esters obtained from Applied Sciences Laboratories, Inc. Carbon-14 content of lipids was determined in a Packard liquid scintillation spectrometer using 0.3% diphenyloxazole in toluene as phosphor-solvent. Radioactivity and content of carbon dioxide were determined according to the method of Fredrickson and Ono (21). Protein-bound I^{131} activity of the proteins precipitated in ethanol–acetone extracts of tissue was determined in a crystal scintillation well counter.

RESULTS

Removal of Palmitate-1-C^{14} from the Blood. The half-time of removal of palmitate-1-C^{14} from the circulation of rabbits was less than 1 min. This was found to be true whether the fatty acid was complexed with lipoprotein-free rabbit serum (22) or with bovine albumin. This made it possible to study the distribution of radioactivity in tissues very soon after injection.

TABLE 1. RECOVERY OF RADIOACTIVITY FROM TISSUE LIPIDS OF 4 FASTED AND 4 RE-FED RABBITS 2 MINUTES AFTER INTRAVENOUS INJECTION OF PALMITATE-1-C^{14}

Tissue	% of Radioactivity Administered (Mean Values and Range)	
	Fasted	Re-fed
Liver	29 (21–39)	37 (30–46)
Kidneys	1.8 (1.2–2.8)	2.6 (1.4–4.4)
Lungs	1.0 (0.8–1.3)	2.1 (0.6–3.1)
Heart	0.5 (0.4–0.8)	1.0 (0.5–1.5)
Skeletal muscle*	10 (8–12)	14 (9–18)
Adipose tissue†	10 (9–11)	8 (3–10)
Plasma	16 (11–21)	5 (2–10)

* Taken as 40% of body weight.

† Taken as 5% of body weight; mean value from four sites used in calculation.

Radioiodinated human-serum albumin was injected intravenously into four fasted and four re-fed rabbits. After 5 min, palmitate-1-C^{14} was given intravenously and a blood sample was obtained exactly 2 min later. The animals were killed immediately by intravenous injection of 5 ml of a 10% aqueous solution of $MgSO_4$. A number of tissues were rapidly removed and extracted immediately in ethanol–acetone. The I^{131} content of the blood plasma and of the proteins precipitated from the ethanol–acetone extracts was determined, and the quantity of blood plasma in the extracted tissues was calculated. The quantity of C^{14} radioactivity in the lipids of 1 ml plasma was determined, and an appropriate correction was made for contamination of tissue extracts by radioactivity in plasma FFA. Plasma volume was calculated from the volume of distribution of I^{131} albumin, and this value was used to calculate the quantity of C^{14} radioactivity remaining in plasma FFA.

Table 1 shows the recovery of radioactivity in tissue lipids. Little difference was observed between the two nutritional states. About 30 per cent of the radioactivity injected was not accounted for; this was presumably present in other tissues or water-soluble metabolites. The distribution of radioactivity in tissue lipids is shown in Table 2. The extent of esterification of the fatty acids varied widely among tissues and with nutritional state. No radioactivity was

TABLE 2. PER CENT OF TOTAL LIPID RADIOACTIVITY CONTAINED IN FREE FATTY ACIDS, TRIGLYCERIDE FATTY ACIDS, AND PHOSPHOLIPID FATTY ACIDS OF RABBIT TISSUES 2 MINUTES AFTER INTRAVENOUS INJECTION OF PALMITATE-1-C^{14}

Tissue	No. of Animals	Free Fatty Acids		Triglyceride Fatty Acids		Phospholipid Fatty Acids	
		Fasted	Re-fed	Fasted	Re-fed	Fasted	Re-fed
		Range		Range		Range	
Liver	4	0	0	60–78	51–80	16–40	20–49
Kidney	4	0	0	38–40	31–33	60–62	66–67
Lung	2	29–37	4–8	13–15	34–35	48–57	56–60
Muscle							
Heart	2	0–1	0–3	35–46	72–80	53–65	20–25
Leg	2	6–7	0	15–26	50–52	68–76	47–48
Back	2	8–20	1–2	18–23	38–52	60–69	46–57
Adipose tissue							
Perirenal	4	86–97	1–41	3–14	59–99		
Omental	4	57–93	0–8	7–43	92–100		
Dorsal scapular	2	83–85	3–5	15–17	95–98		
Subcutaneous	2	67–75	25–28	25–33	72–75		

present in the FFA fraction of liver or kidney and practically none in cardiac muscle under either of the two conditions studied. No measurements of FFA concentration were made. In lung and skeletal muscle, most of the palmitate was esterified in fasted animals, but in re-fed animals esterification was almost complete. In adipose tissue, very little esterification occurred in the fasted state, whereas it was almost complete in the re-fed state except in subcutaneous fat and, in one rabbit, in perirenal fat. There was no appreciable radioactivity in cholesterol esters in any tissue, but both triglycerides and phospholipids became radioactive in tissues other than adipose tissue. Except in liver and kidney, less radioactivity was in phospholipids and more in triglycerides in the re-fed animals.

Hepatic Metabolism of Palmitate. Fasted rabbits were given labeled palmitate intravenously, and serial samples were taken of blood and liver. Liver homogenates were prepared immediately and fractionated as quickly as possible. Two minutes after injection, the specific activity of TGFA was higher in liver mitochondria than in microsomes or supernatant, and it was lowest in floating fat (Table 3). The time required for equilibration of specific activities among these fractions varied, occurring as soon as 45 min after injection when the total liver triglyceride concentration was about 30 μmole per g and as late as 2 hr after injection when concentrations were higher (Table 4).

TABLE 3. RELATIVE ACTIVITIES OF TRIGLYCERIDES AND PHOSPHOLIPIDS IN SUBCELLULAR FRACTIONS OF RABBIT LIVER 2 MINUTES AFTER INTRAVENOUS INJECTION OF PALMITATE-1-C^{14}

	Relative Specific Activities of Fatty Acid Moieties*					
	Fasted			Re-fed		
Exp. No.	1	2	3	4	5	6
Triglycerides						
Floating fat	23	22	4	13	23	14
Supernatant	68	60	154	57	138	23
Microsomes	340	230	106	220	129	82
Mitochondria	390	400	190	240	300	166
Phospholipids						
Supernatant	254	390	1040	220	130	131
Microsomes	105	89	100	88	80	92
Mitochondria	95	71	80	84	98	99

* Specific activity of whole liver = 100.

TABLE 4. SPECIFIC ACTIVITIES OF TRIGLYCERIDES AND PHOSPHOLIPIDS OF SUBCELLULAR FRACTIONS OF LIVER

	Relative Specific Activities of Fatty Acid Moieties*				
	Triglycerides			Phospholipids	
Minutes	Floating Fat	Microsomes + Supernatant	Mitochondria	Microsomes + Supernatant	Mitochondria
Exp. 1		(35 μmole/g)		(34 mg/g)	
15	92	96	169	150	
45	104	99	96	105	105
90		110	92	102	100
150	100	108	93	92	110
240	101	99	99	105	112
Exp. 2		(60 μmole/g)		(43 mg/g)	
3	18	116		325	
15	47	130	147	163	95
45	86	135	108	122	97
90	98	147	94	93	102
120	102	108	91	92	98
Exp. 3		(130 μmole/g)		(36 mg/g)	
5	17	217	560	160	93
15	39	171	390	152	97
45	77	177	290	106	102

* Specific activity of whole liver = 100.

TABLE 5. LIPID CONTENT OF LIVER AND PLASMA IN RABBITS

Tissue	Mean Values and Standard Errors: 14 Fasted Animals	8 Re-fed Animals
Liver		
Weight (% of body weight)	2.3 ± 0.06	3.1 ± 0.16
Triglyceride fatty acids		
μmole per g liver	75 ± 9.5	27 ± 4.8
μmole per kg body weight	1,720 ± 220	840 ± 148
Phospholipids		
mg per g liver	33 ± 1.1	24 ± 2.2
mg per kg body weight	760 ± 24	744 ± 68
Plasma		
Triglyceride fatty acids (d < 1.006)		
μmole per ml	2.07 ± 0.22	1.30 ± 0.27
μmole per kg body weight	83 ± 8.8	52 ± 11
Triglyceride fatty acids (d > 1.006)		
μmole per ml	1.17 ± 0.21	1.03 ± 0.20
μmole per kg body weight	47 ± 8.4	41 ± 8.0

Hepatic TGFA content was higher in fasted than in re-fed animals, whereas phospholipid content was similar (Table 5). The distribution of TGFA in subcellular fractions in six experiments was estimated from the measured values in these fractions and in whole liver. The specific acitivity of TGFA in unbroken cells and debris was close to that of the whole liver, although that of individual fractions varied widely. Therefore, it was assumed that there was no selective loss of triglycerides from the subcellular fractions and that the relative amounts of TGFA actually recovered in the various fractions were proportional to their concentration in whole liver. Thirty-seven to 44% of the radioactivity in the crude homogenate was present in unbroken cells and debris. The quantity recovered in each fraction was corrected accordingly.

TABLE 6. ESTIMATED CONCENTRATIONS* OF TRIGLYCERIDE FATTY ACIDS IN SUBCELLULAR FRACTIONS OF RABBIT LIVER WITH VARYING TRIGLYCERIDE CONTENTS

Exp. No.	Whole Liver	Floating Fat	Supernatant	Microsomes	Mitochondria + Fluffy Layer
1	15.1	5.8	2.3	1.0	6.0
2	20.3	8.6	2.9	1.2	7.6
3	41.7	22.5	3.0	1.1	15.1
4	55.6	35.8	3.4	1.8	14.6
5	100.3	76.5	4.7	1.9	17.2
6	108.2	80.0	7.0	3.0	18.2

* μmole per g wet weight of whole liver.

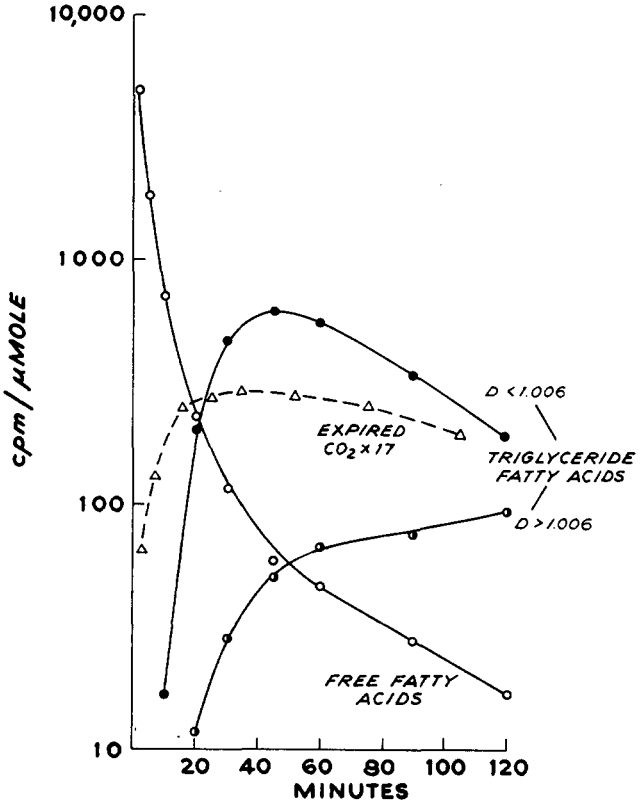

FIG. 1. Specific radioactivity of d < 1.006 lipoprotein, higher density lipoproteins, and expired CO_2 after intravenous injection of palmitate-1-C^{14} into a fasted rabbit. The figures have been corrected to a dose of 10^6 cpm per kg body weight.

The values obtained are given in Table 6. It can be seen that variability of hepatic TGFA concentration was associated largely with variations in the size of the floating fat fraction. TGFA concentration in other fractions also tended to vary directly with that of whole liver, but these differences were considerably less striking. The specific activity of liver phospholipids was highest in the supernatant fraction in the early time periods (Table 3). Equilibration with other fractions required about 45 min (Table 4).

Appearance of Palmitate Radioactivity in TGFA of Plasma. Radioactivity appeared in plasma TGFA about 15 min after injection of labeled palmitate. The specific activity of the TGFA of d < 1.006 lipoproteins rose rapidly and reached a peak in 45–60 min. Their specific activity in higher-density lipoproteins rose much more slowly (Fig. 1). The pattern was similar in fasted and re-fed rabbits, although oxidation of the fatty acid to carbon dioxide was greatly reduced in the re-fed state. The specific activity-time curves for TGFA of liver and d < 1.006 lipoproteins of plasma were compatible with a precursor-product relationship (23) (Fig. 2). In one experiment in which samples

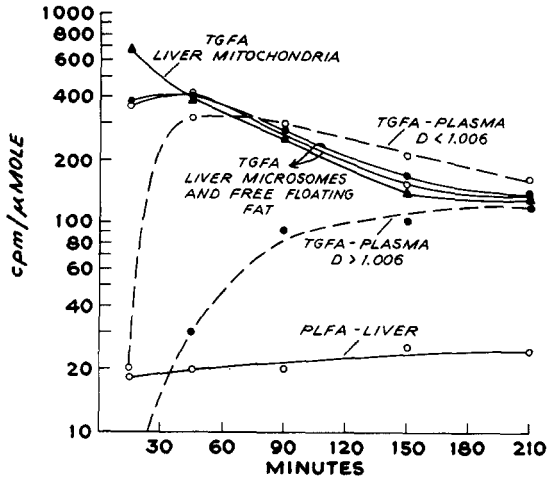

FIG. 2. Specific activities of TGFA of subcellular fractions of liver and plasma lipoprotein fractions in a fasted rabbit after intravenous injection of palmitate-1-C^{14}. Specific activity of hepatic phospholipid fatty acids (PLFA) is also shown (it is assumed that there are two fatty acids per atom of lipid P). Values corrected as in Fig. 1.

of blood and liver were taken from 2 to 8 hr after injection, the specific activity-time curves of TGFA of d < 1.006 lipoproteins and higher-density lipoproteins also bore a precursor-product relationship to one another (Fig. 3). In this experiment, the specific activities of TGFA in subcellular fractions of the liver were similar throughout.

Composition of Triglyceride Fatty Acids. As shown in Table 7, the fatty acid composition of TGFA of liver and of d < 1.006 lipoproteins of plasma was practically identical. TGFA of higher-density lipoproteins contained somewhat less palmitic and more oleic and linoleic acids. Adipose tissue TGFA also contained less palmitic and more palmitoleic and oleic acids than did liver and d < 1.006 lipoproteins. Except for a higher content of myristic acid in liver and plasma of the re-fed animals, no important differences between the two nutritional states were observed. Preliminary analyses show certain differences in the fatty acid composition of TGFA of subcellular fractions of liver. These findings will be reported elsewhere.

Removal of TGFA of d < 1.006 Lipoproteins from the Circulation. After intravenous injection of small quantities of labeled TGFA contained in d < 1.006 lipoproteins, the label rapidly left the circulation. When larger quantities were injected, the incremental concentrations of glyceride glycerol and phospholipids of this fraction decreased at similar rates (Fig. 4). Injections given routinely raised the concentration of TGFA of this fraction about 20%; this was considered to be a tracer dose for purposes of evaluating removal rates. The specific activity-time curves were complex. In fasted rabbits the average initial rate of fall of specific activity had a half-time of 12 minutes, whereas in re-fed animals it was 8 minutes (Fig. 5). The label appeared rapidly in circulating FFA and the specific activity-time curves of FFA roughly paralleled those of TGFA of d < 1.006 lipoproteins (Fig. 6). In fasted animals the specific activity of FFA was about 10% that of TGFA of d < 1.006 lipoproteins. If it is assumed that palmitic acid is a representative tracer for FFA as a whole, then approximately 10% of circulating FFA, or 0.05 μmole per ml plasma, were derived from hydrolysis of circulating TGFA of d < 1.006 lipoproteins. In re-fed rabbits the FFA specific activities were approximately 30% those of TGFA of d < 1.006 lipoproteins, indicating the latter could account for about 0.06 μmole of FFA per ml plasma.

Appearance of Radioactivity in Higher Density Lipoproteins after Intravenous Injection of Labeled TGFA Contained in d < 1.006 Lipoproteins. In the experiments described in the preceding paragraph, radioactivity appeared in TGFA of higher density lipoproteins (>1.006) and rose gradually. After 2–3 hours,

TABLE 7. MOLAR PER CENT COMPOSITION OF METHYL ESTERS OF TRIGLYCERIDE FATTY ACIDS IN BLOOD PLASMA AND TISSUES OF 4 RABBITS

	Fasted				Fasted				Re-fed				Re-fed			
		Lipoproteins				Lipoproteins				Lipoproteins				Lipoproteins		
Fatty Acid	Liver	d < 1.006	d > 1.006	Adipose	Liver	d < 1.006	d > 1.006	Adipose	Liver	d < 1.006	d > 1.006	Adipose	Liver	d < 1.006	d > 1.006	Adipose
14:0	2.1	1.7	1.5	4.1	2.5	2.0	1.9	3.9	4.6	4.1	3.9	4.1	4.5	3.2	3.8	3.4
14:1	1.1	0.8	1.3	0.9	0.7	1.1	0.8	1.1	1.1	1.1	0.8	1.0	1.3	1.3	1.1	1.2
16:0	47.3	48.7	44.3	40.0	47.3	48.5	42.0	37.0	52.3	49.1	45.3	40.0	45.6	45.4	41.1	41.6
16:1	2.8	2.6	1.9	3.6	1.9	2.1	2.8	4.6	2.7	2.5	2.9	5.2	2.7	2.8	2.7	4.1
18:0	4.9	4.5	5.0	5.7	5.1	4.7	5.4	4.8	3.7	5.3	4.9	4.7	5.3	4.9	4.5	4.8
18:1	19.9	20.2	22.0	22.6	18.4	19.4	22.3	24.2	17.8	17.7	19.6	22.0	19.8	18.9	19.9	19.9
18:2	21.4	20.8	21.8	20.5	20.1	20.0	21.7	19.9	14.6	15.8	19.9	18.5	18.4	20.3	23.2	21.2
18:3	1.3	0.6	1.8	2.3	3.5	2.5	3.1	4.4	2.8	4.8	2.4	4.3	2.5	3.4	3.2	3.9

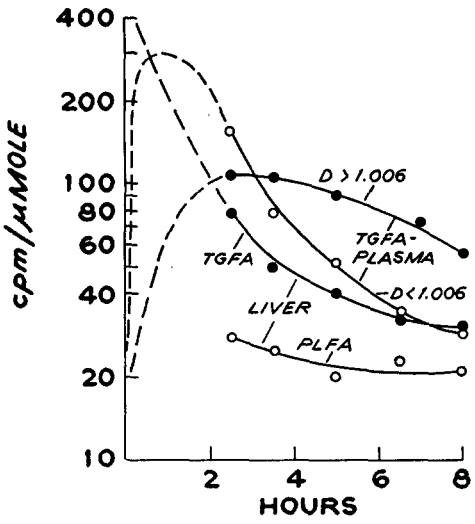

FIG. 3. Specific activities of TGFA of liver and plasma lipoprotein fractions in a fasted rabbit after intravenous injection of palmitate-1-C^{14}. The dotted lines are drawn on the basis of data obtained in 4 other rabbits in which samples were obtained during the first 2 hours after injection. Values calculated and corrected as in Fig. 2.

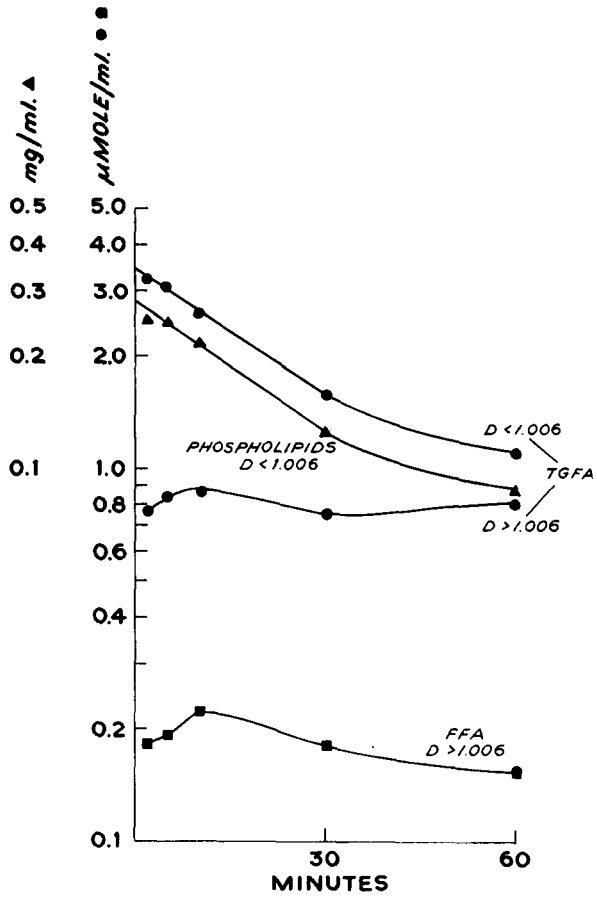

FIG. 4. Concentrations of TGFA and phospholipids of d < 1.006 lipoprotein, TGFA of higher density lipoproteins, and FFA of plasma in a re-fed rabbit (2.43 kg) after intravenous injection of d < 1.006 lipoprotein containing 373 μmoles (about 96 mg) TGFA and 25 mg phospholipid. The initial volume of distribution of the radioactivity injected, based on the plasma radioactivity extrapolated to zero time, was 108 ml (4.45% of body weight). In this experiment the specific activity of FFA averaged about 55% that of TGFA of plasma d < 1.006 lipoprotein.

the specific activities of TGFA in the two fractions were approximately equal.

In order to determine whether radioactivity in TGFA of higher density lipoproteins could have originated by direct transfer within the plasma compartment, d < 1.006 lipoproteins containing radioactive TGFA were incubated with higher density lipoproteins containing unlabeled TGFA for varying intervals. In Figure 7 is shown a comparison of incorporation of radioactivity into TGFA of higher density lipoproteins in one such experiment with that observed when the same radioactive d < 1.006 lipoproteins were injected into a recipient rabbit. It can be seen that radioactivity was transferred *in vitro* but the rate of equilibration of specific activities of TGFA in the two fractions was considerably slower than that observed *in vivo*.

In another experiment, radioactive TGFA in higher density lipoproteins were incorporated into TGFA of d < 1.006 lipoproteins on similar incubation. In these *in vitro* experiments, there was no significant change in TGFA concentrations in the two fractions during incubation. It therefore appears that the transfer of radioactivity was the result of an exchange reaction.

To determine whether this exchange would continue *in vitro* after blood was taken during the *in vivo* experiments, the reaction was studied at various temperatures. No exchange was found during incubation in ice water, so that incorporation of radioactivity in higher density lipoproteins in our *in vivo* experiments was not influenced by continued exchange *in vitro*. The reaction proceeded very slowly at 20–25°. In one experiment, the rates of exchange at 38° and 23° were calculated, assuming that TGFA in d < 1.006 lipoproteins and higher density lipoproteins were homogeneous pools that were completely exchangeable. The values found were 0.0032 per minute at 38° and 0.0008 per minute at 23°.

Distribution in Tissues and Oxidation of TGFA of d < 1.006 Lipoproteins. As shown in Table 8, the distribution in tissue lipids of C^{14} derived from TGFA was markedly affected by the nutritional state. In fasted rabbits, approximately 30% was in hepatic lipids 2 hours after injection. Quantities in other tissues were smaller, and the total recovery of radioactivity in these tissues, plus that in expired CO_2,

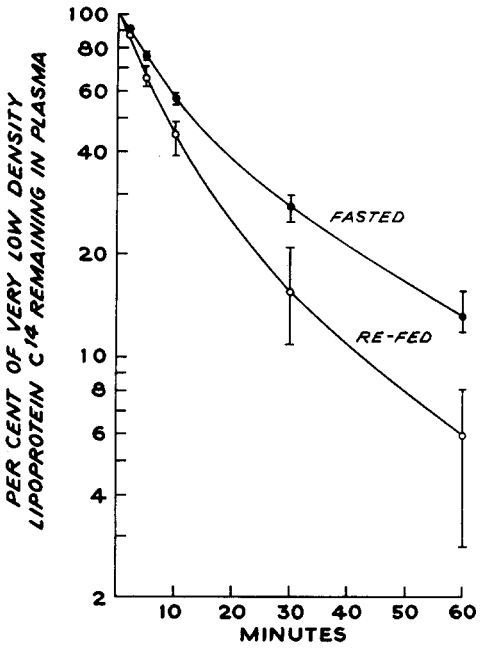

FIG. 5. Disappearance of radioactivity from d < 1.006 lipoproteins after intravenous injection of d < 1.006 lipoprotein containing 36, 38, and 18 μmoles labeled TGFA into 3 fasted rabbits and 25, 36, and 20 μmoles labeled TGFA into 3 re-fed rabbits. The initial concentrations of TGFA of d < 1.006 lipoproteins were 0.97, 1.10, and 1.92 μmole per ml in the fasted rabbits and 0.81, 1.29, and 1.11 in the re-fed rabbits. Average values and range at each time interval are shown.

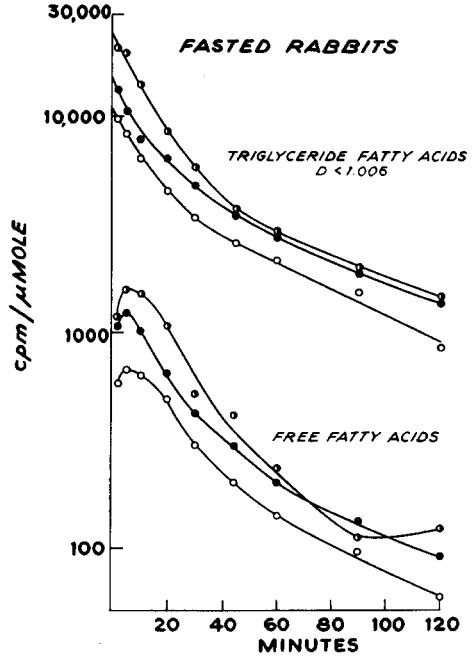

FIG. 6. Specific activities of TGFA of d < 1.006 lipoproteins and FFA in 3 fasted rabbits after intravenous injection of d < 1.006 lipoprotein containing labeled TGFA. The curves for TGFA appear to contain two components with a half-time value for the second component of about 50 minutes.

was about 55%. In re-fed rabbits, only 12% was in hepatic lipids, but adipose tissue lipids contained about 20 times as much as in the fasted rabbits. Total recovery was difficult to assess because of ignorance of the total quantity of adipose tissue. If adipose tissue was 5% of body weight in these rabbits and had a mean specific activity equal to that in the two sites measured, approximately 2 and 40% of the radioactivity injected was present in this tissue in the fasted and re-fed animals respectively. A few measurements in re-fed animals suggest that radioactivity of perirenal and mesenteric fat is similar to that of omental fat, whereas that of fat lying between muscle groups is considerably lower.

Oxidation of injected TGFA was much lower in re-fed than in fasted animals (Table 8 and Fig. 8).

DISCUSSION

The precursor-product relationship between the specific activities of TGFA of liver and plasma d < 1.006 lipoproteins after injection of labeled FFA strongly supports the concept that circulating triglycerides of endogenous origin are derived almost entirely from the liver. Furthermore, it appears that hepatic TGFA are the immediate precursor of TGFA contained in d < 1.006 lipoproteins. This is further supported by the similar fatty acid compositions of TGFA in these two compartments. The subcellular fraction of liver from which TGFA are delivered into circulating d < 1.006 lipoproteins cannot be determined from the present data. In two supplementary experiments in which a constant intravenous infusion of palmitate-1-C^{14} was given, the specific activity of TGFA of d < 1.006 lipoproteins considerably exceeded that of floating fat of the liver after about 1 hour, but was less than that of the other subcellular fractions. This suggests that floating fat serves as a storage reservoir for hepatic TGFA[4] and that TGFA enter plasma d < 1.006 lipoproteins from cell particulates or from the cell sap. The observations of Stein and Shapiro (2) are compatible with this concept, although they found higher specific activities in microsomes than in mitochondria shortly after intravenous injection of labeled palmitic acid.

TGFA of lipoproteins of higher density are probably also derived from the liver (6), but it is not certain that the hepatic TGFA are their immediate precursor. The experimental data demonstrating exchange of

[4] The concept that floating fat represents the hepatic fat depot is further supported by our observations that alterations in hepatic TGFA concentration are mainly due to changes in the concentration of floating fat.

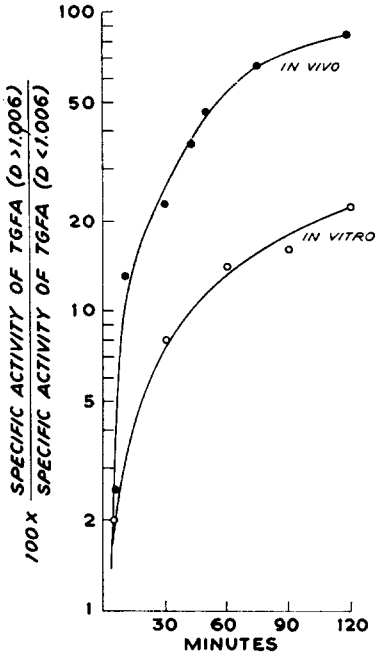

FIG. 7. Appearance of radioactivity in TGFA of higher density lipoproteins after intravenous injection of d < 1.006 lipoproteins containing labeled TGFA (upper curve) and during incubation of aliquots of the same d < 1.006 lipoproteins with unlabeled TGFA in higher density lipoproteins (lower curve). In the *in vivo* experiment, the concentrations of TGFA in d < 1.006 lipoproteins and higher density lipoproteins were 1.20 and 0.52 μmoles per ml respectively. In the *in vitro* experiment, labeled TGFA in d < 1.006 lipoproteins (1.06 μmoles per ml) were incubated at 38° with unlabeled TGFA of higher density lipoproteins (0.60 μmoles per ml) contained in the infranatant fraction of plasma obtained after ultracentrifugation at d = 1.006.

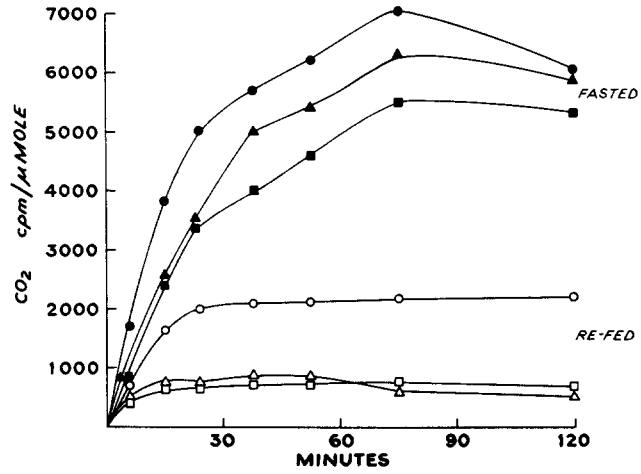

FIG. 8. Specific activity of expired CO_2 after intravenous injection of d < 1.006 lipoproteins containing labeled TGFA into 3 fasted and 3 re-fed rabbits. Values corrected as in Fig. 1.

TGFA radioactivity among lipoprotein fractions *in vitro* suggest that these TGFA may be derived, in part, directly from d < 1.006 lipoproteins. The striking temperature dependence of the exchange reaction suggests that something more than a simple physical process is involved. The fact that the fatty acid composition of TGFA of higher density lipoproteins differs somewhat from that of liver and d < 1.006 lipoproteins is also indicative of a more complex relationship among these TGFA compartments.

Our studies show that consideration of TGFA turnover rates must take into account the fact that TGFA both enter and leave the liver. The rate of transfer of TGFA between the liver and plasma is rapid in relation to their turnover rate, so that changes in specific activity of TGFA of d < 1.006 lipoproteins after injection of labeled FFA clearly represent turnover of a pool of TGFA that includes both plasma and liver. The two components in the specific activity-time curves observed after intravenous injection of labeled d < 1.006 lipoproteins (Fig. 6) probably result largely from a combination of transfer of TGFA from plasma to liver and extrahepatic removal. The data

TABLE 8. RADIOACTIVITY IN LIPIDS OF RABBIT TISSUES 2 HOURS AFTER INTRAVENOUS ADMINISTRATION OF C^{14}-LABELED TRIGLYCERIDES IN VERY LOW-DENSITY LIPOPROTEINS

	Fasted						Re-Fed					
	Exp. No.						Exp. No.					
	1	2	3	1	2	3	4	5	6	4	5	6
Tissue	*cpm/g**			*% of dose*			*cpm/g*			*% of dose*		
Liver	9,200	14,300	10,800	24	38	28	3,820	3,980	5,450	10	10	14
Kidney	1,730	3,240	3,200	1.0	1.5	1.5	1,120	1,420	1,190	0.5	0.7	0.6
Heart	1,750	1,410	1,450	0.3	0.3	0.3	1,260	1,740	2,590	0.3	0.5	0.6
Skeletal muscle	190	110	110	6.8	3.5	3.6	240	210	180	7.8	7.3	7.5
Omental fat	720	350	220				8,200	11,200	12,700			
Dorsal scapular fat	640	300	210				6,300	5,700	4,070			
Expired CO_2†				16	15	12				4	4	2

* Values corrected to a dose of 10^6 cpm per kg body weight.

† Cumulative radioactivity during the 2 hours after injection.

of Laurell (24) and of Stein and Shapiro (25) are also consistent with rapid transfer of TGFA between liver and plasma, but they do not permit precise evaluation of the rates involved. Most of the hepatic-plasma d < 1.006 lipoproteins pool of TGFA is in the liver itself (Table 5). Disappearance of isotope from this pool is complex, presumably because of recycling of label within the liver, from extrahepatic tissues, or both. A similar situation exists in human subjects (9). A further complication in interpretation of specific activity-time curves of plasma TGFA arises from the fact that several TGFA pools exist within the liver itself. Whereas these pools equilibrate rapidly when hepatic TGFA concentrations are normal, this may not be the case when they are high.[5] Our data suggest that the rate of equilibration of TGFA of floating fat with other subcellular fractions is inversely related to its quantity (Table 4). If so, slow equilibration of this pool with other hepatic TGFA pools in the presence of fatty liver may further complicate the interpretation of turnover rates.

With these problems in mind, we have considered, as a first approximation, the period of rapid fall of TGFA specific activity in liver and plasma after injection of labeled palmitic acid to be a measure of the turnover rate of TGFA in the liver-plasma pool. In the experiment shown in Figure 3, the liver-plasma compartment contained 1.02 mmole of TGFA per kg body weight, and TGFA specific activity in liver and plasma were decreasing with a half-time of about 90 minutes (turnover time about 130 minutes). This gives a turnover rate of 0.47 mmole per kg × hr. Similar calculations in the experiment shown in Figure 2 give a value of 0.57 mmole per kg × hr. In this experiment 32% of the injected FFA radioactivity was found in liver TGFA 15 minutes after injection. Thus, assuming no lipogenesis and assuming that 32% of FFA turnover is due to conversion to TGFA in the liver-plasma pool, the FFA turnover should have been 0.57/0.32 or 1.78 mmole per kg × hr. Since the plasma concentration of FFA was 1.2 mmole per liter, assuming plasma volume to be 40 ml per kg, the total plasma content of FFA was 0.048 mmole per kg. This gives a turnover time for FFA of 1.62 minutes and a half-time of 1.12 minutes. This is a reasonable value and suggests that the TGFA turnovers calculated from the data are of the right order of magnitude.

The question of the site of this turnover remains to be considered. In the basal state, the liver accounts for about 30% of the oxygen consumption of the animal (26). The potential energy yield from FFA transport is roughly equal to total caloric needs (8). Therefore, if the liver uses FFA as its major energy source in the postabsorptive state, complete oxidation within the liver must be a major fate of FFA entering the liver (26). Ketoacids probably arise from FFA entering the liver as well, and provide another pathway of utilization. The results of the present study demonstrate an additional pathway—transport of hepatic TGFA to peripheral tissue in d < 1.006 lipoproteins.

An estimate of *maximal* turnover of TGFA due to transport to extrahepatic tissues can be made from the rate of removal of injected TGFA of d < 1.006 lipoproteins in fasted and re-fed rabbits. The actual quantity is undoubtedly considerably less than this because the rate of removal of injected TGFA of d < 1.006 lipoproteins depends on transfer of TGFA of plasma to the liver as well as on extrahepatic removal. The initial half-times were 12 and 8 minutes in fasted and re-fed rabbits respectively (Fig. 5). Given a mean plasma TGFA content of 0.083 and 0.052 mmole per kg body weight in fasted and re-fed rabbits respectively (Table 5), the calculated maximal turnover rates are 0.29 and 0.27 mmole per kg × hr. In fasted animals, the calculated turnover rate of the liver-plasma pool of TGFA is about 0.5 mmole per kg × hr (see above). Therefore, assuming a steady state, other mechanisms, such as hepatic oxidation and ketogenesis, must have been responsible for much of the turnover occurring in the fasting state. In the re-fed animals, FFA turnover was considerably reduced, so that extrahepatic removal of TGFA may have been quantitatively more important. This is supported by the observation that under these conditions injected TGFA were removed more rapidly from the blood and were deposited primarily in adipose tissue. However, the extent of hepatic lipogenesis under these conditions is unknown. More precise evaluation of the question of the relative importance of intra- and extrahepatic turnover of hepatic TGFA in various states of nutrition awaits further experimentation.

In our experiments, TGFA levels in the liver and plasma d < 1.006 lipoproteins were generally lower in re-fed than in fasted rabbits. Presumably, reduced influx of FFA and augmented extrahepatic removal of TGFA in the re-fed animals outweighed the effects of augmented hepatic lipogenesis and reduced oxidation. Consideration of such factors should assist in understanding the genesis of certain types of fatty liver and hypertriglyceridemic states.

Our observation that the tissue distribution of FFA is not influenced markedly by nutritional state

[5] Bezman, A., P. Nestel, J. M. Felts, and R. J. Havel, unpublished experiments.

is consistent with the concept that uptake of FFA by tissues is limited by blood flow (27, 28) rather than by availability of fatty acid binding sites or chemical conversions in tissues. Such is not the case for TGFA. After injection of chylomicrons labeled in the TGFA moiety, Bragdon and Gordon (29) found much more label in lipids of adipose tissues of fed than of fasted rats. Our data show that uptake of TGFA of $d < 1.006$ lipoproteins in adipose tissue is similarly influenced by nutritional state in rabbits. Since Bragdon and Gordon failed to observe differential deposition of labeled FFA in adipose tissue of fed and fasted rats, they concluded that chylomicron triglycerides entered adipose tissue without prior hydrolysis. However, rapid hydrolysis of chylomicron TGFA accompanies their removal from the blood in extrahepatic tissues (6), and several investigators have found that the activity of lipoprotein lipase, which specifically catalyzes the hydrolysis of TGFA associated with lipoproteins, is much greater in adipose tissue of fed than in that of fasted rats (30–32). Furthermore, it is likely that the enzyme is, at least in part, in the capillary wall (33, 34). Therefore, it appears to us at least as likely that localization of TGFA in adipose tissue of fed animals is a result of augmented local hydrolysis of TGFA in the adipose tissue capillary; high concentrations of FFA thus produced facilitate their uptake into the cytoplasm of the adipose tissue cell.

Rapid hydrolysis of TGFA of $d < 1.006$ lipoproteins and of chylomicrons (22) accompanies their removal from the blood and uptake of TGFA of both moieties in adipose tissue is augmented in the fed state. This suggests strongly that the mechanisms for removal of exogenous (chylomicron) and endogenous TGFA are very similar.

We found that the capacity of adipose tissue to esterify fatty acids is markedly influenced by nutritional state, as other investigators have shown in *in vitro* studies (35). Lack of glycerokinase activity (36) in this tissue is probably the most important determinant of this phenomenon, since glycerophosphate production depends on utilization of glucose. The rate of α-glycerophosphate production presumably controls not only the rate of FFA mobilization from adipose tissue, but also the extent of esterification of FFA derived from TGFA in the adipose tissue capillary. Thus the large quantities of fatty acids made available by augmented lipoprotein lipase activity in adipose tissue of the fed animal are rapidly stored as TGFA within the cell. The role of lipoprotein lipase activity and esterifying capacity of adipose tissue in the incorporation of TGFA of $d < 1.006$ lipoproteins will be considered further in a subsequent report.

In liver and kidney, esterification of FFA was rapid and complete in both fasted and re-fed rabbits. In the rat (36) and rabbit (37),[6] these two tissues have high glycerokinase activities that enable them to phosphorylate the large amounts of glycerol released from adipose tissue in the fasted state. However, cardiac muscle in these two species (36, 37)[6] has very low glycerokinase activity, so that, in this tissue, other mechanisms must be responsible for the rapid esterification we observed in fasted animals. It is clear that the enhanced esterification we observed in skeletal muscle and lung of re-fed rabbits may be an important controlling factor in the extent of oxidation of both circulating FFA and TGFA. The recent studies of Fritz (38) on the metabolism of FFA by the isolated rat diaphragm accord with this concept.

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