Relationship of lipoprotein lipase activity to triglyceride uptake in adipose tissue

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ABSTRACT Fasted rats injected with actinomycin or fed glucose show increased lipoprotein lipase activity of epididymal adipose tissue. Data from the actinomycin-treated animals showed a direct correlation between the lipoprotein lipase activity and the uptake of lipoprotein triglyceride by the epididymal fat pad in vitro and in vivo. Data from the animals fed glucose confirmed these findings in vitro. These data strongly suggest that lipoprotein lipase plays a major role in triglyceride deposition in adipose tissue.

KEY WORDS lipoprotein lipase · triglyceride uptake · fat deposition · adipose tissue · actinomycin D · glucose feeding · very low density lipoprotein · rat

It has been noted often that the enzyme lipoprotein lipase appears to play a role in the transport of triglyceride molecules into adipose tissue, and, therefore, may be a significant factor in the control of fat deposition (1–5). This concept is based on several observations, as follows. First, the ratio of isotopic activities of triglycerides isolated from adipose tissue of animals injected with doubly labeled cylomicrons indicated that the material injected was markedly hydrolyzed (5–8). Second, studies on adipose tissue from fed and fasted animals showed a positive correlation between the uptake of triglyceride (9–12) and lipoprotein lipase activity (1, 2, 13, 14). Third, inhibitors of lipoprotein lipase activity also inhibited the uptake of triglyceride fatty acid in adipose tissue (15). Fourth, other data indicate that the transport in vitro of triglyceride fatty acids of very low density lipoprotein (VLDL) into adipose tissue of the rabbit varies directly with lipoprotein lipase activity (16). However, no data have provided a satisfactory answer as to whether the observed relationship between enzyme activity and triglyceride uptake is one of cause and effect or only the reflection of a prior change in a mutually controlling metabolite.

Recently, we showed that lipoprotein lipase activity in epididymal adipose tissue of a fasted rat can be increased significantly within a relatively short time period by administration of glucose by intubation or by treatment of the rat with actinomycin D (17). In the present investigation these two methods of inducing lipoprotein lipase activity were studied in an attempt to determine whether a correlation between the enzyme activity and triglyceride uptake exists in adipose tissue.

METHODS

All rats (male Sprague-Dawley, Charles River Breeding Laboratory, 160–210 g) were deprived of food but had free access to water for 22 hr before being divided into three groups. One group was refed by intubation 2 ml of 50% glucose (w/v), three times at 1 hr intervals, and used 1 hr after the third feeding. The second experimental group was given, in a single intraperitoneal injection, 125 μg of actinomycin D (kindly supplied by Dr. G. E. Boxer, Merck, Sharp & Dohme) in 0.5 ml of 0.9% NaCl (w/v); these animals were studied 8 hr later. The control animals, the third group, were fed water by intubation or injected with 0.5 ml of 0.9% NaCl (w/v), and killed at times corresponding to the appropriate experimental group.

A single epididymal fat pad from each rat was made into an acetone powder for determination of the lipolytic activity of that tissue. The adipose tissue was homogenized at ambient temperature in 15 ml of acetone in a TenBroeck glass tissue grinder and filtered on a Buchner

Abbreviations: VLDL, very low density lipoprotein(s); TGFA, triglyceride fatty acids.

funnel with 4.25 cm Whatman No. 50 filter paper. The precipitate was washed briefly with small aliquots first of acetone and then of ethyl ether. The air-dried powders were stored in a desiccator at −20°C for not longer than 7 days and then assayed.

Lipolytic activity was determined as follows. For the assay the total acetone powder from a single fat pad was extracted with 0.2 ml of 0.025 M NH_4OH in an ice bath for 1 hr, after which the mixture was centrifuged at 35,000 *g* for 10 min at 0–4°C. 0.1 ml of the supernatant fraction was incubated at 37°C with 0.05 ml of 1 M $(NH_4)_2 SO_4$, 0.4 ml of 10% bovine albumin (pH 8.5), 0.2 ml of a mixture (1:1) of 4% Ediol (Schenlabs Pharmaceuticals, Inc., New York) plus serum from fasted human subjects (the mixture having been previously incubated at 37°C for 30 min), and 0.25 ml of 0.025 M NH_4OH; the final pH of the mixture was 8.5 (18). After a 10 min incubation the reaction was stopped by addition of 4 ml of isopropyl alcohol–3 N H_2SO_4 39:1. Free fatty acids were extracted and their quantity was determined by titration (19). The protein concentration of the initial NH_4OH extract was also determined (20).

Labeled lipoprotein triglyceride for intravenous injection and for incubation of adipose tissue in vitro was obtained from donor rats, fasted 22 hr, from the same population as the experimental animals. To prepare labeled VLDL, we injected 27 μc of palmitic acid-1-^{14}C (27 mc/mmole, Lot No. 31-238-17, New England Nuclear Corp. Boston, Mass.,), complexed to rat serum (21), into the tail vein of unanesthetized donor rats. The animals were decapitated 25 min later, and the blood was collected and allowed to clot for 1 hr at 16°C. Serum was obtained by centrifugation at 2,000 *g* for 10 min. Then 5 ml of 0.9% NaCl (w/v) was layered carefully over 4 ml of serum, and the VLDL were separated by centrifugation at 63,000 *g* for 15 min at 16°C in the No. 50 rotor of a Model L-2 Spinco centrifuge. The upper, 2 cm layer was collected, diluted with 0.9% NaCl (w/v), and stored at 16°C. The ^{14}C-VLDL for intravenous injection contained approximately 0.01 μc/ml in the triglyceride fraction.

Radioactive incubation medium was prepared by a less complicated procedure, which gave a maximum yield of labeled triglyceride with minimal manipulation and delay. Since preliminary experiments suggested that the incorporation of labeled free fatty acid into serum triglyceride was increased by ether anesthesia, these donor rats were lightly anesthetized with ethyl ether during injection of palmitic acid-1-^{14}C rat serum complex (30 μc; specific activity, 9.25 mc/mmole; New England Nuclear Corp., Lot No. 102-267-24). The rats were decapitated 30 min later. Serum was collected and diluted 1:1 with Krebs-Ringer bicarbonate buffer (pH 7.4). In this experiment, in contrast to the previous one, VLDL were not isolated. The ^{14}C-VLDL serum medium contained 0.038 μc/ml, with a triglyceride concentration of 0.17 mg/ml. In both isotopic preparations, 85% of the total lipid radioactivity was contained in the triglyceride fraction as determined by thin-layer silicic acid chromatography of the neutral lipids (22). Both were used within 3 hr of preparation.

Uptake of triglyceride by epididymal adipose tissue was measured in vivo and in vitro. In one experiment, 0.5 ml of ^{14}C-VLDL was injected into the tail vein of unanesthetized rats of the control group and of the two experimental groups which had been either glucose-refed or actinomycin-treated. 10 min after injection the rats were decapitated and the epididymal fat pads were immediately excised. One fat pad was homogenized in 15 ml of $CHCl_3$–CH_3OH 2:1 and filtered. Unlabeled palmitic acid was added to an aliquot of this filtrate and the mixture was dried at 70°C. Radioactivity of the total lipid extract was assayed in scintillator gel (23) in a Packard Tri-Carb liquid scintillation counter. In a second experiment animals from the three groups were decapitated, the epididymal fat pads were excised, and one fat pad was incubated for 2 hr in 2 ml of ^{14}C-VLDL serum preparation at 37°C in a Dubnoff shaker in an atmosphere of O_2–CO_2 95:5. In addition, fat pads from comparable animals were shaken for 10 sec in the same volume of medium to mimic approximately the degree of adsorption of radioactivity to the tissue, as suggested by Bezman, Felts, and Havel (16). After either incubation or brief shaking the tissues were rinsed three times in 0.9% NaCl (w/v), blotted, and homogenized in 15 ml of $CHCl_3$–CH_3OH 2:1. ^{14}C uptake was determined as described above. In both experiments the contralateral fat pad from each rat was made into an acetone powder as described above for comparison of lipolytic activity with isotopic uptake.

The quantities of free fatty acid (19) and glycerol (24, 25) present in the medium at the beginning and end of the incubation of the epididymal adipose tissue of the experimental and control groups were measured. The procedure for the adipose tissue incubation described in the preceding paragraph was repeated, with the exception that the incubation medium (pH 7.4) consisted of Krebs-Ringer bicarbonate buffer with 5% bovine albumin only.

RESULTS

Effect of Actinomycin D on Adipose Tissue Lipases

The lipolytic activity extracted from adipose tissue of actinomycin-treated rats was inhibited by protamine and NaCl, and stimulated by heparin. In addition, the absence of serum from the incubation mixture caused a large decrease in lipase activity (Table 1). These characteristics are associated with lipoprotein lipase and not with other adipose tissue lipases.

TABLE 1 CHARACTERIZATION OF ACTINOMYCIN D-INDUCED LIPASE

Condition of Assay	% Activity
Complete System	100
− Serum	35
+ Protamine Sulfate (5×10^{-5} M)	85
+ NaCl (0.3 M)	50
+ Heparin (58 μg/ml)	117

Rats, previously fasted for 22 hr, were injected with 125 μg of actinomycin D. 8 Hr later, acetone powders were prepared from the epididymal adipose tissue of the rats. Lipase activity was determined from an extract made with 0.025 M NH_4OH The complete assay system consisted of 0.05 ml of 1 M $(NH_4)_2SO_4$, 0.4 ml of 10% bovine albumin (pH 8.5), 0.2 ml of 4% Ediol plus serum (1:1) (the mixture previously incubated at 37°C for 30 min), 0.25 ml of 0.025 M NH_4OH, and 0.1 ml of enzyme extract, final pH 8.5. The assay tubes were incubated at 37° C for 10 min. Free fatty acids were determined before and after incubation.

TABLE 2 LIPOPROTEIN LIPASE ACTIVITY AND FFA ACCUMULATION FROM FAT PADS OF FASTED CONTROLS AND OF ACTINOMYCIN-TREATED AND GLUCOSE-REFED RATS

Fasted Controls		Actinomycin Treated		Glucose-Refed	
Lipoprotein Lipase*	Free Fatty Acid†	Lipoprotein Lipase	Free Fatty Acid	Lipoprotein Lipase	Free Fatty Acid
0.96	2.0	1.8	2.2	2.8	0.35
1.1	0.62	2.7	1.5	3.0	0.25
1.3	1.2	2.9	0.39	4.8	0
1.6	1.6	3.0	0.97	4.9	0
1.9	1.3	3.2	1.4	5.6	0.13
2.1	0.67	3.6	0.79	6.0	0
		4.5	1.4		
		4.5	0.70		
		7.5	0.44		

FFA, free fatty acid (s).

One fat pad from each rat was incubated in Krebs-Ringer bicarbonate buffer (pH 7.4) with 5% bovine albumin for 2 hr at 37°C. FFA concentrations of the medium was measured before and after incubation of the tissues. Lipolytic activity was determined from 0.025 M NN_4OH extracts of actone powders from the contralateral fat pads. For details of the assays see Table I. Values given are for individual animals. The means are expressed in Fig. 1.

* μmoles of FFA per mg of protein per hr.

† μmoles of FFA per g of tissue per hr.

Actinomycin did not affect the endogenous lipolysis of stored triglyceride as measured by release of glycerol from the isolated epididymal adipose tissue (Fig. 1). Comparison of glycerol concentrations of the medium indicated that total hydrolysis of endogenous triglyceride in adipose tissue of the actinomycin-treated rats was of the same order as that of saline-injected rats fasted for the same duration of time. Thus, endogenous lipolysis was neither stimulated nor inhibited by the antibiotic (Fig. 1). That refeeding glucose also caused no significant change in glycerol release suggests that this lipase is unaffected by this variation of the nutritional state.

Typically, when adipose tissue from carbohydrate-fed rats is incubated in vitro, free fatty acids do not accumulate in the medium, whereas they do accumulate when adipose tissue from fasted rats is used. This difference is due, in part, to the fact that fatty acids are reesterified much faster by adipose tissue of fed than of fasted rats (26). The accumulation of free fatty acids in the incubation medium was the same for fat pads from fasted and actinomycin-treated animals (Table 2). In contrast, the free fatty acid accumulation from adipose tissue of the glucose-refed rats was markedly less. Since glycerol accumulation was the same for all three groups, variation in the FFA accumulation in the medium probably reflects different rates of reesterification by the tissue associated with the experimental treatments. We infer from these data that reesterification of free fatty acids by adipose tissue from actinomycin-treated rats proceeds at a rate similar to that in adipose tissue of fasted rats and more slowly than in adipose tissue of glucose-refed animals. Although free fatty acid accumulation is inversely correlated with lipoprotein lipase activity when adipose tissues from fasted and refed rats are compared, statistical analysis showed no significant correlation between free fatty acid accumulation in the medium and lipoprotein lipase activity in the tissue in the fasted and actinomycin-treated

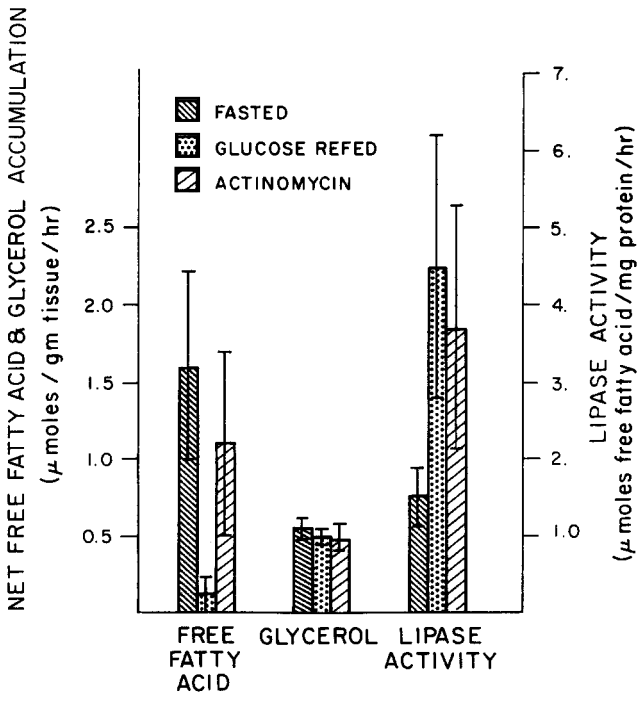

FIG. 1. Comparison of net free fatty acid and glycerol release, and lipoprotein lipase activity in epididymal adipose tissue of fasted, actinomycin-treated, and glucose-refed rats. One fat pad from each rat was incubated in Krebs-Ringer bicarbonate buffer (pH 7.4) with 5% bovine albumin for 2 hr at 37°C. Free fatty acid and glycerol concentrations of the medium were measured. Lipolytic activity was determined from 0.025 M NH_4OH extracts of acetone powders from the contralateral fat pads. For details of the assays see Table 1. The actinomycin-treated group consisted of nine rats, the other two groups of six each. Values given are the mean ±SD.

groups (correlation coefficient = 0.59; $P > 0.05$). It should be noted that in this experiment (Table 2), all except one of the rats treated with actinomycin responded with increased lipoprotein lipase activity.

Time Course of Lipoprotein Lipase Induction

Lipase activity increased initially at a more rapid rate after glucose refeeding than after actinomycin D injection (Figs. 2, 3). After the third glucose feeding, lipoprotein lipase activity in the epididymal adipose tissue increased for 4 hr. By 6 hr it was no longer increasing but was still considerably greater than the fasting level. 4 hr after actinomycin injection into fasted rats lipase activity was not significantly increased. This activity then rose rapidly to a maximum at 8 hr and fell markedly by 12 hr, though still not to the levels of the control rats. The fasted control values decreased during the 24 hr period.

After the regimen of glucose feeding described, lipoprotein lipase activities increased significantly in almost 100% of the experimental rats at 1 hr after the third feeding. However, the increase in enzyme activity following actinomycin treatment was unpredictable during the series of experiments. In any group of animals injected with the antibiotic, the percentage that showed a significant increase in lipoprotein lipase activity ranged from 50 to 100%. With either inducer, the degree of the increase varied; therefore, each group of experimental animals has been compared with its parallel control group.

Uptake of ^{14}C-TGFA In Vivo and In Vitro

10 min after intravenous injection of ^{14}C-VLDL into glucose-refed, actinomycin-injected, and appropriate control rats, the epididymal adipose tissue of the refed animals contained twice as much radioactivity as that of its corresponding control group fed water, while the adipose tissue of the actinomycin-treated fasted group had taken up an intermediate amount of ^{14}C. At the same time lipoprotein lipase levels of the glucose-refed group showed approximately a 4-fold increase compared with the fasted controls. In the actinomycin-treated rats enzyme activity fell between the values for the other two groups and corresponded in an almost linear fashion with ^{14}C uptake by the adipose tissue (Fig. 4). The correlation between enzyme activity and ^{14}C uptake was highly significant (correlation coefficient = 0.81; $P < 0.01$).

The pattern of triglyceride uptake by the isolated epididymal adipose tissue of three similar groups of rats corresponded to that described above (Fig. 5). All the glucose-refed animals showed significantly higher lipoprotein lipase levels than the fasted control rats, with a ^{14}C uptake about twice as great (correlation coefficient = 0.75; $P < 0.01$). With one exception, the rats in which lipase was increased by actinomycin treatment also

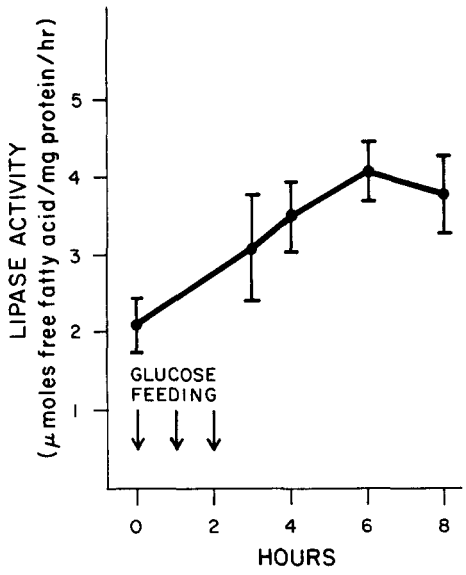

FIG. 2. Induction of lipase activity by glucose feeding. Rats fasted for 22 hr were fed 2 ml of 50% glucose (w/v) by intubation at the times indicated by the arrows. Lipase activity was determined as described previously (17). The mean value ±SD is given for six rats per group, except for the 8 hr group which consisted of four rats.

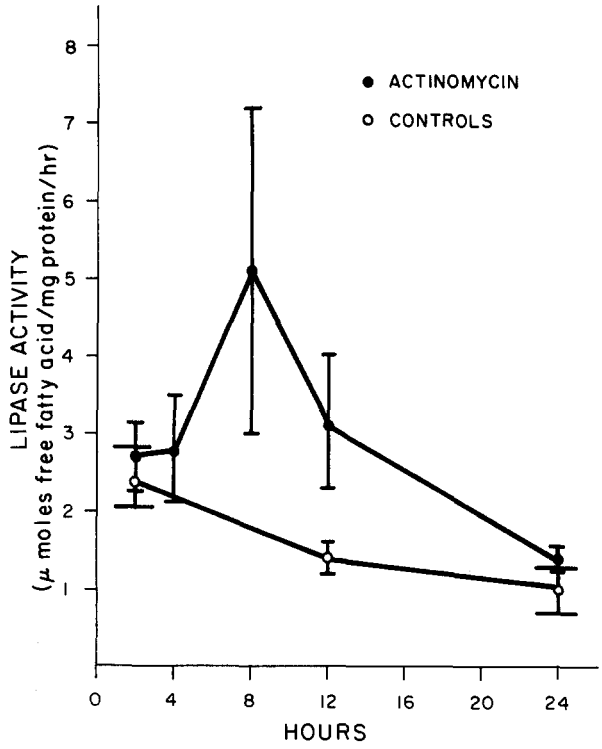

FIG. 3. Induction of lipase activity by actinomycin D treatment. After a 22 hr fast, rats were injected intraperitoneally with 125 μg of actinomycin D in 0.5 ml of 0.9% NaCl (w/v) at zero time. Lipolytic activity was determined from 0.025 M NH_4OH extracts of acetone powders from the fat pads. All values represent the mean ±SD from five animals per group except at 24 hr after actinomycin treatment, where the value is the mean for four rats.

showed increased ^{14}C uptake compared with saline-injected controls. Generally, enzyme activity corresponded well with isotope uptake. The amount of ^{14}C adsorbed to the tissue was about 0.1% of the amount of isotope found in the tissue after incubation and was the same for both fasted and refed rats. This value is considerably lower than other reported values (15, 16). The lower adsorption observed in the present study may reflect the smaller surface of cut tissue exposed to the medium. In other studies in which adsorption has been estimated, tissue slices were used, whereas whole fat pads with only one cut edge at the proximal margin of each pad were used here.

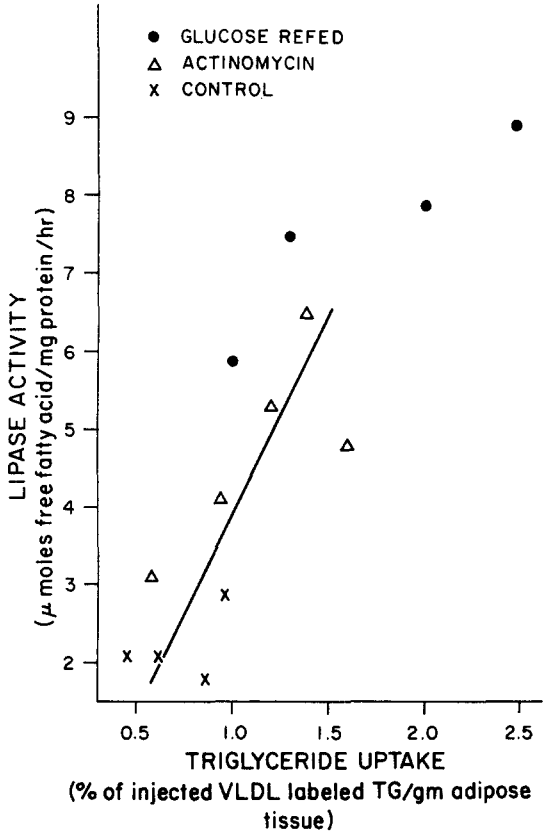

FIG. 4. Uptake of labeled triglyceride, in vivo, as a function of lipoprotein lipase activity. Rats were injected via the tail veil with ^{14}C-VLDL triglyceride prepared from donor animals injected with labeled palmitate. All rats were fasted for 22 hr. The refed rats were intubated with 2 ml of 50% glucose (w/v) three times at 1 hr intervals and studied 1 hr after the third feeding. Experimental rats were injected intraperitoneally with 125 μg of actinomycin D and studied 8 hr later. Controls were injected with saline and studied at times corresponding to the experimental groups.

Uptake of triglyceride-^{14}C was determined by measurement of the radioactivity in the total lipid extract from one epididymal fat pad. Lipase activity of the contralateral fat pad was assayed in an 0.025 M NH_4OH extract from acetone powder. Each point represents a single animal. A line representing a least squares fit of the data is shown. The data from the refed animals have not been included in the computation of the least square fit because of the difficulty in interpretation discussed in the text.

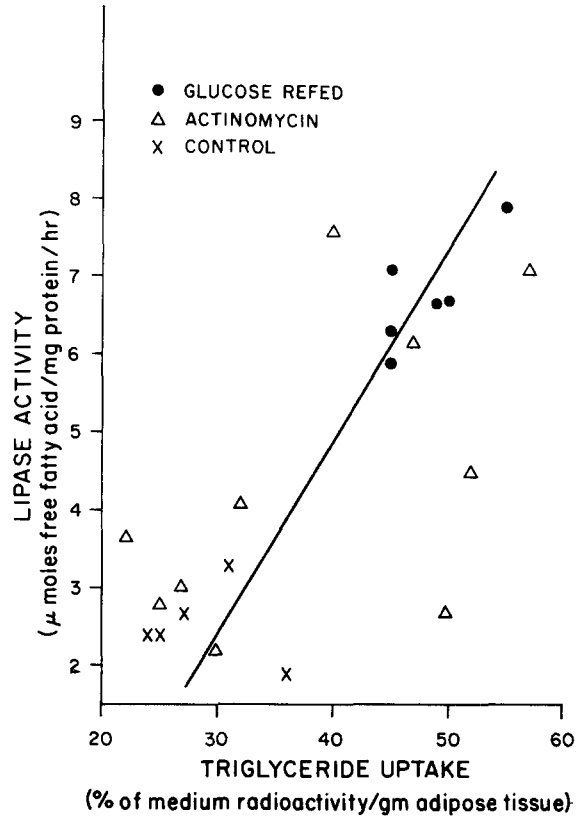

FIG. 5. Uptake of labeled triglyceride, in vitro, as a function of lipoprotein lipase activity. Donor animals were injected via the tail vein with palmitate-^{14}C and the serum was collected 30 min later. The incubation medium consisted of labeled serum diluted 1:1 with Krebs-Ringer bicarbonate buffer (pH 7.4). All rats were fasted for 22 hr. The refed rats were intubated with 2 ml of 50% glucose (w/v) three times at 1 hr intervals and studied 1 hr after the third feeding. Other experimental rats were injected intraperitoneally with 125 μg of actinomycin D and studied 8 hr later. Controls were given either water by intubation or saline by injection, and studied at times corresponding to the experimental groups. Uptake of triglyceride-^{14}C was calculated by measurement of the radioactivity in the total lipid extract of one epididymal fat pad after a 2 hr incubation at 37 °C. Lipase activity of the contralateral fat pad was assayed from an 0.025 M NH_4OH extract from acetone powder. Each point represents a single animal. A line representing a least squares fit of all the data is shown.

DISCUSSION

In the present study we employed two distinct methods of increasing lipoprotein lipase activity—acute glycose refeeding and treatment with actinomycin—to test the hypothesis that triglyceride uptake is correlated with this enzyme activity in adipose tissue. A positive correlation would support the concept that this enzyme activity is essential for triglyceride deposition in fat depots. Such a correlation has been inferred by other investigators (1, 2, 13–16) in studies in which the nutritional state of the animal has been altered. Except in the study by Bezman et al. (16), which attempted to relate lipoprotein lipase activity to triglyceride uptake in adipose tissue of chronically

fasted and refed rabbits, this relationship has been based upon measurements of either triglyceride uptake or lipoprotein lipase activity but not of both simultaneously. All the studies, including that of Bezman et al. (16), have the deficiency that the animals were eating intermittently and that no measurements were made prior to 24 hr after initial feeding. Under the latter conditions the time sequence of metabolic responses is difficult to determine. In contrast to chronic feeding, the use of acute glucose refeeding permits the measurement of early metabolic changes in adipose tissue—specifically, lipolysis of endogenous triglyceride, reesterification of free fatty acids, and uptake of triglyceride and lipoprotein lipase activity, the latter two being of primary interest.

In this study, the antibiotic actinomycin D has been used to explore a mode of regulation of lipoprotein lipase activity that probably differs from that of glucose-refeeding. The mechanism of actinomycin action involves the inhibition of DNA-dependent RNA synthesis within the cell (27). In an earlier study in this laboratory we used the antibiotic to determine whether inhibition of RNA synthesis would prevent the increase in lipoprotein lipase activity following acute glucose refeeding (17). Surprisingly, even in the absence of glucose, actinomycin increased rather than decreased lipoprotein lipase activity in adipose tissue. Eagle and Robinson had observed the same phenomenon in experiments in vitro (28). They proposed that increased lipoprotein lipase activity following actinomycin treatment is "most readily explained if synthesis of clearing factor lipase (lipoprotein lipase) occurs on a stable RNA template and its destruction is mediated through the action of an unstable, actinomycin-sensitive form of RNA." A number of other enzymes have been reported to be elevated by the antibiotic (29), possibly by the same mechanism.

In the present study only one supposition has been made regarding the mechanism by which actinomycin enhances lipoprotein lipase activity, namely, that it does not act by increasing carbohydrate metabolism. If this supposition holds, the use of actinomycin to increase lipoprotein lipase activity would allow one to divorce changes in this enzyme activity from congruent alterations in tissue esterifying activity. The validity of the assumption is substantiated by our finding that the rapid reesterification of free fatty acid that accompanies glucose refeeding is absent from the tissues of fasted rats injected with actinomycin (Fig. 1). This finding is of major importance since this new tool, actinomycin, appears to have eliminated a complication present in all previous attempts to demonstrate the primacy of lipoprotein lipase activity in the regulation of triglyceride uptake by fat tissue.

Our data show that after actinomycin injection, increases in lipoprotein lipase activity in rat epididymal adipose tissue are accompanied by significant increases in triglyceride uptake by this tissue both in vivo and in vitro. Specifically, we observed that in almost all actinomycin-treated rats that responded with increased lipoprotein lipase activity, triglyceride uptake was greatly enhanced. On the other hand, when actinomycin injection failed to increase lipoprotein lipase activity, no increase in triglyceride uptake was found. This correlation between triglyceride uptake and lipoprotein lipase was further substantiated by our studies of glucose-refed rats, which showed increased triglyceride uptake in vitro, accompanied by increased lipoprotein lipase activity. Since the variables affected by actinomycin treatment are unknown, these data are inferential only. They do, however, provide strong evidence that the uptake of lipoprotein triglyceride by epididymal adipose tissue is critically related to the level of the enzyme lipoprotein lipase in the tissue.

Interpretation of our present study was complicated by the finding that the plasma triglyceride pool size of the glucose-refed animals was markedly lower than that of the controls.[1] Thus, the dilution of the isotope upon injection into the glucose-refed rats was considerably less than in the fasted controls. Therefore, this experiment does not allow a simple comparison of triglyceride uptake by the adipose tissue of refed and fasted rats in vivo. However, plasma triglyceride pool size of the actinomycin-treated rats was the same as that of the corresponding controls. Comparison of triglyceride-^{14}C uptake by all three groups in vitro is valid since no complication of isotopic dilution existed.

The difficulty of comparing data from isolated tissue with those obtained in vivo has been discussed by several authors (15, 16). In our experiments this consideration is pertinent since the active enzyme may be located in the capillary bed and might be more readily accessible to substrate in vivo than in vitro. However, the data obtained under the two conditions are in excellent agreement and strongly implicate lipoprotein lipase as a regulator of fat deposition.

Manuscript received 7 October 1966; accepted 24 January 1967.

This investigation was supported, in part, by Public Health Service Research Grant 4706 from the National Institute of Arthritis and Metabolic Diseases.

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