

The liberation of lipoprotein lipase by heparin from adipose tissue incubated *in vitro*

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SUMMARY

Epididymal fat tissues of rats, incubated *in vitro* in the presence of heparin, have been found to release lipoprotein lipase into the medium. This release occurs rapidly and shows little dependence upon the composition of the medium. The tissues of carbohydrate-fed rats are more active with respect to lipoprotein lipase liberation than those of fasted animals.

The administration of heparin parenterally to animals or man has been shown to cause the appearance in plasma of a "clearing factor" with the ability to cause the clearing of lactescence (1, 2); heparin itself added to plasma *in vitro* is without effect. This factor has been characterized by Korn (3, 4) and Korn and Quigley (5, 6) and renamed lipoprotein lipase, an enzyme which has a specificity for hydrolysis of triglycerides contained in soluble lipoproteins. Free fatty acids liberated by this reaction have been shown to inhibit the lipase activity and an acceptor such as serum albumin is needed for the reaction to proceed (7). Methods for the assay of biological preparations for lipoprotein lipase activity have been standardized by Korn (8), and depend upon the measurement of hydrolysis of a triglyceride substrate.

The presence of lipoprotein lipase in extracts prepared from rat and chicken adipose tissue has been shown by Korn and Quigley (5, 6). That peripheral tissues release lipoprotein lipase on perfusion with heparin-containing blood was shown by Jeffries (9); it therefore seemed possible that adipose tissue might be an important source of lipoprotein lipase in the intact animal. The present experiments were undertaken to determine the ability of heparin to elicit the release of lipoprotein lipase from surviving adipose tissue incubated *in vitro*.

METHODS

The animals used in these experiments were young male rats of the Sprague-Dawley strain, weighing from 150 to 220 g. They were sacrificed by an abrupt blow on the head, and the two epididymal fat bodies were quickly excised. These tissues were placed in 25 ml. Erlenmeyer flasks, each containing approximately

1 ml. of Krebs-Ringer phosphate buffer per 100 mg. wet weight of tissue, and incubated at 37°C with gentle agitation in a Dubnoff metabolic shaking incubator. Heparin, albumin, and other agents were added to the incubation system of one epididymal fat body from each rat, as indicated in the descriptions of the individual experiments; the contralateral tissue served as a control. The lipase activity of an aliquot of each incubation medium was assayed for its ability to produce free fatty acids from a coconut oil substrate; one unit of lipoprotein lipase was considered to be that amount of lipase which would release one μ mole of fatty acid per hour. The substrate was prepared by mixing 8 parts of a 10 per cent solution of bovine serum albumin (Armour Fraction V), adjusted to pH 8.7 by titration with ammonium hydroxide, 1 part of a 4 per cent coconut oil emulsion prepared by dilution of Ediol¹ with water, and 1 part of fresh pooled human serum, and incubating the mixture for 30 minutes at 37°C. An aliquot (0.5 ml.) of the solution to be assayed for lipoprotein lipase was mixed with 0.5 ml. of this substrate (giving a final pH of 8.5), and incubated for one hour at 37°C. The free fatty acids evolved were determined by the method of Dole (10), modified to the extent that Nile blue A was used in place of thymol blue for the titrations. The use of a glass-stoppered centrifuge tube for the enzyme-substrate mixture during the lipase assay made it possible to extract the free fatty acids without further transfer of the sample. The addition of the isopropanol-heptane extraction mixture served as a conven-

¹ Ediol (Schenley Laboratories, Lawrenceburg, Ind.) is an aqueous fat emulsion containing 50 per cent coconut oil, 12.5 per cent sucrose, 1.5 per cent glyceryl monostearate, and 2 per cent polyoxyethylene sorbitan monostearate.

ient means of terminating the lipolytic reaction. Preliminary experiments indicated that under these conditions free fatty acid evolution proceeded at a constant rate for 2 hours, and was a linear function of the enzyme concentration.

RESULTS

Rate of Release of Lipase in the Presence of Heparin. The incubation of tissue in the presence of heparin was accompanied by the rapid release of lipase into the medium. In order to follow the time-course of enzyme liberation, an experiment was performed in which epididymal fat bodies (one from each of six fed rats) were pooled and incubated in 30 ml. of Krebs-Ringer phosphate buffer, to which had been added sodium heparin (128 units per mg., U.S.P.) in a concentration of 30 μ g. per ml. The six contralateral tissues were incubated under identical conditions but for the omission of heparin. At the intervals plotted in Figure 1, 1.0 ml. of each medium was withdrawn for duplicate lipase assays; the volume of medium was not replaced. Although the medium in which the tissues were incubated without heparin did not show measurable lipolytic activity, lipase was found in the heparin-containing medium in as little as 2 minutes. As may be seen in Figure 1, this activity increased rapidly and then declined on further incubation. In a similar experiment in which the medium contained bovine serum albumin, it was noted that the decline of activity was postponed. In subsequent experiments in which the effects of other factors were tested, the adipose tissue was incubated in the experimental medium for a 40-minute period.

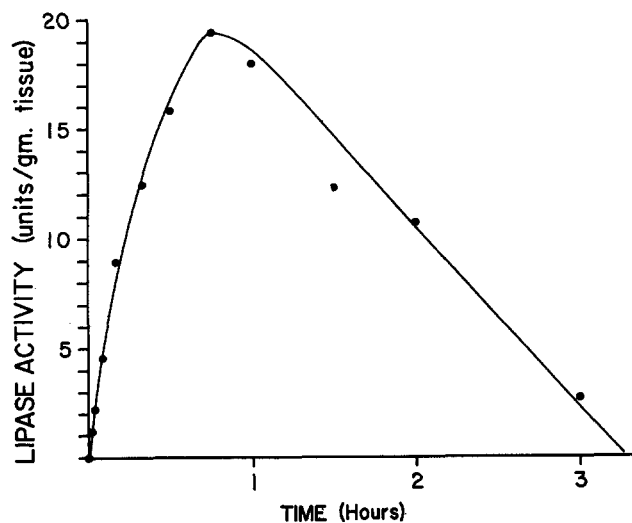


Fig. 1. The liberation of lipoprotein lipase by heparin from adipose tissue into Krebs-Ringer phosphate buffer per unit of time.

Demonstration of the Identity of Adipose Tissue Lipase. As a means of demonstrating that the lipolytic enzyme released into the medium by surviving adipose tissue was identical to lipoprotein lipase, the effects of some factors known to influence the activity of lipoprotein lipase were investigated. The results of these studies are presented in Table 1. Sodium chloride solution, 1 M, and protamine sulfate, which have been shown to be inhibitors of lipoprotein lipase, were also effective in inhibiting the activity of the enzyme released by adipose tissue. Sodium fluoride, on the other hand, which is known to inhibit pancreatic lipase but not lipoprotein lipase, had little or no effect. It will be observed that the inhibition produced by 1 M sodium chloride, while considerable, was not as complete as that reported by Korn (3) in experiments in which glycerol production was used as an index of enzyme activity. When free fatty acid production is measured, however, it has never been possible to show total inhibition of the activity of postheparin plasma by 1 M sodium chloride.²

The requirements of lipoprotein lipase for optimum activity on artificial fat emulsions were shown to apply also to the enzyme released from adipose tissue. Small amounts of fresh serum in addition to albumin markedly increased the lipolytic activity (see Table 2). In this experiment, of course, no fresh serum was added to the substrate mixture in the control incubation. The optimum pH for the activity of the lipase was shown to be in the range of 8.5 to 8.7, in good agreement with the figures reported for lipoprotein lipase by Korn and Quigley (6).

Finally, the ability of calcium phosphate gel to adsorb the enzyme was demonstrated. As may be seen in Table 3, the gel adsorbed the lipase completely, and a good recovery of a partially purified enzyme could be obtained by subsequent elution. This method of purifying lipoprotein lipase from postheparin plasma has been described by Nikkilä (11).

Conditions for the Release of Lipoprotein Lipase by Adipose Tissue. As noted above, there was no demonstrable release of lipoprotein lipase in the absence of added heparin. At heparin concentrations as low as 10 μ g. per ml. some liberation of lipase could be observed. The optimal heparin concentration was found to be 30 μ g. per ml.; at higher levels heparin itself has been found to inhibit the action of lipoprotein lipase (3), and to interfere with the subsequent enzyme assay.

Since many polyvalent anions have a heparinlike effect in eliciting the release of lipoprotein lipase into the circulation of the intact animal (8, 12, 13), an

² H. S. Gates and R. S. Gordon, Jr., unpublished data.

TABLE 1. EFFECTS OF INHIBITORS ON ADIPOSE TISSUE LIPASE

Inhibitor	Concentration	Percentage of Inhibition of Lipolysis
NaCl	1 M	83
NaF	0.2 M	7
Protamine sulfate	20 mg. per ml.	57

experiment was performed to test the effect of one such agent on the isolated adipose tissue. Sodium poly-metaphosphate, in a concentration of 30 μ g. per ml., was found to be effective, though less active than heparin, in stimulating the output of lipase from the tissue.

Experiments were also designed to test the effects of variations in the composition of the incubation medium on the liberation of lipase from adipose tissue, heparin being present in all cases at the optimal concentration of 30 μ g. per ml. Differences in lipase liberation when albumin was added to the medium, when bicarbonate was substituted for phosphate, when simple isotonic saline (buffered with albumin) was substituted for the Krebs-Ringer solution, or when the incubation was carried out under anaerobic conditions, were not statistically significant at the 5 per cent level.

Comparison of Adipose with Other Tissues. The effectiveness of epididymal adipose tissue in releasing lipoprotein lipase into the incubation medium suggested that fat tissue might be chiefly responsible for the liberation of lipase into the plasma after injection of heparin into the intact animal. It therefore seemed desirable to compare the effectiveness of adipose tissue *in vitro* with that of other tissues from the same animal. A rat fed *ad libitum* was sacrificed, and the epididymal fat bodies, mesenteric fat, aorta, diaphragm, and slices of liver, kidney, spleen, and heart were incubated in Krebs-Ringer phosphate buffer containing 30 μ g. of heparin per ml. The two samples of adipose tissue (epididymal and mesenteric) were most active in liberating lipase into the medium. The contrast was heightened when activity was expressed as units of lipase released per mg. tissue protein (estimated by biuret reaction). In subsequent experiments these differences were studied in greater detail with a larger number of rats.

Effect of Nutritional State of Donor Rats. In preliminary experiments, variations in the amount of lipoprotein lipase released under similar conditions

suggested that differences in the nutritional state of the donor rat might be important in determining the amount of lipase released during incubation of the tissue with heparin *in vitro*. It had also been observed that the adipose tissue of fasted rats yielded less lipoprotein lipase than that of rats fed *ad libitum*, when the enzyme was prepared from extracts of acetone powders according to Korn (8). An experiment was therefore devised to emphasize the contrast between tissues from fasted and fed donor animals. Three rats were kept overnight with access to water but no food, and three others were given rat chow *ad libitum* and, in addition, had access to water containing 10 per cent sucrose. The epididymal fat bodies were removed from these rats and incubated in Krebs-Ringer phosphate buffer containing heparin at the usual concentration of 30 μ g. per ml. In this study the contralateral tissues could not be used as controls, so that fasted and fed rats were compared. The differences observed were striking (Table 4): the tissues from the fed animals were much more active than those from fasted ones. Identical experiments were carried out to investigate the possibility that the nutritional state of the

TABLE 2. STIMULATION OF LIPOLYSIS BY ADDED SERUM (0.1 ml. serum per ml. substrate)

Experiment	Lipase Activity Without Serum	Lipase Activity With Serum
1	4.8	36.5
2	5.1	36.4
3	2.5	18.2
4	2.3	23.3

donor animal might influence the production of lipoprotein lipase by tissues other than epididymal fat. The results with mesenteric fat, diaphragm, and slices of myocardium and kidney are also summarized in Table 4. Only the samples of adipose tissue yielded differences that were statistically significant at the 5 per cent level.

DISCUSSION

The ability of adipose tissue to release lipoprotein lipase into the incubation medium on stimulation with heparin *in vitro* cannot be considered surprising in view of the demonstration by Korn and Quigley (5, 6) that this tissue is rich in enzyme activity, and the recent results of Robinson and Harris (14), which attest to the ease and rapidity of the release of lipoprotein lipase *in vivo*. The most noteworthy features

TABLE 3. ADSORPTION OF LIPASE BY CALCIUM PHOSPHATE GEL *

Fraction	Lipase	Protein	Specific Activity
	<i>units/ml.</i>	<i>mg./ml.</i>	
Original Incubation Medium	5.0	0.24	21
Supernatant from Gel Adsorption	0.1	0.17	0.6
Enzyme Eluted from Gel	3.3	0.026	127

* 5 ml. of tissue incubation medium were adsorbed with 0.5 ml. gel. The gel was washed with 0.1 M sodium oxalate, and the enzyme then eluted with 5 ml. 0.04 M sodium citrate.

of the present investigation are felt to be, first, the promptness of the liberation of lipase from adipose tissue by heparin and the apparent lack of any co-factor requirements and, second, the marked effect of the nutritional state of the donor animal in determining the activity and availability of lipase in the tissue.

It has been found in these studies that lipoprotein lipase is released into the medium when heparin or a heparin analogue (polymetaphosphate) contacts surviving adipose tissue *in vitro*. The composition of the medium and the availability of oxygen to support tissue respiration appear to be of no importance. In the light of the data of Korn and Quigley (5, 6), who have shown the existence of preformed enzyme in adipose tissue, it seems likely that the role of heparin is to attack the bond which normally causes the lipase to remain in the tissue, and thus make it free to dissolve into the medium. Heparin is not alone in possessing this ability, but it has been found (8, 12, 13) that many polyvalent anions may elicit the release of lipoprotein lipase *in vivo*. It may therefore be reasonable to suggest that lipoprotein lipase is normally held to cell surfaces primarily by electrostatic forces, perhaps acting between some cationic group on the cell surface and some polyvalent anionic grouping on the enzyme itself. The presence of small amounts of lipoprotein lipase in blood plasma under normal circumstances (15) is compatible with the hypothesis that the binding of the enzyme to cell surfaces is an equilibrium that normally allows slight dissociation.

The second important finding, and one which was unexpected, was the striking difference between tis-

ues from fed and fasted animals with respect to the amount of lipoprotein lipase which could be liberated. As has been shown in previous studies (16), adipose tissue has a physiologic role in the release of stored fat into the blood stream as free fatty acid. It was anticipated that starvation, the nutritional state associated with maximum release of fatty acids, would be associated with maximal lipoprotein lipase release. The actual result was quite the contrary, and lipoprotein lipase activity was observed to be lower in fasted tissue, either when it was liberated into an incubation medium by the action of heparin, or when it was directly isolated from the tissue, without added heparin, by the preparation of an acetone powder.

It has long been suspected, but never rigidly proved, that lipoprotein lipase plays an important role in the normal processes of removal of chylomicron fat from the circulating plasma (16). One more indication that this hypothesis is correct may be obtained by comparing the present results with the data of Bragdon and Gordon (17), in which it is shown that the *in vivo* uptake of chylomicron fatty acids by adipose tissue is greater in the carbohydrate-fed than in the fasted rat. The liberation of free fatty acid from adipose tissue, which has been shown to be an important means for the mobilization of depot fat when it is required as a

TABLE 4. EFFECT OF NUTRITIONAL STATE ON RELEASE OF LIPASE BY RAT TISSUES

Tissue	Lipase Activity Released *	
	Fed Rats	Fasted Rats
	<i>units/gm. wet tissue</i>	<i>units/gm. wet tissue</i>
Epididymal Fat	40.1 ± 10.0	1.2 ± 0.49
Mesenteric Fat	21.4 ± 1.0	0.45 ± 0.39
Diaphragm	5.9 ± 0.73	4.1 ± 1.5
Heart Slices	10.0 ± 2.2	9.1 ± 1.6
Kidney Slices	4.1 ± 1.7	2.7 ± 0.57

* The values given are the means ± one standard deviation.

source of energy, might also be a function of the lipoprotein lipase contained in adipose tissue. This suggestion, however, is not consistent with the observations of this study, as the adipose tissues of fasted animals, which are most active in releasing free fatty acids (18), are just those that have the least demonstrable enzyme activity, whereas the adipose tissues of carbohydrate-fed animals, which do not release free fatty acids, are richest in lipoprotein lipase.

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