J. Lipid Research, October, 1962
Volume 3, Number 4

Relation between incorporation of triglyceride fatty acids and heparin-released lipoprotein lipase from adipose tissue slices*

ALYCE BEZMAN,† JAMES M. FELTS,‡ and RICHARD J. HAVEL§

Cardiovascular Research Institute and Department of Medicine, University of California School of Medicine, San Francisco, California

[Received for publication May 28, 1962]

SUMMARY

The mechanism of the incorporation of plasma triglyceride fatty acids (TGFA) into adipose tissue was investigated. Slices of adipose tissue from rabbits in different nutritional states were incubated under various conditions with plasma very low-density lipoproteins (d <1.006), in which the TGFA had been biologically labeled with palmitate-1-C^{14}. Lipoprotein lipase activity, released into a heparin-containing medium, was assayed in the same tissues. The results show that the incorporation of TGFA into the slices is dependent on the nutritional state of the animal and is positively correlated with the lipoprotein lipase activity released from the tissue under the influence of heparin, which in turn probably correlates with the total lipoprotein lipase activity of the tissue.

Recent studies have shown that the liver takes up intact triglyceride molecules from the blood (1, 2). There is some evidence that chylomicrons are incorporated into the hepatic parenchymal cell by pinocytosis (3). The process by which triglycerides are incorporated into other tissues is less clearly understood. Bragdon and Gordon (4) and Havel *et al.* (5) demonstrated that the distribution of triglyceride fatty acids (TGFA) of intravenously injected chylomicrons and very low-density lipoproteins depends on the nutritional state of the animal. Greater amounts of TGFA were found in the adipose tissue of fed animals than in that of fasted animals. The activity of lipoprotein lipase, an enzyme thought to facilitate the movement of circulating TGFA from the blood into extrahepatic tissues (6, 7), is also considerably greater in the adipose tissue of fed animals (8).

This study was designed to investigate the mechanism of the uptake of triglycerides into adipose tissue cells. Slices of adipose tissue obtained from rabbits in three nutritional states were incubated with lipoproteins of density less than 1.006 in which the TGFA had been previously labeled with palmitate-1-C^{14}. Our results indicate that there is a direct correlation between uptake of TGFA into adipose tissue and the amount of lipoprotein lipase released from it when heparin is added to the medium, supporting the concept that hydrolysis of TGFA facilitates their incorporation into the adipose tissue cell.

METHODS

Preparation of Labeled Lipoproteins. Lipoproteins of density less than 1.006 (d <1.006 lipoproteins), in which the TGFA were labeled with palmitate-1-C^{14}, were prepared according to the method of Havel *et al.* (5); 98% of the C^{14} was present in TGFA. The TGFA content of these preparations was determined by analysis of glyceride glycerol (9).

Preparation of Tissue. New Zealand white rabbits weighing 2–3 kg were maintained on Nunes rabbit pellets. The animals were fasted for 3 days (fasted), fed *ad libitum* (fed), or fasted for 3 days and re-fed for 1 day (re-fed). They were killed by a blow on the head,

† Postdoctoral Research Fellow, U.S. Public Health Service.
‡ Senior Research Fellow, U.S. Public Health Service.
§ Established Investigator, American Heart Association.
* Supported by grants from the National Heart Institute, U.S. Public Health Service (H-2554 and H-6285); the American Heart Association (No. 59-G-28); the Monterey and Napa County Heart Associations; the Raschen-Tiedemann Fund allocated by the Committee on Research of the University of California School of Medicine, San Francisco; and the Committee on Research of the Academic Senate of the University of California.

TABLE 1. RELATION BETWEEN NUTRITIONAL STATE AND THE INCORPORATION OF TGFA OF d <1.006 LIPOPROTEINS INTO SLICES OF LIVER AND ADIPOSE TISSUE*

Tissue	Nutritional State	Percentage of C^{14}-Labeled TGFA in Tissue Lipids at End of Incubation		
		Expt. 1	Expt. 2	Expt. 3
Liver	Fasted	11	7	6
	Fed	12	9	6
	Re-fed	15	7	8
Adipose tissue	Fasted	3	5	5
	Fed	8	10	13
	Re-fed	19	16	..

* Incubations were carried out for 1 hr. Each flask contained 4.5 ml of buffer, 0.5 ml of d <1.006 lipoproteins in 0.15 M NaCl solution, and either 500 mg of liver slices or 200 mg of adipose tissue slices. Expt. 1, 0.36 μmoles of TGFA per flask; Expt. 2, 0.80 μmoles of TGFA per flask; Expt. 3, 5.8 μmoles of TGFA per flask.

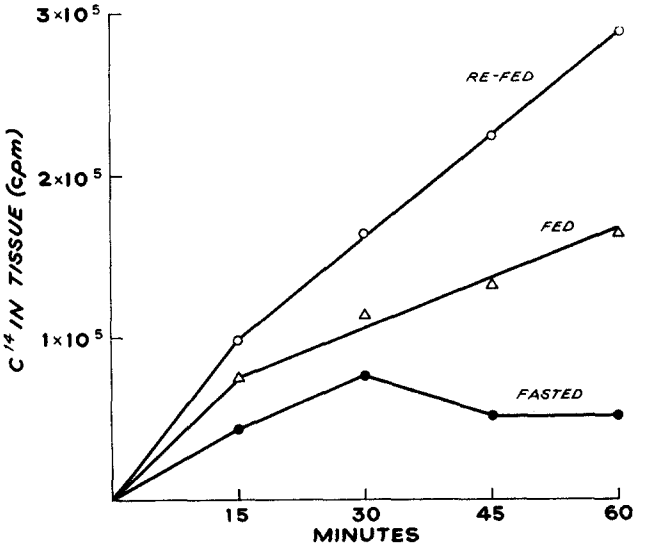

FIG. 1. Relation between nutritional state and the rate of incorporation of TGFA of d < 1.006 lipoproteins into adipose tissue. Each flask contained 4.5 ml of buffer, 0.5 ml of d < 1.006 lipoprotein solution (0.86 μmoles of TGFA), and 200 mg of adipose tissue. Values represent means of duplicate determinations in single experiments.

and the liver and perirenal adipose tissue were quickly removed. The liver was placed immediately in cold Krebs-Henseleit buffer (10), and the adipose tissue was placed in buffer at room temperature. Slices approximately 0.5 mm thick were prepared with a McIlwain tissue slicer, rinsed in buffer, blotted, and weighed.

Incubation of Tissue with Labeled Lipoprotein. Tissue slices (200 mg of adipose tissue or 500 mg of liver) were placed in 50-ml Erlenmeyer flasks containing 4.5 ml of Krebs-Henseleit buffer and 0.5 ml of labeled d <1.006 lipoproteins in 0.15 M NaCl at pH 7.4. The flasks were aerated with 95% oxygen and 5% carbon dioxide, sealed with a serum stopper, and incubated at 37.5° in a Dubnoff metabolic shaker for 1 hr, unless noted otherwise. The total volume was kept to 5.0 ml. All determinations were done in duplicate.

Analysis of Radioactivity. At the end of incubation, the tissue was separated from the incubation medium by filtration through Whatman No. 1 filter paper in a Büchner funnel. The tissue and filter paper were washed three times with 10 ml of 0.15 M NaCl. After separation, the lipids were extracted from the tissue and medium with at least 25 vol of alcohol–acetone 1:1 (v/v). Samples of the lipid extracts were taken up in 0.3% diphenyloxazole in toluene for analysis of C^{14} in a Packard liquid scintillation spectrometer. To correct for radioactivity that might adhere to the tissue, comparable slices of liver or adipose tissue were placed in incubation flasks containing buffer and d <1.006 lipoproteins and mixed for 10 sec, and the tissue and medium were then separated as described above. The radioactivity recovered in the tissue after this procedure was subtracted from that found after incubation. This correction factor was similar in tissues from fasted, fed, and re-fed animals when the same concentration of d <1.006 lipoproteins was used. The magnitude of the correction, expressed as a percentage of the initial medium radioactivity, was generally found to be inversely related to the absolute amount of d <1.006 lipoproteins added to the incubation flasks.

In preliminary experiments, lipid classes were separated from the total lipid extracts by chromatography on silicic acid columns (5). Since more than 96% of the radioactivity in lipids of tissues and media was found either in TGFA or free fatty acids (FFA), subsequent extracts were only separated into acidic and neutral lipids by the method of Borgström (11).

Adipose tissue lipids were separated on silicic acid by thin-layer chromatography. A solvent system, consisting of ether–heptane–glacial acetic acid 60:40:2 by volume, separated phospholipids, monoglycerides, free cholesterol, diglycerides, FFA, and triglycerides. Radioactivity was determined by scanning the glass plates with an automatic scanner equipped with a thin end-window Geiger tube. Lipid components on the developed chromatogram were visualized by spraying the plate with 18 N sulfuric acid and charring on a hot plate. R_f values of these compounds were compared with R_f values of known compounds.

Assay of Lipoprotein Lipase Activity. Lipoprotein lipase activity was assayed by a modification of the method of Cherkes and Gordon (8). Slices (500 mg) of

adipose tissue or liver were placed in Erlenmeyer flasks with and without 160 μg of heparin in 5 ml of Krebs-Henseleit buffer at pH 7.4. The tissue was incubated at 37.5° in a Dubnoff metabolic shaker for 1 hr. One-half milliliter of the incubation medium was then transferred to a test tube containing 0.5 ml of a mixture of 8 parts 10% crystalline bovine albumin in 0.15 M NaCl adjusted to pH 8.6 with ammonium hydroxide, 1 part fresh human serum, and 1 part 4% coconut oil emulsion prepared by diluting Ediol (Schenley Laboratories) with water. This mixture was incubated for 1 hr at 37.5° in a Dubnoff metabolic shaker. Free fatty acids were determined by a modification of Dole's method (12). Maximal release of lipoprotein lipase from the incubated adipose tissue slices occurred at 1 hr, and the release of FFA in the assay system was found to be linear up to 75 min. These results are in keeping with those of Cherkes and Gordon (8). The quantity of FFA released during the assay when heparin was absent from the medium was subtracted from that released during the assay when heparin was included. One unit of lipoprotein lipase activity is defined as that amount that produces 1 μmole of FFA per gram of tissue per hour.

RESULTS

Incorporation of Labeled TGFA of d <1.006 Lipoproteins into Adipose Tissue and Liver. Table 1 shows that the nutritional state of the animal had little effect on incorporation of radioactivity into liver lipids. However, adipose tissue from re-fed animals incorporated $4^1/_2$ times as much radioactivity as that from fasted rabbits. The incorporation of radioactivity into adipose tissue from fed and re-fed animals was a linear function of time between 15 and 60 min (Fig. 1). Incorporation was faster in re-fed than in fed animals; uptake in tissues from fasted animals was very small.

Since the concentration of d <1.006 lipoproteins in all incubation flasks reported in Fig. 1 was the same, the correction factor for adsorbed radioactivity in all instances was the same (0.5×10^5 counts/min or 6% of the C^{14}-labeled d<1.006 lipoproteins added to the incubation flasks).

Regardless of the amount of TGFA in the incubation medium, incorporation of TGFA was always greatest in the re-fed state (Fig. 2). At TGFA concentrations of 0.86–5.2 μmoles/flask, about 50% of the radioactivity in tissues of fed and re-fed animals was in FFA. In tissues from fasted animals, only 10–20% was in FFA. In all dietary states, more than 95% of the C^{14} remaining in the medium was in TGFA. When the lipid in the tissue extracts was fractionated by thin-layer

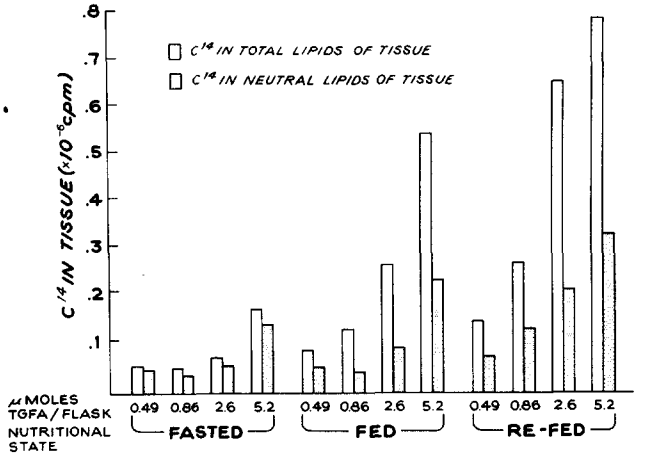

FIG. 2. Relation between nutritional state and incorporation of TGFA of d <1.006 lipoproteins into adipose tissue at varying concentrations of TGFA. Incubations were for 1 hr at 37.6°. Each flask contained 4.5 ml of buffer, 0.5 ml of d <1.006 lipoprotein solution, and 200 mg of adipose tissue. Values represent means of duplicate determinations.

chromatography and scanned, the C^{14} was found to be almost exclusively in FFA and triglycerides.

Total recovery of C^{14} in the tissue and medium ranged from 90–100% with an average of 95% in fasted animals and 92% in fed and re-fed animals. It is therefore unlikely that losses due to differences in oxidation could account for the results noted in Figs. 1 and 2.

Correlation Between Lipoprotein Lipase Activity and Incorporation of Labeled TGFA. Fig. 3 shows a positive correlation between the incorporation of radioactivity into adipose tissue and the activity of heparin-released lipoprotein lipase in samples of the same tissue. The slope as determined by regression analysis is 0.58 ($0.002 > P > 0.001$). Lipoprotein lipase activity in the liver was insignificant.

Exchange Reactions Between FFA and TGFA. To determine whether incorporation of radioactivity into adipose tissue represented an exchange reaction or net uptake of TGFA, the following studies were done. (1) Adipose tissue from re-fed rabbits was incubated for 1 hr with unlabeled d <1.006 lipoproteins and albumin-bound palmitic acid-1-C^{14}; less than 1% of the radioactivity in the medium was in the form of neutral lipids (Table 2). (2) Adipose tissue was pre-incubated for 30 min with albumin-bound palmitic-1-C^{14}, washed three times with 5 ml of 0.15 M NaCl, and then re-incubated with unlabeled d <1.006 lipoproteins; less than 1% of the radioactivity present in neutral lipids of the pre-labeled tissue was found in TGFA of the medium. Thus, it appears that our findings are not the results of exchange of fatty acids; i.e., the hydrolysis catalyzed by lipoprotein lipase is not reversible under the conditions of our experiments.

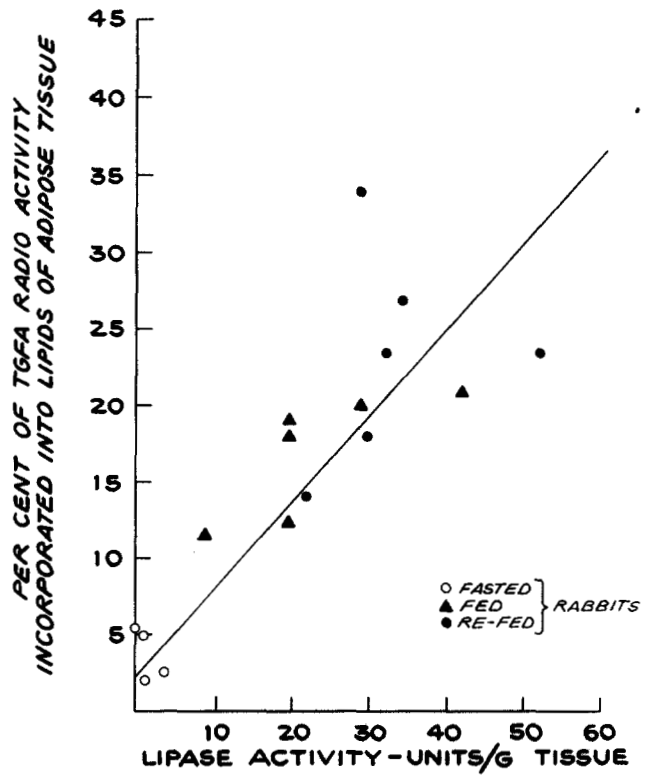

FIG. 3. Relation between lipoprotein lipase activity and incorporation of TGFA into adipose tissue. Five hundred milligrams of adipose tissue was taken for measurement of incorporation of TGFA. Both samples were taken from the same piece of perirenal adipose tissue. Concentration of TGFA per flask varied from 0.86 to 1.75 μmoles. Each point represents mean of duplicate determinations.

DISCUSSION

There is much indirect evidence suggesting that lipoprotein lipase plays a significant role in the removal of triglycerides in chylomicrons and very low-density lipoproteins from the blood. Bragdon and Havel (13) demonstrated that protamine sulfate, a known inhibitor of lipoprotein lipase, increased the concentration of triglycerides in plasma of fasted rats and decreased the rate of removal from the blood of intravenously injected chylomicrons. It has also been shown that the movement of chylomicron TGFA into extrahepatic tissues is accompanied by extensive hydrolysis (14). One form of idiopathic hyperlipemia is associated with a defect in the release of lipoprotein lipase into the blood by heparin (13). Our results suggest that the ability of adipose tissue to incorporate TGFA *in vitro* is also related to the activity of lipoprotein lipase, measured here in terms of the amount of enzyme released into a heparin-containing medium. Since the incorporation of TGFA was closely correlated with the activity of "releasable" lipoprotein lipase, it is likely that triglyceride hydrolysis is a rate-limiting step in this process.

TABLE 2. INCORPORATION OF RADIOACTIVITY INTO TGFA OF INCUBATION MEDIUM FROM LABELED FFA OF MEDIUM OR TGFA OF TISSUE

Expt. No.	Radioactivity in Tissue		Radioactivity in Medium	
	FFA	TGFA	FFA	TGFA
	cpm		*cpm*	
1A*	5830	64,080	207,600	1050
1B	4650	60,880	202,750	850
2A†	1510	22,080	750	225
2B	1590	25,220	600	175

* Flasks contained 4.5 ml of buffer, 0.5 ml of solution of d <1.006 lipoproteins (5.2 μmoles of TGFA), and 0.86 μmoles of palmitate-1-C^{14} (360,000 cpm) complexed to albumin. One-hour incubation.

† Tissues were preincubated with 0.86 μmole of palmitate-1-C^{14} (360,000 cpm) complexed to albumin. After 30 min, the tissues were washed three times with buffer. They were then incubated with 4.5 ml of buffer, 0.5 ml of solution of d <1.006 lipoproteins (5.2 μmoles of TGFA) for 1 hr.

It is important to appreciate that, in such an *in vitro* system, TGFA adhere to the adipose tissue slices, apparently by a process of simple adsorption. For this reason, we subtracted the radioactivity found in the tissue 10 sec after mixing the tissue and lipoprotein from that found after incubation. The results shown in Fig. 1, which indicate that there was little uptake of TGFA by tissue from fasted rabbits after the first 15 min and that more was taken up in the fed and re-fed tissue during the first 15 min, suggest that the correction for adsorption was inadequate or that there is some incorporation of TGFA into adipose tissue by other processes.

In our experiments, about 50% of the TGFA incorporated into adipose tissue from fed and re-fed animals was in FFA. These results differ from those of other workers (15, 16), who found a very small fraction of the tissue radioactivity in FFA when adipose tissue from fed animals was incubated with albumin-bound FFA. This discrepancy might be due to the absence of a fatty acid acceptor in the medium of our system. It is possible, however, that adipose tissue metabolizes TGFA and FFA by different pathways.

The lack of any effect of nutritional state on incorporation of TGFA into liver (Table 1) is consistent with our failure to detect lipoprotein lipase activity in this tissue. These results are in accord with those of Borgström and Jordan (1) and of Stein and Shapiro (2), who showed that intact triglycerides are incorporated into the liver. It has previously been pointed out that chylomicrons and d <1.006 lipoproteins have direct access to the absorptive microvillous surface of the hepatic parenchymal cell through pores in the sinusoidal endothelium (6, 7). The absence of lipoprotein lipase

in the liver might also be expected since the liver lacks a true capillary bed.

If, as has been suggested (6), lipoprotein lipase is present on the endothelial surface of the capillary, study of the incorporation of TGFA into adipose tissue in our *in vitro* system is subject to the criticism that the lipoproteins are not exposed to this enzyme to the same degree as in the intact animal. Our results suggest that sufficient capillary surface was exposed or that lipoprotein lipase is present on the surface of the adipose cell.

Although the *in vitro* studies presented here correlate well with results of several studies *in vivo* (13, 14, 17), they differ from those of Rodbell (18), who incubated the epididymal fat pad of rats with C^{14}-labeled TGFA in chylomicrons and artificial fat emulsions. His study suggested that chylomicron triglycerides are incorporated intact into adipose tissue, perhaps by a process of pinocytosis. The reason for the disparity between his results and ours may be found in the following differences in technique: (1) he used the intact epididymal fat pad of the rat, and, under these circumstances, less lipoprotein lipase may have been exposed to the substrate; (2) his conclusions were based on the incorporation of triglycerides in an artificial emulsion rather than in a lipoprotein; (3) he used much higher concentrations of TGFA in the medium; and (4) he did not correct for TGFA adsorbed to the tissue immediately after its addition to the incubation flask.

REFERENCES

1. Borgström, B., and P. Jordan. *Acta Soc. Med. Upsalien.* **64**: 185, 1959.
2. Stein, Y., and B. Shapiro. *J. Lipid Research* **1**: 326, 1960.
3. Ashworth, C. T., V. A. Stembridge, and E. Sanders. *Am. J. Physiol.* **198**: 1326, 1960.
4. Bragdon, J. H., and R. S. Gordon, Jr. *J. Clin. Invest.* **37**: 574, 1958.
5. Havel, R. J., J. M. Felts, and C. M. Van Duyne. *J. Lipid Research* **3**: 297, 1962.
6. Havel, R. J. *Am. J. Clin. Nutr.* **6**: 662, 1958.
7. Robinson, D. S., and J. E. French. *Pharmacol Rev.* **12**: 241, 1960.
8. Cherkes, A., and R. S. Gordon, Jr. *J. Lipid Research* **1**: 97, 1959.
9. Carlson, L. A. *Acta Soc. Med. Upsalien.* **64**: 208, 1959.
10. Umbreit, W. W., R. H. Burris, and J. F. Stauffer. *Manometric Techniques and Tissue Metabolism.* Minneapolis, Burgess Publishing Co., 1957, p. 149.
11. Borgström, B. *Acta Physiol. Scand.* **25**: 111, 1952.
12. Trout, D. L., E. H. Estes, and S. J. Friedberg. *J. Lipid Research* **1**: 199, 1960.
13. Bragdon, J. H., and R. J. Havel. *Am. J. Physiol.* **177**: 128, 1954.
14. Havel, R. J., and A. Goldfien. *J. Lipid Research* **1**: 102, 1959.
15. Bally, P. R., G. F. Cahill, B. Leboeuf, and A. E. Renold. *J. Biol. Chem.* **235**: 333, 1960.
16. Dole, V. P. *J. Biol. Chem.* **236**: 3121, 1961.
17. Havel, R. J., and R. S. Gordon, Jr. *J. Clin. Invest.* **39**: 1777, 1960.
18. Rodbell, M. *J. Biol. Chem.* **235**: 1613, 1960.