Incorporation of lipoprotein-borne triglycerides by adipose tissue in vitro

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SUMMARY Rat adipose tissue was shown to take up triglycerides (TG) upon incubation with isolated human or rat serum lipoproteins. In the physiologic TG concentration range, the uptake in 3 hr was proportional to TG concentration in the medium, without regard to the nature of the TG carrier (lipoproteins of different density classes or chylomicrons). At low TG concentrations an increase in fractional uptake was found.

The TG incorporated were found partly in the fat layer and partly dissolved in an aqueous tissue compartment. When doubly labeled TG (fatty acid-C^{14}, glycerol-H^3) were used, the TG of the soluble compartment retained the initial C^{14}/H^3 ratio of radioactivity, were released in part from the tissue upon reincubation in protein-free medium, and were still contained in intact lipoproteins, immunochemically identical with the original lipoproteins of the medium. On the other hand, the TG in the fat layer had undergone partial transesterification, as inferred from the increase in the ratio of isotope radioactivity. TG elaborated within the tissue by esterification of free fatty acids or by synthesis from glucose were not released into any of several media investigated, so that the release mentioned above does not represent a physiologic mechanism for surrender of tissue fat.

It is concluded that incorporation of lipoprotein-borne TG into adipose tissue proceeds in two stages, at first in the intact lipoprotein into a soluble compartment, followed by a shift into the fat droplet, during which some exchange of TG glycerol takes place. The efficiency of the latter stage appeared to determine the over-all rate of uptake of lipoprotein TG, as concluded from kinetic studies of uptake into the two tissue compartments and from the effects of age, nutritional conditions, and temperature.

Uptake and partition of TG in the tissue compartments, as well as the extent of transesterification, were also studied in adipose tissues of different anatomic sites of the rat, guinea pig. rabbit, cat, and dog.

KEY WORDS adipose tissue · lipoprotein · triglyceride · metabolism · uptake · rat · age · nutrition · anatomic site · chylomicrons · guinea pig · rabbit · cat · dog · lipoprotein labeling

MOST RESEARCH on the removal and metabolism of triglycerides (TG) has hitherto been carried out with chylomicrons or emulsified fat particles (1–4). Although the bulk of the postprandial increment in TG is transported in plasma and initially taken up by the liver as chylomicrons or lipomicrons of density <1.006, the subsequent recycling and uptake by nonhepatic tissues continue at normal plasma TG levels, through lipoproteins of higher density. Quantitatively, the continuous redistribution of TG as constituents of lipoproteins elaborated in the liver seems more important than the transiently prominent transport of particulate TG. This study is therefore concerned with the ability of lipoproteins to serve as donors of TG to adipose tissue, with the mechanism of uptake of lipoprotein TG, and with certain influences affecting the uptake.

METHODS

Trace amounts of TG of high specific activity were introduced into lipoproteins by solubilizing them with the aid of small amounts of Tween 80 and mixing them with human or rat serum as described elsewhere (5). The TG used were: tripalmitin-(carboxyl)-1-C^{14} and triolein-1-C^{14} purchased from the Radiochemical Centre, Amersham, Bucks., England, trilinolein-1-C^{14} from Applied Science Laboratories, Inc., State College, Pa., and a mixture of C^{14}-carboxyl-labeled triolein with 2-H^3-glyceryl-labeled triolein from Amersham. The introduction of the TG tracers into the lipoproteins was confirmed by electrophoresis of the serum on strips of cellulose acetate, on which almost all of the radioactivity moved with β-, α_2-, and α_1-lipoprotein fractions and virtually none remained at the origin. The lipoproteins were isolated from the serum by successive ultracentrifugations at densities of 1.019, 1.063, and 1.21, adjusted by the addition of solid KBr (6). After 14–18 hr at

TABLE 1 UPTAKE OF TG BY RAT EPIDIDYMAL ADIPOSE TISSUE FROM HOMOLOGOUS AND HUMAN LIPOPROTEINS

TG Source and Label	Uptake (Units)
Human lipoproteins d = 1.019–1.063 labeled with:	
Triolein-1-C^{14}	52 ± 4
Trilinolein-1-C^{14}	57 ± 3
Tripalmitin-1-C^{14}	50 ± 3
+ sodium pyrophosphate (0.01 M)	27 ± 1
+ Triton X-100 (0.1%)	29 ± 2
Rat lipoproteins d = 1.019–1.063 labeled with:	
Tripalmitin-1-C^{14}	107 ± 11
+ sodium pyrophosphate (0.01 M)	72 ± 4
+ Triton X-100 (0.1%)	64 ± 4

The uptake is expressed in units of mμmoles TG recovered in 100 mg tissue after 3 hr of incubation per micromole of medium TG. The actual medium concentration of lipoprotein TG ranged from 0.8 to 1.0 μmole/ml. No significant change of specific activity was observed during the period of incubation. Standard errors of the mean are given for 10 and 6 experiments with human and rat lipoproteins respectively.

140,000 × *g* in rotor 40.3 of a Model L Spinco ultracentrifuge, the lipoproteins were separated by slicing the tube, and the KBr was removed by dialysis at 4° for 24–48 hr against Krebs-Ringer phosphate buffer, pH 7.4, from which $CaCl_2$ had been omitted. The lipoproteins were filtered through Whatman No. 42 paper and diluted with buffer to the desired TG concentration before incubation with the tissues. In several experiments human postprandial serum was labeled and chylomicrons were isolated from it by the method of Bragdon (7).

Adipose tissues were excised from rats after decapitation and from guinea pigs, cats, rabbits, and dogs under pentothal anesthesia. The tissues were cut into several longitudinal sections weighing 100 mg, and incubated in the buffer described above, with shaking, for 10 min at 37°. After this preliminary incubation, which was found necessary to remove any loose fat droplets exuded during the preparation, 3–5 pieces were transferred for 3 hr incubation in air in 1.5–2.5 ml of the lipoprotein solutions. At the end of incubation, the tissues were briefly but thoroughly rinsed in a large volume of buffer and either homogenized for fractional centrifugation of cellular components (see below), or directly extracted by grinding in the heptane–isopropanol mixture of Dole (8). Similarly, aliquots of the incubation medium were taken for extraction. The extract was separated into the upper heptane phase containing TG and free fatty acids (FFA) and the lower isopropanol phase containing free glycerol. FFA were removed from the heptane layer by shaking with an equal volume of 0.1 N NaOH in 40% aqueous isopropanol and then reextracted into fresh heptane by acidification. Radioactivity was measured on aliquots of the heptane fractions in a Packard Tricarb Liquid Scintillation Spectrometer. Other aliquots of the respective heptane fractions were saved for hydrolysis and chemical determination of TG-glycerol by a modification of the method of Lambert and Neish (9) or titration of FFA (8). The solid residue of the Dole extract was taken for the determination of tissue protein content according to the method of Lowry et al. (10).

Centrifugal fractionation was carried out after homogenization of the tissue at 20–24° in Krebs-Ringer buffer supplemented with 0.2 M NaF in order to inhibit the lipolytic activity in the homogenate (11). The homogenate was promptly chilled and centrifuged for 30 min at 10,000 × *g* and 2°. The floating, solidified fat layer was freed of residual cytoplasmic particles by a short resuspension at 37° in another volume of buffer (with NaF) and repeated centrifugation in the cold, and finally extracted with Dole's mixture. The infranatant solutions were combined and the particles, including cell debris, nuclei, mitochondria, and microsomes, were precipitated by ultracentrifugation for 2 hr at 100,000 × *g*. The precipitate (washed once) and the supernatant solution were then extracted with Dole's solvent mixture and the TG content and radioactivity determined.

Lipoproteins were identified among the components of the soluble fraction of tissue homogenate by the double diffusion technique of Ouchterlony (12). The soluble fraction prepared after incubation of adipose tissue with human lipoproteins was concentrated by dialysis against 25% polyvinylpyrrolidone, and diffusion was carried out for 4–5 days against rabbit antihuman lipoprotein serum (Behringwerke A.G., Marburg/Lahn, Germany).

RESULTS

Preliminary Testing of the Methods

In Table 1 are shown the tissue uptakes of TG from lipoproteins isolated from homologous and human serum, and from human lipoproteins labeled with different TG tracers. The nature of the tracer appeared to be without influence on the fraction of TG taken up by adipose tissue: tripalmitin, triolein, and trilinolein were thus equally representative of the total lipoprotein TG. This conclusion was further supported by the observation that the specific activity of the variously labeled medium TG did not change appreciably during the incubation, indicating that unlabeled tissue TG were not released, nor were TG of composition other than the tracer itself taken up preferentially from the medium.

Rat adipose tissue incorporated a larger fraction of medium TG from homologous lipoprotein than from human lipoprotein. Human serum was, however, a

TABLE 2 UPTAKE OF TG FROM DIFFERENT LIPOPROTEINS

Lipoprotein TG (μmoles/ml)	Tissue TG Uptake (Units) from Lipoproteins of Density Class				
	<1.006 (Chylomicrons)	<1.019	1.019–1.063	1.063–1.21	Whole Serum
0.15		98 (2)	114 ± 9 (4)	95 ± 8 (5)	
0.25		60 (2)		73 ± 11 (9)	
0.53			52 ± 4 (12)	58 ± 6 (6)	
0.82		41 ± 2 (5)			45 ± 3 (10)
1.10			48 ± 2 (10)	50 ± 4 (6)	40 ± 3 (10)
1.55	36 ± 4 (4)	44 ± 2 (29)			
1.97					42 ± 4 (4)
2.5	42 ± 3 (5)	36 ± 5 (8)	42 ± 3 (5)		
3.7		50 ± 6 (4)			
6.3		31 ± 4 (4)			

Units of TG uptake as in Table 1. The lipoproteins were isolated from human fasting serum and chylomicrons from postprandial serum after a fat-rich meal. Tripalmitin-1-C^{14} or triolein-1-C^{14} was used as label. The values presented are means ± SEM for the number of experiments given in parentheses.

readily available and richer source of lipoprotein TG, so that most of the subsequent experiments were carried out with human lipoproteins. The effects of various factors on the rate of uptake were similar in magnitude with the lipoproteins of both species, as exemplified in Table 1 by the reduction of TG uptake in the presence of inhibitors. Only negligible lipolysis of medium TG occurred during the incubation of adipose tissue with human lipoproteins; with rat lipoproteins the extent of lipolysis was slightly higher but did not exceed 2% of the medium TG. This implies that the exchange of TG glycerol (see below) takes place after incorporation into the tissue. Of the total tissue radioactivity after 3 hr of incubation, TG always accounted for at least 90%, and the balance was recovered as FFA. These results of TG recovery are higher than those of Bezman et al. (13), who found an equal distribution of TG and FFA radioactivity after uptake of labeled lipoproteins of density <1.006 by rabbit adipose tissue, but similar to those of Rodbell with rat adipose tissue and chylomicrons (2).

Table 2 shows that the fraction of the medium TG taken up by the tissue did not change markedly over a wide range of TG concentration, from about 0.8 to 6.0 μmoles/ml, and this practically constant uptake seemed independent of the nature of the lipoprotein carrier. The same was found also when chylomicrons or unfractionated serum served as TG carrier. However, at TG concentrations below 0.5 μmole/ml, which is less than one-third of the normal (fasting) concentration of human serum, the fraction of medium TG taken up rose. This was seen with all three classes of lipoproteins. As pointed out in a previous study (5), the magnitude of uptake may vary with different lipoprotein preparations, as well as with the metabolic state of the adipose tissue, but the relationship to TG concentration remains clearly evident.

The possibility of surface adsorption of TG was tested by immersing the tissue in labeled lipoproteins of various density classes for 1–3 min at 27° and rinsing it in Krebs-Ringer buffer for 30 sec. An uptake of less than 2% of the value at 3 hr was found, which did not justify the subtraction of a nonspecific adsorption blank (13).

Tissue Distribution and Partial Release of TG Taken up

To compare the fate of TG incorporated from lipoproteins with that of TG synthesized within the tissue from FFA, sections of adipose tissue were incubated either with lipoproteins containing doubly labeled TG, or with albumin-bound palmitate-1-C^{14}, and then homogenized and subjected to fractional centrifugation (Table 3). With palmitate virtually all of the newly esterified TG were found in the floating fat layer, representing the fat droplet compartment of the tissue. The radioactivity and TG content of the "soluble fraction" and of the particles were very low, but the specific activity of the TG in these compartments was 4–7 times higher than in the fat layer. As discussed previously (14), this was taken to indicate that the newly formed TG were shifted, after esterification on the particles, into the relatively inactive storage compartment in the fat droplets. After incubation with doubly labeled lipoprotein TG, about two-thirds of the C^{14}-labeled fatty acid moiety was found in the fat layer, but as much as one-third was recovered in the soluble fraction, and small quantities were associated with the particles. The specific activity of the fatty acid moiety of tissue TG was highest in the soluble fraction, lowest in the fat layer, and intermediate in the particles. The ratio of radioactivity of the fatty acid moiety (C^{14}) to that of the glycerol moiety (H^3) in the TG of the supernatant fraction was similar to that of the original medium, but was higher in the particles and highest in the

TABLE 3 FRACTIONATION OF ADIPOSE TISSUE AFTER UPTAKE OF LIPOPROTEIN TG OR ALBUMIN-BOUND FFA

	Esterification of Palmitate-1-C^{14}			Uptake of Lipoprotein TG			
	Distribution of Radioactivity		Specific Activity	Distribution of Radioactivity (C^{14})		Specific Activity (C^{14})	Isotope Ratio $\frac{C^{14}}{H^3}$
	cpm/100 mg	%	*cpm/μmole TG*	*cpm/100 mg*	%	*cpm/μmole TG*	
Fat layer	146,000	99	1360	1045	69.1	10.4	3.92
Soluble fraction	180	0.01	6100	410	27.2	29,800	1.15
Particles	880	0.05	9260	55	3.7	518	1.42

Pooled sections of rat epididymal adipose tissue weighing 500 mg were incubated for 2 hr in 2.5 ml of palmitate-1-C^{14} of specific activity 1.5 × 10^6 cpm/μeq in 4% solution of bovine albumin, or with 2.5 ml of human lipoprotein, d = 1.019–1.063, containing doubly labeled triolein of specific activity in the C^{14} labeled fatty acid moiety of 77,000 cpm/μmole and isotope ratio C^{14}/H^3 = 1.13. For separation of fat layer, soluble fraction, and particles see Methods.

fat layer. This fact pointed to an exchange of glycerol to be connected with the shift of TG from the soluble compartment into the fat droplets. Since the isotope ratio in the fat layer did not increase sufficiently to account for total lipolysis and loss of glycerol from the transferred TG, the possibility was tested that the H^3 radioactivity was due to a nonspecific adsorption onto the fat layer of the original lipoprotein molecules. The fat layer was resuspended in a solution of unlabeled lipoproteins at 37°, and separated again by centrifugation at 4°. The C^{14}/H^3 ratio did not increase appreciably through this washing procedure. Since a substantial increase in the isotope ratio would be expected as a result of removal of lipoproteins with the original label, the persistence of glycerol-labeled TG in the fat layer was taken to indicate that glycerol exchange occurred only in part, in the TG assimilated by the tissue.

Since both the specific activity and the isotope ratio of the TG in the soluble fraction of adipose tissue were close to those of the medium TG, it was inferred that the soluble portion of the tissue might include a compartment directly exchanging with medium lipoproteins. The reversibility of TG incorporation and the possibility of release of intact TG into the surrounding medium were therefore explored. Table 4 shows that adipose tissue containing TG synthesized from radiopalmitate or radioglucose did not surrender appreciable quantities of label, whether incubated with albumin, lipoproteins of various density classes, or even with lipoproteins whose acceptor capacity for TG had been augmented by partial delipidation. On the other hand, adipose tissue labeled by incorporation of lipoprotein TG during 2 hr released 15–25% of the amount taken up on subsequent reincubation for 1 hr (Table 5).

TABLE 4 LACK OF RELEASE OF ADIPOSE TG LABELED BY INCUBATION WITH PALMITATE OR GLUCOSE

	Initial Incubation in Labeling Medium		Reincubation in Acceptor Medium	
	Source of Label	Radioactivity of Tissue TG	Nature of Acceptor Medium	Released TG Radioactivity
		cpm		*cpm*
1	Palmitate-1-C^{14}	161,000	Bovine albumin, 4% in Krebs-Ringer buffer	180
2		159,000	d < 1.019 lipoprotein, 1.8 μmoles TG per ml	76
3		145,000	" " , delipidated	68
4		181,000	d = 1.019–1.063 lipoprotein, 0.9 μmole TG per ml	55
5		164,000	" " , delipidated	128
6		206,000	d = 1.063–1.21 lipoprotein, 0.25 μmole TG per ml	262
7		211,000	" " , delipidated	900
8	Glucose-U-C^{14}	288,000	Whole serum	620
9		17,000	d = 1.063–1.21 lipoprotein	36
10		14,000	" " delipidated	40

In vitro labeling was carried out by 2 hr incubation of a pool of 100-mg pieces of rat epididymal adipose tissue in solutions of albumin-bound palmitate-1-C^{14} or glucose-U-C^{14}. The tissues were then rinsed thoroughly and incubated for 15 min in non-labeled palmitate or glucose solutions respectively, and each section was transferred to 1 ml of the specified acceptor medium for further 2 hr of incubation. Medium and tissue TG radioactivities were then determined, taking care to exclude the radioactivity of FFA and free glycerol.

In vivo labeled rat epididymal adipose tissue (Experiments 9 and 10) was obtained 12 hr after intragastric administration of 10 μc of glucose-U-C^{14}.

Partial delipidation of isolated lipoproteins was performed by gentle shaking with ether in the cold according to Avigan (15). Between 30 and 70% of the normal lipoprotein TG content was removed.

TABLE 5 PARTIAL RELEASE OF ADIPOSE TISSUE TG AFTER UPTAKE OF DOUBLY LABELED LIPOPROTEIN TG

	Initial Incubation in Labeling Medium		Reincubation in Acceptor Medium			
	Tissue TG Radioactivity			Medium TG Radioactivity		
	cpm (C^{14} + H^3)	$\frac{C^{14}}{H^3}$	Nature of Medium	cpm (C^{14} + H^3)	Tissue Radioactivity Released	$\frac{C^{14}}{H^3}$
1	3360	2.42	Buffer	508	15.1	0.82
2	2760	2.82	Bovine albumin 4%	492	17.8	0.88
3	2160	2.02	d = 1.019–1.063 lipoprotein	539	24.9	0.86
4	3470	2.98	Whole serum	516	14.8	0.94

Pooled tissue sections were incubated for 2 hr in human serum lipoprotein of d = 1.019–1.063 containing doubly labeled triolein of isotope ratio C^{14}/H^3 = 0.80. After thorough rinsing, the tissues were incubated for 3 min in buffer and each section (100 mg) was transferred for 1-hr incubation in 2 ml of the unlabeled acceptor medium. The medium was then extracted in Dole's solution and the TG radioactivity determined, taking care to exclude FFA and free glycerol radioactivity. Tissue TG radioactivity is the sum of the activity remaining after reincubation and that released into the acceptor medium.

This release did not differ markedly in magnitude whether buffer, whole serum, lipoproteins, or albumin was employed as acceptor. The C^{14}/H^3 ratio in the released TG radioactivity was close to that of the labeling medium, in contrast to the isotope ratio of the TG radioactivity retained by the tissue, which was markedly higher.

The above observations suggested that the released TG were still in the form of a soluble complex with protein, so that the presence of external acceptor protein was superfluous. To ascertain the identity of the soluble carrier of the released TG and of the carrier of TG recovered in the soluble fraction, immunochemical tests were performed (Fig. 1). In both cases precipitation arcs were obtained with human lipoprotein antiserum, showing that the TG carrier recovered from tissue was identical with the original medium lipoprotein. The soluble fraction recovered from a freshly excised rat tissue showed no reaction with the antiserum.

To investigate whether the release of lipoproteins into an external medium was the result of transfer from a definite tissue compartment, the time course of the release was studied. As seen in Table 6, the TG released into the external medium at 90 min of reincubation were mostly derived from the soluble compartment. Fractionation of the tissue after 90 min of reincubation revealed that the soluble compartment was not rendered completely void of TG but still contained about one-sixth of the total tissue TG radioactivity which, by contrast with that in the fat layer, was at the initial C^{14}/H^3 ratio. Comparison with the tissue fractionated prior to reincubation indicated that no release from the fat layer compartment had occurred. Of the TG radioactivity surrendered by the tissue during the 90 min, about one-third was released in 5 min, the rest at a gradually decreasing rate. Even several changes of the accepting medium during the release did not hasten the outflow from adipose tissue (unpublished experiments), and 20–40% of the TG originally recovered in the soluble fraction of the tissue appeared to remain tightly bound, although still at the initial C^{14}/H^3 ratio.

Distribution of TG Taken up by Adipose Tissue Under Different Tissue and Medium Conditions

The effect of concentration of lipoprotein TG in the medium on the size of the soluble compartment of adipose tissue is illustrated in Fig. 2. As hinted before in Table 2, the fraction of medium TG taken up by the

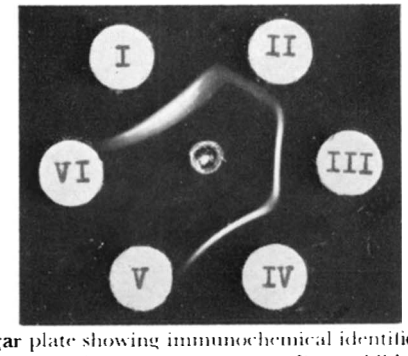

FIG. 1. Agar plate showing immunochemical identification of TG carriers of soluble tissue compartment. Rat epididymal adipose tissue sections weighing 1 g were incubated for 3 hr in human lipoproteins of density 1.019–1.063 and took up about 0.3 μmole of lipoprotein TG. In a control experiment 1 g of tissue was incubated in buffer. After rinsing, 500 mg of tissue was homogenized and the soluble fraction isolated and concentrated (see Methods). Another 500 mg of tissue was reincubated in buffer for 1 hr and the released TG carrier concentrated likewise. Antihuman β-lipoprotein serum was placed in the center well. The other wells contained: I, Medium lipoprotein, 0.84 μmole TG per ml. II, Soluble fraction of the tissue after incubation with the lipoprotein. III, TG carrier collected in buffer during reincubation of the tissue. IV, Medium lipoprotein, diluted 1:3. V, Soluble fraction of the tissue after incubation in buffer instead of lipoprotein solution (control of II). VI, Buffer after reincubation of tissue which had not been brought in contact with lipoproteins (control of III). Note the fusion of precipitation arcs opposite wells II and III with those opposite wells I and IV.

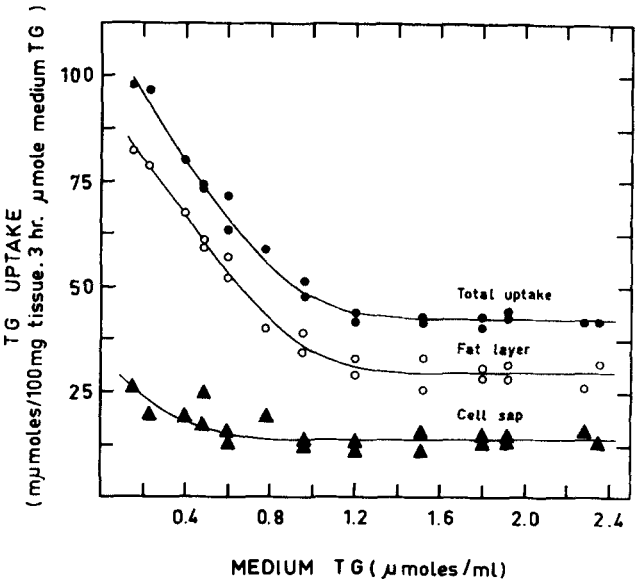

FIG. 2. Partition of TG radioactivity among centrifugal fractions of rat epididymal adipose tissue following incubation at different concentrations of lipoprotein TG. Human lipoproteins, d< 1.063, labeled with triolein-1-C^{14} were used. For separation of tissue fat layer and soluble fraction see Methods.

whole tissue decreased initially with rising medium TG concentration up to about 1 μmole/ml, and then remained practically constant. Likewise, above this level the amounts of TG partitioning into the fat layer and the soluble compartment rose proportionately with the medium TG concentration, so that the fractional uptake by both compartments remained constant. At low medium TG concentration, the soluble compartment appeared, however, to comprise a smaller fraction of the TG taken up.

Table 7 records that the TG uptake by the whole tissue, at constant medium TG concentration, rose with time but not in a linear fashion. This was caused by an uneven rise in the uptake into the two tissue compartments. In the fat layer the uptake of TG was directly proportional to the time of incubation, whereas in the soluble compartment the rise was proportional to time only during the first 90 min of incubation and later leveled off. Consequently, the fat layer comprised an increasing portion of the TG taken up. The C^{14}/H^3 isotope ratio in the soluble compartment remained practically unchanged throughout the incubation period, while in the fat layer it rose gradually, indicating continuing shift and transesterification of the incorporated TG.

Table 8 demonstrates that on lowering the incubation temperature, the uptake of lipoprotein TG decreased markedly. The reduction of uptake was seen in both compartments of adipose tissue, but was particularly associated with loss of transesterification capacity, especially at 0°, as is evident from a very limited increase in the isotope ratio of the TG. Similar influence of temperature was observed on the uptake of particulate fat (2).

The effects of age, the nutritional condition of the rat, and the in vitro addition of glucose, on the rate of uptake of lipoprotein TG are shown in Table 9. Compared with the animals weighing 150–200 g, which were used in all other experiments, the total uptake of lipoprotein TG was higher in the tissues of young animals (less than 100 g) and lower in those of older animals (over 400 g). The extent of transesterification of the newly taken up TG was greater in the young animals and smaller in the older ones, as judged from the change in the ratio of C^{14}/H^3 radioactivity of the doubly labeled TG. These

TABLE 6 TIME COURSE OF RELEASE OF LIPOPROTEIN TG FROM ADIPOSE TISSUE

	Tissue TG Radioactivity after Prelabeling				TG Radioactivity in the Acceptor Medium		
	Fat Layer		Soluble Compartment				
Incubation Time	Cpm ($C^{14}+H^3$)	$\frac{C^{14}}{H^3}$	Cpm ($C^{14}+H^3$)	$\frac{C^{14}}{H^3}$	Cpm ($C^{14}+H^3$)	% of Total Release	$\frac{C^{14}}{H^3}$
min							
0	19,320	2.96	11,470	0.98			
5					3060	32.8	1.03
10					4720	50.6	1.02
15					5460	57.6	0.98
30					6460	69.3	1.01
60					8340	89.4	1.09
90	20,400	3.08	3,890	0.96	9320	100.0	1.11

Pooled 100 mg sections of rat epididymal adipose tissue weighing 1 g were incubated for 2 hr in 5 ml of d < 1.063 lipoprotein containing doubly labeled TG of isotope ratio 0.92. After thorough rinsing in unlabeled lipoprotein and buffer, five sections of the tissue were transferred for a second incubation in 5 ml of buffer from which 0.5 ml samples were taken at specified time intervals. At the start and at the end of the second incubation the tissues were homogenized and the distribution of TG radioactivity between the fat layer and soluble compartment was determined.

TABLE 7 TIME COURSE OF TG UPTAKE INTO FAT LAYER AND SOLUBLE COMPARTMENTS OF ADIPOSE TISSUE

Incubation Time	Total Tissue Uptake (Units)	Fat Layer			Soluble Compartment		
		Units	% of Total	$\frac{C^{14}}{H^3}$	Units	% of Total	$\frac{C^{14}}{H^3}$
min							
45	10.7	6.1	57.0	1.90	4.6	43.0	1.12
90	19.7	11.7	59.4	2.18	8.0	40.4	1.14
180	32.7	20.2	61.8	3.01	12.5	38.2	1.18
360	57.7	43.0	74.6	3.73	14.7	25.4	1.17

Units of uptake correspond to mμmoles of TG taken up by 100 mg tissue per micromole of medium TG at the specified time interval, calculated on the basis of initial specific activity of the C^{14}-labeled fatty acid moiety of the TG. For each time interval a pool of 5 tissue sections weighing 500 mg was incubated in lipoproteins of d < 1.063, containing 0.92 μmole TG per ml of C^{14}/H^3 isotope ratio 1.04.

changes in activity with age were accompanied by a reduction of tissue protein. The distribution of the TG radioactivity between the soluble compartment and the fat layer was similar in the case of the large- and intermediate-sized animals. Addition of glucose to the medium induced an accelerated transesterification and shift into the fat layer of the tissue, but the total uptake of lipoprotein TG was not significantly increased.

The rate of TG incorporation was significantly reduced by fasting and enhanced by refeeding of the experimental animals. These changes were larger than the alterations in tissue protein content associated with the feeding regime. After 3 days of fasting, both the proportion of lipoprotein TG taken up which shifted into the fat layer of the tissue as well as the extent of transesterification was markedly decreased. Upon refeeding for 1 day, there was a rapid rebound in the esterification capacity as evidenced by the high value of the isotope ratio of the assimilated TG, although the partition of the newly absorbed TG in the tissue did not yet reach the normal proportion.

Uptake of Lipoprotein TG by Adipose Tissues of Various Species

In Table 10 the amounts of lipoprotein TG taken up in 3 hr are listed for adipose tissues from different anatomic sites of several animals. It should be borne in mind that human lipoproteins were brought in contact with adipose tissues of other species, so that the efficiencies of uptake by adipose tissues from different sites of the same animal, rather than from different species, should be compared. In the rat the order of activity in adipose tissues seems to be mesenteric > epididymal > perirenal > subcutaneous, as judged from the rate of uptake and the extent of transesterification. In the guinea pig the mesenteric and perirenal tissues incorporated more lipoprotein TG than did the epididymal and subcutaneous tissues, although the extent of transesterification was much the same in all tissues. The perirenal adipose tissue of the rabbit seemed the most active as far as the magnitude of uptake was concerned; the other tissues did not show clear-cut differences. In the cat and dog the perirenal and subcutaneous tissues exhibited a higher rate of uptake than did the mesenteric, though the esterification capacity of the subcutaneous tissue was low. In these animals also the orbital and paw fat pads have been examined. These relatively inert tissues exhibited a TG uptake intermediate between subcutaneous and mesenteric, but the esterification capacity was even lower than in the respective subcutaneous tissues and a larger proportion was retained in the soluble compartment of the tissue.

TABLE 8 EFFECT OF TEMPERATURE ON THE UPTAKE OF LIPOPROTEIN TG BY ADIPOSE TISSUE

Temperature	Total Tissue Uptake (Units)	Fat Layer			Soluble Compartment		
		Units	% of Total	$\frac{C^{14}}{H^3}$	Units	% of Total	$\frac{C^{14}}{H^3}$
37°	43.5	33.4	76.8	4.42	10.1	23.2	1.51
20°	25.2	18.0	71.5	3.16	7.2	28.5	1.48
0°	6.5	3.0	46.2	1.90	3.5	53.8	1.40

Sections of rat epididymal adipose tissue weighing 500 mg were incubated in 2.5 ml of a solution of lipoprotein of d<1.063, containing 1.2 μmoles/ml of TG, labeled with triolein of isotope ratio C^{14}/H^3 of 1.30. Units of TG uptake as in Table 1.

TABLE 9 EFFECT OF RAT AGE AND NUTRITIONAL CONDITION AND OF GLUCOSE ADDITION ON THE UPTAKE OF LIPOPROTEIN TG BY RAT ADIPOSE TISSUE

Animal Series and Weight	Total Tissue Uptake (Units)	Uptake into Fat Layer			Tissue Protein Concentration (mg/100 mg wet weight)
		Units	% of Total	$\frac{C^{14}}{H^3}$	
Fed ad lib. 150–200 g	37 ± 3	26 ± 2	70	4.1 ± 0.3	1.24 ± 0.03
		$P = 0.02$		$P = 0.02$	
As above, medium supplemented with 3 mg/ml glucose	42 ± 3	35 ± 2	83	5.6 ± 0.4	
Fed ad lib. 75–100 g	63 ± 6	53 ± 4	84	7.2 ± 0.4	1.57 ± 0.03
Fed ad lib. 400–800 g	30 ± 4	20 ± 2	67	3.3 ± 0.2	0.75 ± 0.04
Fasted 3 days 150–200 g	24 ± 4	10 ± 2	42	2.5 ± 0.2	1.67 ± 0.04
Refed 1 day incl. 10% glucose in drinking water after 3 days of fasting 150–200 g	63 ± 5	38 ± 4	60	5.4 ± 0.5	1.41 ± 0.05

Units of TG uptake as in Table 1, calculated on the basis of the specific activity of the C^{14}-labeled fatty acid moiety of the lipoprotein TG. The tissues were incubated in lipoproteins of d <1.063 containing doubly labeled triolein with isotope ratio $C^{14}/H^3 = 1.10$ and TG concentration 1.06 μmoles/ml. The values given are means ± SEM of 8 experiments. Total tissue uptake is the sum of the uptake into the fat layer and soluble fractions of the tissue, as determined separately. The effect of glucose was assayed on paired tissues of the same animals.

DISCUSSION

Mechanism of Incorporation

The results indicate that lipoproteins of various density classes may serve as carriers of TG to adipose tissue and that the incorporation of TG proceeds in two stages: an uptake of intact lipoprotein molecules into a compartment recovered in the soluble part of tissue homogenate, and a subsequent esterification, which involves lipolysis and partial exchange of the glycerol moiety. The rearrangement of the TG during the second stage seems to be closely associated with an intracellular transport process, resulting in a loss of TG solubility and a shift into the fat droplet of the cell. The capacity to perform the latter function appears to determine the over-all rate of TG incorporation. This is exemplified by kinetic studies of the uptake into the two compartments, and by the enhancement of the shift into the fat layer brought about by favorable nutritional conditions of the donor animal and the presence of glucose, or by a rise in incubation temperature from 0° to 37°.

It cannot be decided on the basis of the present method of experimentation to what extent the lipoprotein carriers of TG recovered with the soluble components of the tissue actually penetrated into the fat cells in an intact state, or were trapped in the extracellular spaces of the tissue, however small they may be. That they might have resided in part in the extracellular spaces is suggested by the rapid initial rate of egress of a portion of the labeled lipoproteins when the incubation medium was replaced. On the other hand, most of the lipoprotein TG in the soluble fraction was

TABLE 10 COMPARISON OF TG UPTAKE AND DISTRIBUTION IN ADIPOSE TISSUES FROM DIFFERENT ANATOMIC SITES OF FIVE SPECIES

	Uptake of Doubly Labeled TG by Adipose Tissues of														
	Rat			Guinea Pig			Rabbit			Cat			Dog		
	Total Uptake (Units)	% in Fat Layer	$\frac{C^{14}}{H^3}$	Total Uptake (Units)	% in Fat Layer	$\frac{C^{14}}{H^3}$	Total Uptake (Units)	% in Fat Layer	$\frac{C^{14}}{H^3}$	Total Uptake (Units)	% in Fat Layer	$\frac{C^{14}}{H^3}$	Total Uptake (Units)	% in Fat Layer	$\frac{C^{14}}{H^3}$
Epididymal	48	72	5.5	29	59	2.3	23	43	1.5		—			—	
Mesenteric	70	75	6.0	42	53	2.9	12	50	2.1	24	50	2.1	28	44	2.2
Perirenal	38	68	3.9	41	61	2.6	36	46	1.9	35	51	2.4	61	49	2.4
Subcutaneous	30	54	2.5	34	60	2.3	25	49	1.4	31	41	1.5	55	31	1.5
Paw		—			—			—		25	44	1.3	47	34	1.3
Orbital		—			—			—		19	47	1.4	41	28	1.3

Units of TG uptake as in Tables 1 and 9. The tissues were incubated in human serum containing doubly labeled triolein with radioactivity ratio $C^{14}/H^3 = 1.07$. The TG concentration was 1.42 μmoles/ml. The values are means of 3 experiments.

less available for release; possibly this major portion was associated with adipose cells. Though this remains to be proven, it is pertinent that numerous cellular functions as demonstrated on whole adipose tissue have been confirmed when investigated by Rodbell with isolated adipose cells (16). The same author has suggested earlier (2) that the uptake of particulate fat by adipose tissue involves a membrane process described as pinocytosis, and there is electron microscopic evidence for such a mechanism of fat uptake in liver parenchymal cells (17) and in the intestines (18). If the present results warrant the extension of the concept of uptake by pinocytosis also to lipoproteins, their presence in the soluble part of adipose tissue homogenate need not be interpreted as penetration of lipoprotein molecules inside the cell, so as to mix freely with the cytoplasm. Since the lipoproteins may be engulfed by the pinocytotic vesicles, the rupture of these elements upon homogenization would result in admixture of their soluble contents with the cell sap fraction of the tissue. How accurately this model of TG assimilation, evolved from in vitro studies, reflects the physiologic uptake of lipoprotein TG has yet to be studied.

The extent of tissue uptake was found to be related to TG concentration in the medium rather than to the nature of the carrier (Table 2), in spite of the pronounced variations in TG content and in the molecular size of the lipoproteins of different density classes or chylomicrons. It is suggested, therefore, that the "sites of uptake" on adipose tissue cells adapt to the available TG carrier and are able to accommodate either a small number of large particles rich in TG, such as chylomicrons, or a large number of small molecules low in TG, such as lipoproteins of density 1.063–1.21.

In the physiologic range of serum TG concentration the partition of TG between the two compartments of adipose tissue remained practically constant. However, at low medium TG concentration the fractional uptake increased (Table 2 and Fig. 2) and the partition of TG within the tissue was altered in favor of the fat layer compartment, perhaps because of a more efficient transesterification at lower substrate concentration. An inverse relationship between chylomicron TG concentration and uptake by adipose tissue was also observed by Rodbell (2) and by French and Morris (19), though over a higher concentration range of chylomicrons, and has been interpreted as characteristic of pinocytotic processes.

The uptake of labeled TG by adipose tissue may be considered as a net "one way movement," since negligible changes in the specific activity of medium TG during the incubation were found. Special care was taken in this respect to preincubate the tissue before the immersion in labeled lipoproteins, in order to eliminate any nonspecific exudation of TG from cut tissue edges. Further attempts to demonstrate significant release from adipose tissue of TG synthesized by the tissue from labeled fatty acids or glucose were unsuccessful, which may be understood if it is assumed that the assimilated or synthesized FFA are esterified in proximity to the cell fat droplets, into which the newly elaborated TG are promptly incorporated. FFA seem to be removed from the external albumin carrier by direct trapping on the sites of esterification and thus are not likely to require any transporting vehicle within the cell (20). On the other hand, the slower uptake of lipoprotein-borne TG is partially reversible (Table 6), since the TG stay for some time enmeshed within the structure of the lipid–protein complex and only slowly lose the ability to leave the tissue, presumably following a selective removal from the carrier. Rodbell reported that particulate fat once taken up by the tissue is not removable by repeated washings or reincubation (2). This is not necessarily contradictory, since already during the first stage of uptake, chylomicron TG may readily dissociate from their minute protein stabilizer and quickly lose the freedom of movement in an aqueous environment.

Although some lipoprotein could be reextracted into the medium immediately after incorporation, this probably represents only its transient existence during the incorporation process, and not a mechanism of importance during the physiologic surrender of adipose fat. For the TG, recovered from the tissue through a reversal of doubly labeled lipoprotein TG, were always at an isotope ratio close to the original value, i.e., prior to transesterification and storage in the tissue fat droplets; and, further, the release of TG was not induced on contact of media with tissue containing TG labeled by biosynthesis from external precursors. As far as the outflow of tissue fat is concerned, it must be effected through a separate pathway, namely TG lipolysis followed by liberation of FFA and glycerol.

Influence of Age and Nutritional State of the Animal and Anatomic Site of the Adipose Tissue

With advancing age of the rats, the reduction in the rate of TG uptake was about twofold (Table 9), and appeared proportional to the changes in the concentration of cytoplasmic protein. By way of comparison, the decline in fatty acid synthesis from radioacetate, as observed by Benjamin et al. (21), in rat epididymal fat pad was 30- to 40-fold in the corresponding age span. This may signify a selective loss of enzymes concerned with fatty acid synthesis, and an increasing dependence on the supply of preformed TG for the replenishment of fat stores.

The nutritional influences on the uptake of lipoprotein TG (Table 9) paralleled those on the uptake of particu-

late TG (2, 13, 22, 23). Fasting and refeeding, or in vitro addition of glucose, affected mainly the stage of glycerol exchange and shift into the storage compartment. The cleavage of the newly incorporated TG, required for this stage of assimilation, seems linked to the availability of lipoprotein lipase under various nutritional conditions (22, 23). Although the activity of this enzyme, as determined by heparin-induced in vitro release from rabbit perirenal tissue, was indeed found positively correlated with the rate of TG incorporation (13), some observations obscure this relationship. The effect of several inhibitors of lipoprotein lipase on the uptake of lipoprotein TG by rat adipose tissue was not well related to the degree of enzyme inhibition in solution (24). Inconsistencies are apparent between the reported activity of lipoprotein lipase in adipose tissues of different anatomic sites (25, 26), and the TG uptake as listed in Table 10. For example, rat mesenteric adipose tissue exhibits a higher TG uptake than does epididymal, while the order of lipoprotein lipase activity in these tissues is in the reverse direction (25). Also, since the nutritional conditions affect the reesterification capacity of adipose tissue in the same direction as lipoprotein lipase activity, the enhanced exchange of TG glycerol upon refeeding may be due at least in part to the increased supply of α-glycerophosphate (14, 26, 27). Better comparison of the capacity for TG uptake by different adipose tissues will be possible after extension of our knowledge of the intercellular structure of adipose tissues, which may affect the rate of transport of TG carriers toward the cell, and after full understanding of the importance of the *intra*cellular activity of lipoprotein lipase (16). Apart from the quantitative relation of TG uptake to the activity of this enzyme, the role of lipoprotein lipase in the process of cellular assimilation requires a more exact definition, as suggested by the observations of this study that the average change of the doubly labeled TG as they passed into the fat droplet indicated a replacement of glycerol in only about 60% of the molecules. Should this be construed to indicate that intact TG molecules may be assimilated into the fat layer in part, or that the shift into the fat layer does not necessitate a complete cleavage to glycerol in a large proportion of molecules? If the latter possibility should prove true, the incorporation of TG into adipose tissue would be less dependent on the elaboration of α-glycerophosphate than is the assimilation of FFA.

The metabolic characteristics of adipose tissue from different anatomic sites in experimental animals may be variously compared on the basis of assays of lipogenetic activity or capacity to esterify or release FFA upon hormonal and physiologic stimulation (28). From the data of Table 10 it is apparent that the differences in the rate of uptake of TG are not pronounced among the various tissues. Even in the adipose tissues cushioning the paws or the orbital cavity of cats and dogs, which are known to exhibit low metabolic activity (29), substantial TG uptake was found, although the extent of partitioning into fat layer and transesterification were low. Among other species certain tissues seemed particularly active, such as the perirenal adipose tissue of the rabbit or the mesenteric tissue of the rat, but the extent of the shift into the fat layer was not always well correlated with the total amount of TG taken up and with the extent of transesterification. The partition between the soluble tissue compartment and fat layer compartment seemed to be a characteristic property of a given tissue, though in general the proportion shifting into the fat layer was highest in the rat and decreased in the order guinea pig > rabbit > cat > dog.

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