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THE EFFECT OF NUTRITIONAL STATE ON LIPOPROTEIN LIPASE ACTIVITY IN ISOLATED RAT ADIPOSE TISSUE CELLS

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SUMMARY

Lipoprotein lipase was studied in adipose tissue cells isolated by collagenase treatment of parametrial fat pads obtained from rats fasted 3 days or rats fasted 3 days and refed 1 day. Lipoprotein lipase was associated with fat cells but not with stromal-vascular cells. Refeeding brought about an increase in the level of lipoprotein lipase in the isolated fat cells. Fat cells obtained from refed rats responded to heparin added *in vitro* by release of lipoprotein lipase into the incubating media. No such effect of heparin was observed with fat cells of fasted rats or with stromal-vascular cells of rats of either nutritional state. The addition of collagenase to the lipoprotein lipase assay system inhibited the production of free fatty acid. These results demonstrate that in adipose tissue the major site of occurrence and regulation of lipoprotein lipase activity is the fat cell. The possibility that the enzyme acts at or within the capillary wall cannot be excluded since any enzyme present outside the adipocyte itself might have been rendered inactive by the collagenase preparation during the process of cell isolation.

INTRODUCTION

Adipose tissue contains lipoprotein lipase[1], an enzyme which specifically hydrolyzes triglycerides present in chylomicrons and low-density lipoproteins[2] and apparently plays an important role in their uptake by that tissue[3–6]. ROBINSON AND FRENCH[7] proposed that lipoprotein lipase is located on the capillary surface. A few recent reports are basically in line with this viewpoint[8–10]. To the contrary, in isolated adipose tissue cells RODBELL[11] found lipoprotein lipase associated exclusively with fat cells and not with stromal-vascular cells. Therefore, the exact site of action of lipo-

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protein lipase is still uncertain. RODBELL AND SCOW[12] recently postulated that the fat cell synthesizes and secretes lipoprotein lipase but that the site of chylomicron hydrolysis may be the vascular endothelial cell. If so, it would be reasonable to expect that, under conditions of high lipoprotein triglyceride uptake and increased level of lipoprotein lipase, some enzyme activity would be associated with the stromal-vascular cell fraction of adipose tissue. We therefore investigated the activity and localization of lipoprotein lipase in adipose tissue cells isolated from rats following a 3-day fast and 20 h of refeeding, a dietary regimen known to greatly increase both the uptake of serum lipoproteins by adipose tissue *in vitro* and the lipoprotein lipase content of the tissue[5]. In our studies with cells we also tested another feature of lipoprotein lipase observed in intact tissue[4], namely, its release *in vitro* by the addition of heparin.

METHODS

(a) Animals and dietary regimen

Adult female Long–Evans rats (body wt. 150–200 g) were divided into two groups: fasted and refed. The animals of the fasted group were deprived of food for 3 days with free access to water. The rats of the refed group were first subjected to the same fasting regimen for 3 days and then allowed to eat *ad libitum* a stock diet for 18–20 h with 10% sucrose as the drinking solution. The rats were exsanguinated by cardiac puncture under light ether anesthesia. In almost all experiments animals from both groups were sacrificed on the same day.

(b) Isolation of cells

Parametrial fat pads were quickly excised and kept at room temperature in Krebs–Ringer phosphate buffer[13] containing half the recommended concentration of calcium and 1 mg/ml of glucose. Pooled adipose tissue, blotted and weighed, was used for separation of fat from stromal-vascular cells following treatment with collagenase (Worthington Biochemical Corp., Freehold, N.J.) essentially as described by RODBELL[14]. The above phosphate buffer, containing also 4.5% Fraction V bovine albumin (Nutritional Biochemicals, Cleveland, Ohio), was used instead of the glucose- and albumin-containing Krebs–Ringer bicarbonate buffer called for in RODBELL's procedure. The pH of the buffer was adjusted to 7.4 with NaOH after addition of the albumin. Microscopic examination of a drop of suspension of fat cells smeared over a glass slide and stained with methylene blue revealed that fat cells containing nuclei and virtually free of stromal-vascular cells were obtained by this procedure. The fat cells responded to insulin by an increase in glucose uptake; the values were similar to those reported by RODBELL[14].

(c) Homogenization of cells

The isolated fat and stromal-vascular cells were washed 3 times with 10 ml of albumin-free buffer (the same as used for cell isolation, but containing no albumin). After the last wash the cells were centrifuged briefly (400 $\times$ *g* for 20–30 sec) and the top oil layer (in the case of fat cells) and any remaining buffer were removed. The plastic centrifuge tube and its content were then weighed. Cells so obtained were quickly transferred with 0.25 M sucrose into a Potter–Elvehjem homogenizing tube, placed in crushed ice and homogenized (4 strokes with a Teflon plunger attached to

the Precision Instruments varied-speed stirrer, set at speed No. 2, cooling the tube in ice between each two strokes). The amount of sucrose used for transferring and homogenization was 10–15 ml/g with the fat cells and 15–20 ml/g with the stromal-vascular cells. The homogenates were centrifuged at 1000 × *g* in a refrigerated centrifuge (4° for 10 min). The aqueous layer was transferred into a graduated centrifuge tube in crushed ice and conc. NH_4OH was added to obtain a final concn. of about 0.025 M. Aliquots (0.5 ml) of homogenates so prepared were tested for enzyme activity as described under (*f*). Stromal-vascular cell homogenates were assayed directly after addition of NH_4OH.

(d) Enzyme release

Cells were washed twice (see (*b*)) with glucose- and albumin-containing phosphate buffer. After the last wash approximately equal amounts of cells were dispensed, with the aid of a plastic rod, into two tared 20-ml plastic scintillation counting vials (Packard) filled with 3 ml of the buffer. The weight of dispensed cells was measured by the difference between weight of the vial before and after addition of the cells. To one vial 0.75 ml of 0.9% NaCl was added (control), to the other 0.75 ml of a solution prepared by diluting heparin (Upjohn Co., Kalamazoo, Mich.) with 0.9% NaCl (1:10). The final concentration of heparin was 20 U.S.P. units per ml. The vials were incubated for 1 h at 37° with gentle shaking. After incubation the samples were transferred to plastic centrifuge tubes, centrifuged at 400 × *g* for 1 min at room temperature, and the media separated from the cells. Aliquots (0.5 ml) of media were tested for enzyme activity as described in (*f*). In some experiments cells, too, were saved, washed 3 times with albumin-free buffer, weighed and homogenized as described in (*c*).

(e) Intact tissue

In experiments with intact tissue whole parametrial fat pads were incubated in glass 25-ml erlenmeyer flasks with albumin-free buffer (10 ml/g tissue). One fat pad was incubated in the presence of heparin (final concn. 3 U.S.P. units per ml) and the contralateral fat pad without heparin (control). After 1 h of incubation at 37° the tissues were removed with forceps and aliquots of media (0.5 ml) tested for enzyme activity. The tissue was cut into small pieces and homogenized in 10 vol. of 0.25 M sucrose as described for isolated fat cells. Enzyme assays were performed on 0.5-ml aliquots of the homogenates.

(f) Lipoprotein lipase activity

The composition of substrate mixture for the assay was: 15% Ediol (Riker Laboratories, Northridge, Calif.) in water, 0.1 ml; 25% solution of Fraction V bovine albumin in Tris buffer (adjusted to pH 8.5 with conc. NH_4OH), 0.2 ml; 0.05 M Tris-HCl buffer (Sigma Chemical Co., St. Louis, Mo.) (pH 8.5), 0.4 ml; serum, obtained from fasted rats on the day of experiment, 0.05 ml; and distilled water or 4.0 M NaCl, 0.25 ml. The substrate mixture was preincubated at 37° for 15 min. To 1.0 ml of substrate 0.5 ml of either homogenate or incubating media were added and the incubations were carried out at 37° in 20 mm × 150 mm test tubes. The reaction was terminated at zero time or after a 1-h incubation by addition of a mixture of isopropanol–heptane–0.75 M H_2SO_4 (40:10:1, by vol.) and free fatty acids determined by the me-

thod of DOLE AND MEINERTZ[15]. Enzyme assays were carried out at least in duplicate. The drop in pH of the assay system after 1 h of incubation was not more than 0.2 unit. The production of free fatty acids was found to be linear during 1 h of incubation and proportional to the added fat cell homogenate in the range of 0.25–2.5 ml of homogenate added to 1.0 ml of substrate. With 0.5 ml of homogenate the production of free fatty acids (1-h incubation) leveled off with 0.5 ml of the above substrate.

(g) Additional analytical procedures

The triglyceride content of the oil layer recovered after incubation of fat cells in the presence of heparin and of the fat cake obtained after the removal of water phase of fat cell homogenates was determined as follows: The lipids of the oil and cake were extracted by the procedure of DOLE AND MEINERTZ[15]. Aliquots (1.0 ml) of the upper phase were evaporated under the stream of N_2 and refluxed in a water bath at 60° for 1 h with 4% KOH in ethanol (saponified sample) or with ethanol (unsaponified sample). Tripalmitin (Calbiochem, Los Angeles, Calif.) dissolved in heptane (10 mg/ml) was used as the standard and was subjected to the above extracting and saponification procedure. The amount of triglyceride fatty acid was calculated by the difference in fatty acid content between the saponified and unsaponified sample.

Protein was determined in homogenates of tissue or isolated cells by the method of LOWRY *et al.*[16] using crystalline bovine plasma albumin as the standard.

RESULTS

Lipolytic activity in homogenates of isolated fat cells was approximately twice as high in the refed as in the fasted state (Table I). The activity was inhibited approx. 67% by 1.0 M NaCl, an inhibitor of lipoprotein lipase activity[4,17]. No significant in-

TABLE I

THE EFFECT OF NUTRITIONAL STATE ON LIPOPROTEIN LIPASE ACTIVITY IN HOMOGENATES OF FAT CELLS AND STROMAL-VASCULAR CELLS

The experiments were performed 9 times with cells of rats from both the refed and fasted groups (12–16 rats per group) and twice with cells of refed rats only. Enzyme assays were carried out in 3–5 replicates with fat cell homogenates and 2–4 replicates with stromal-vascular cell homogenates. All values are means ± S.E.; the number of determinations is shown in parentheses.

Nutritional state	*Isolated cells*		*Lipoprotein lipase activity (μequiv free fatty acids per mg protein per h)*	
	Type	*Homogenized per experiment (g)*	*Substrate with serum*	*Substrate with serum + NaCl**
Refed	Fat cells	3.31 ± 0.41 (11)	3.26 ± 0.22 (11)**	1.08 ± 0.13 (10)***
	Stromal-vascular cells	0.58 ± 0.07 (9)	0.21 ± 0.04 (9)	0.19 ± 0.06 (6)
Fasted	Fat cells	2.88 ± 0.54 (9)	1.68 ± 0.10 (9)**	1.30 ± 0.11 (8)
	Stromal-vascular cells	0.49 ± 0.04 (6)	0.31 ± 0.09 (6)	0.20 ± 0.10 (5)

* Final concn. 1.0 M.

** The difference between these two values is significant ($P < 0.01$).

*** The inhibition by NaCl is significant ($P < 0.01$) only with fat cell homogenates obtained from refed rats.

hibition by NaCl was observed in cells of fasted rats. The portion of the lipolytic activity inhibited by NaCl was approx. 8 times higher in homogenates of fat cells isolated from refed than fasted rats. Some lipolytic activity was detected also in homogenates prepared from stromal-vascular cells, but the addition of 1.0 M NaCl did not bring about any appreciable inhibition. No effect of nutritional state on lipolytic activity could be demonstrated in homogenates of these cells.

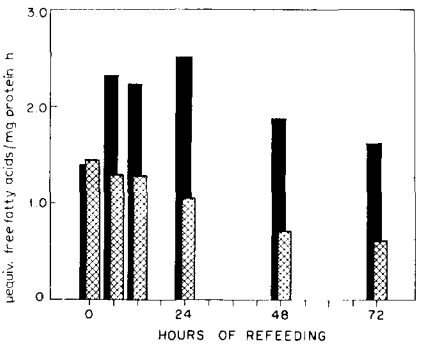

Fig. 1. Effect of the duration of the refeeding period on lipoprotein lipase activity in homogenates of isolated fat cells. The experiment was performed twice; each time an approximately equal number of rats was used (8–9 rats per group). Each column is the average of the two experiments (in each experiment the enzyme assays were carried out in quadruplicate). Black columns represent the enzyme activity measured with the standard substrate mixture, cross-hatched columns the enzyme activity determined in the presence of 1.0 M NaCl.

In Fig. 1 the change in lipoprotein lipase activity of fat cell homogenates is shown in relation to the duration of refeeding following a 72-h fast. Fasting and refeeding periods were scheduled so that all groups were sacrificed on the same day to minimize day-to-day variations. Lipoprotein lipase increased after 6 h of refeeding; the peak activity was reached after 24 h. Thereafter the activity declined slowly, remaining well above the fasting value up to 72 h. The lipolytic activity of fat cell homogenates not inhibited by the presence of 1.0 M NaCl progressively declined with the duration of refeeding.

The omission of serum from the substrate mixture inhibited the lipolytic activity of homogenates of fat cells from refed (46%) but not from fasted rats (Table II). The effect of other inhibitors of lipoprotein lipase[4,17,18] is also shown in Table II. In the presence of serum, sodium chloride and protamine sulfate inhibited the lipolytic activity in fat cell homogenates from refed rats 58 and 50%, respectively. These substances did not inhibit the reaction in the absence of serum or with fat cell homogenates prepared from fasted rats, either in the presence or absence of serum. The lower inhibition of lipoprotein lipase by omission of serum in our experiments (46%) than in RODBELL's[11] (90%) might be accounted for by an increase in lipases other than lipoprotein lipase during the 3-day period of fasting. As shown in Fig. 1 the lipolytic activity not inhibited by NaCl was high after 3 days of fasting. Although this activity declined steadily during the 72 h of refeeding, it was still high at 24 h, the approximate period of refeeding routinely used in our experiments.

Our next experiments were designed to investigate the release of lipoprotein

TABLE II

EFFECT OF INHIBITORS ON LIPOLYTIC ACTIVITY IN HOMOGENATES OF FAT CELLS ISOLATED FROM FASTED AND REFED RATS

Cells were isolated from parametrial fat pads pooled from all rats of the same group (15 rats per group; amount of cells: 2.7 g fasted, 2.1 g refed). Substrate mixtures without serum were prepared by replacing serum with an equivalent amount of distilled water. Inhibitors were added to the substrate mixture during the preincubation period. The values are averages of triplicate enzyme assays.

Additions	*Lipolytic activity (μequiv free fatty acids per mg protein per h)*			
	Refed		*Fasted*	
	Substrate with serum	*Substrate without serum*	*Substrate with serum*	*Substrate without serum*
None (control)	3.65	1.98	2.61	2.98
NaCl (1.0 M)	1.52 (58%)*	1.83 (8%)	2.42 (7%)	2.98 (0%)
Protamine sulfate (10 μg/ml)	1.83 (50%)	2.15 (0%)	2.24 (14%)	2.61 (12%)
NaF (0.2 M)	2.89 (21%)	0.91 (54%)	2.24 (14%)	1.68 (44%)
Eserin sulfate (5 μM)	3.35 (8%)	1.22 (38%)	2.05 (21%)	1.77 (41%)

* % inhibition.

lipase by the addition of heparin *in vitro*. In our experiment with intact tissue, as in those reported by CHERKES AND GORDON[4] and BEZMAN, FELTS AND HAVEL[5], the amount of enzyme released by heparin was considerably higher with adipose tissue obtained from the fed than fasted rats (30.6±5.3 *vs.* 5.4±2.6 μequiv free fatty acids/g tissue per h, expressed as the difference between heparin-incubated and control tissue). In initial experiments with isolated adipose tissue cells it was found that stromal-vascular cells did not respond to heparin. Although some lipolytic activity could be

TABLE III

RELEASE OF LIPOPROTEIN LIPASE FROM ISOLATED FAT CELLS BY HEPARIN

The experiments were performed 4 times with cells of rats from fasted and refed groups (12–16 rats per group), and twice with cells of refed rats only. Enzyme assays were carried out in triplicate. NaCl was present in the final concn. 1.0 M. All values are means ± S.E.; the number of observations is shown in parentheses.

Nutritional state	*Number of separate experiments*	*Total number of rats*	*Enzyme release*		*Lipoprotein lipase activity (μequiv free fatty acids per g cells per h)*	
			Fat cells incubated (g)	*Heparin (final concn.)*	*Substrate with serum*	*Substrate with serum + NaCl*
Refed	6	74	0.95 ± 0.15	20 U/ml	13.14 ± 1.50 (6)*†	2.50 ± 1.32 (3)
			1.10 ± 0.18	None	5.46 ± 1.58 (6)*	3.46 ± 0.58 (3)
Fasted	4	66	1.37 ± 0.56	20 U/ml	4.08 ± 1.24 (4)†	2.92 ± 1.10 (3)
			1.40 ± 0.47	None	4.68 ± 1.48 (4)	3.92 ± 1.88 (3)

* The difference between these two values is significant ($P < 0.01$).
† The difference between these two values is significant ($P < 0.005$).

detected in the incubating media, neither the addition of heparin nor the nutritional state of the animal affected the amount of activity released. In addition, the lipolytic activity liberated from these cells was not inhibited to any significant extent by 1.0 M NaCl. Fat cells isolated from refed but not fasted animals responded to heparin added *in vitro* by liberation of significantly more lipolytic activity than in the absence of heparin ($P<0.01$) (Table III). The amount of lipolytic activity released from fat cells isolated from refed rats in the presence of heparin was significantly higher ($P<0.005$) than from fat cells isolated from fasted rats. The lipolytic activity released by heparin from fat cells obtained from refed rats was inhibited to a large extent (about 80%) by 1.0 M NaCl. The lipolytic activity released into the media by fat cells obtained from refed rats in the absence of heparin and from fasted rats did not exhibit a great inhibition by NaCl. The heparin-released activity inhibited by NaCl was approx. 9 times higher in the fat cells obtained from refed than fasted rats.

TABLE IV

CELL DISRUPTION AND LIPOPROTEIN LIPASE RELEASE FROM ISOLATED FAT CELLS DURING THE INCUBATION WITH HEPARIN

Fat cells isolated from parametrial fat pads pooled from 31 refed rats (18.65 g of tissue) were incubated in the presence of heparin (5 vials) or without heparin (5 vials). NaCl was present in the final concn. 1.0 M. For further details see text. All values are means ± S.E.

Condition	*Number of determinations*	*Amount of cells incubated (g)*	*Triglyceride (mmole)*		*Lipoprotein lipase activity (μequiv free fatty acids per g cells per h)*	
			*In oil layer**	*In fat cake***	*Substrate with serum*	*Substrate with serum + NaCl*
Heparin (20 U/ml)	5	0.52 ± 0.02	0.062 ± 0.006	0.188 ± 0.021	18.84 ± 1.26	5.62 ± 0.60
Control (no heparin)	5	0.58 ± 0.04 N.S.****	0.079 ± 0.007 N.S.	0.203 ± 0.021 N.S.	9.40 ± 2.46 $P < 0.02$	3.94 ± 0.78

* Recovered at the end of the 1-h incubation period.

** Fat cake refers to fat collected after homogenization and centrifugation of fat cells following the termination of the 1-h incubation period.

*** The corresponding vertical pairs of values (Heparin *vs.* Control) were compared with the *t* test; N.S. = not significant.

The results of the experiment shown in Table IV and Fig. 2 indicate that the effect of heparin upon the appearance of lipoprotein lipase in the incubation media cannot be explained by an enhancement of cell breakage. The triglyceride content of the top oil layer recovered by means of mild suction applied to plastic tubing following centrifugation of the cell suspension at the end of the 1-h incubation period was taken as a measure of cell breakage. The triglyceride content of the oil layer was not affected by the presence of heparin, but the lipoprotein lipase activity was approximately twice as high in the presence of heparin.

The data in Fig. 2 show also that the heparin-stimulated release was not accompanied by a drop in the lipoprotein lipase activity remaining in homogenates of fat cells after incubation. Therefore, the total lipoprotein lipase activity detected in the

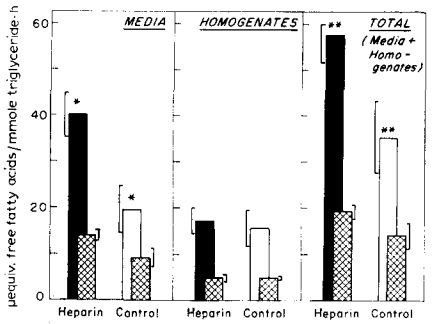

Fig. 2. The effect of heparin on the amount of lipoprotein lipase released into the media during a 1-h incubation period and the amount present in the fat cells after incubation. For experimental details see text and Table V. The enzyme activity is expressed per total triglyceride (in 'oil layer' + in 'fat cake'; for explanation see footnotes to Table V). Each column is the mean value of 5 determinations. Vertical lines alongside each column indicate the 2 S.E. Black columns represent the enzyme activity of fat cells incubated in the presence of heparin, white columns the activity of cells incubated without heparin. Cross-hatched columns represent the enzyme activity measured in the presence of 1.0 M NaCl. The difference between the values marked with * and** are statistically significant at the level of $P < 0.025$ and $P < 0.05$, respectively.

TABLE V

INHIBITION OF LIPOLYSIS (pH 8.5) BY COLLAGENASE

Tissue for all experiments was obtained from refed rats. In Expts. 1 and 2 lipoprotein lipase was released by incubation of parametral fat pads with heparin as described in METHODS. In Expt. 1 the enzyme was released from tissue (5.0 g) pooled from 10 rats. In Expt. 2 the enzyme was released from individual fat pads (0.20 ± 0.05 g) obtained from 4 rats. In Expt. 3 homogenates were prepared from 2.77 g of fat cells isolated from 7.75 g of parametral fat pads pooled from 11 rats. Before beginning the enzyme assay 0.5-ml aliquots of enzyme-containing media or homogenate were preincubated for 15 min at 37° with or without the additions listed. Final concentration of collagenase was 10 mg/ml and that of heparin 3 U/ml. The data of Expts. 1 and 2 are means ± S.E.; the number of determinations is shown in parentheses. The data of Expt. 3 represent the averages of triplicate enzyme assays.

Expt. No.	*Source of enzyme*	*Additions to the enzyme during pre-incubation*	*Lipolytic activity (μequiv free fatty acids per g tissue or cells per h)*	
			Substrate with serum	*Substrate with serum + NaCl*
1	Released from tissue	None	32.02 ± 1.30 (5)	
		Collagenase	10.73 ± 3.31 (5)	
		Heparin	28.01 ± 1.29 (5)	
		Collagenase + heparin	13.28 ± 4.20 (5)	
2	Released from tissue*	None	57.07 ± 17.16 (4)	14.33 ± 10.05 (4)
		Collagenase	18.00 ± 8.74 (4)	12.63 ± 4.75 (4)
3	Fat cell homogenate	None	13.28	5.25
		Collagenase	7.90	5.05

* 4 contralateral fat pads (0.22 ± 0.05 g) of these animals were incubated under identical conditions but without heparin. The media showed a lipolytic activity of 18.98 ± 8.86 μequiv free fatty acid per g per h.

whole system was significantly higher ($P<0.05$) in samples of cells incubated in the presence of heparin than in control samples. Similar results were obtained with intact parametrial fat pads. While there is no obvious explanation for this observation, it is possible that heparin has a stabilizing action on the enzyme. It has been suggested that heparin has a role in the synthesis of lipoprotein lipase[6,19,20], but it is questionable whether such an action could be important in our experiments since they were of such short duration.

When lipoprotein lipase activity originally present in the intact fat tissue was compared with that determined simultaneously in undigested tissue fragments, isolated fat cells and stromal-vascular cells, we recovered more than 75% of the tissue protein, but less than 50% of the lipoprotein lipase activity. No activity was detected in the media in which the cells were isolated. When the possibility of a direct interference by the collagenase preparation on lipoprotein lipase activity (released from intact adipose tissue by heparin) was tested by preincubation of the enzyme-containing media with collagenase, a pronounced inhibition was observed in the production of free fatty acid in the enzyme assay (Table V). The addition of heparin to the enzyme assay system did not reverse the inhibition (Expt. 1). The addition of 1.0 M NaCl did not bring about a further reduction in free fatty acid production (Expt. 2). Collagenase was found to exhibit an essentially very similar effect on free fatty acid production when assays were carried out with homogenates of isolated fat cells as the source of enzyme (Expt. 3).

DISCUSSION

The term 'lipoprotein lipase' was first proposed by KORN[17] and emphasizes the preferential hydrolysis, by this enzyme, of lipoprotein triglycerides[2]. However, for various reasons this term has not been adopted generally[21,22]. As used in this paper, lipoprotein lipase refers to the lipolytic activity measured at pH 8.5 with an emulsion of simple triglycerides (Ediol) preincubated with serum and inhibited by 1.0 M NaCl.

Lipolytic activity was associated predominantly with fat cells and not with stromal-vascular cells both in fasted and refed animals. To a large extent the lipolytic activity in fat cells of refed rats could be ascribed to lipoprotein lipase. The lipolytic activity in fat cells of fasted rats and in stromal-vascular cells of both refed and fasted rats, on the other hand, could almost entirely be ascribed to lipases other than lipoprotein lipase. Further indication that the lipolytic activity of fat cells isolated from fasted rats did not represent lipoprotein lipase activity was the observation that neither the omission of serum nor the addition of protamine sulfate had any effect on the level of activity in homogenates of those cells.

The finding that lipoprotein lipase was associated with the isolated fat cell and not with the stromal-vascular cells confirms RODBELL's observation[11]. In addition, our data show that this was the case even in refed rats, *i.e.*, under conditions when fat tissue is known to exhibit the highest uptake of chylomicron and lipoprotein triglycerides[5]. Refeeding increased the level of lipoprotein lipase in fat cells six-fold but had no effect on the stromal-vascular cells. Furthermore, our data show that lipoprotein lipase was released from fat cells of refed but not from fasted rats and that no release was observed from stromal-vascular cells obtained from rats of either nutritional state. Although these observations appear to be in conflict with the view that

the site of action of lipoprotein lipase is the vascular endothelial cell, they actually are not in the light of our observation that the preparation of collagenase used for the isolation of cells, when added *in vitro* to lipoprotein lipase released from fat pads by heparin, was found to inhibit the production of free fatty acids in subsequent enzyme assay. If the inhibition of free fatty acid production in the enzyme assay is assumed to represent the result of the action of collagenase (or of impurities present in the preparation of collagenase) on lipoprotein lipase, then any lipoprotein lipase accessible to the collagenase preparation in the course of tissue digestion would be rendered inactive. If any lipoprotein lipase in the tissue is present along capillaries, then this portion of the enzyme is conceivably extracellular, or attached to cells at a place accessible to, or present in a form readily affected by, the collagenase preparation. If this is the case, it could account for the failure in our experiments, as well as in RODBELL's[11], to find any appreciable lipoprotein lipase activity in association with the stromal-vascular cell fraction of adipose tissue digested by collagenase. These experiments, therefore, are not inconsistent with the view that lipoprotein lipase is synthesized in the fat cells of rat adipose tissue, but acts at sites along the capillary wall.

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