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The lipoprotein lipase of white adipose tissue

Studies on the intracellular distribution of the adipocyte-associated enzyme

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1. The separation of rat epididymal adipocytes into plasma-membrane, mitochondrial, microsomal and cytosol fractions is described. The fractions, which were characterized by marker-enzyme analysis and electron-micrographic observation, from the cells of fed and 24 h-starved animals were used to prepare acetone/diethyl ether-dried powders for the measurement of lipoprotein lipase activities. 2. The highest specific activities and proportion of recovered lipoprotein lipase activity were found in the plasma-membrane and microsomal fractions. The two fractions from the cells of fed rats showed similar activities and enrichments of the enzyme, these activities being higher than the plasma-membrane and lower than the microsomal activities recovered from the cells of starved animals. 3. Chicken and guinea-pig anti-(rat lipoprotein lipase) sera were prepared, and an indirect labelled-second-antibody cellular immunoassay, using ^{125}I-labelled rabbit anti-(chicken IgG) or ^{125}I-labelled sheep anti-(guinea-pig IgG) antibodies respectively, for the detection of cell-surface enzyme was devised and optimized. 4. The amount of immunodetectable cell-surface lipoprotein lipase was higher for cells isolated from fed animals than for cells from 24 h-starved animals, when either anti-(lipoprotein lipase) serum was used in the assay. The amount of immunodetectable cell-surface lipoprotein lipase fell further when starvation was extended to 48 h. 5. The lipoprotein lipase of plasma-membrane vesicles was shown to be a patent activity and to be immunodetectable in a modification of the cellular immunoassay. 6. Although the functional significance of the adipocyte surface lipoprotein lipase is not known, the possibility of it forming a pool of enzyme en route to the capillary endothelium is advanced.

INTRODUCTION

Lipoprotein lipase (EC 3.1.1.34) is the extrahepatic enzyme responsible for the hydrolysis of plasma lipoprotein triacylglycerol (Hamosh & Hamosh, 1983). Changes in tissue lipoprotein lipase activities, which in general correlate closely with changes in the rate of triacylglycerol uptake by tissues (Rogers & Robinson, 1974; Cryer *et al.*, 1976; Scow *et al.*, 1976; Linder *et al.*, 1976; Vernon & Clegg, 1985), occur in response to a variety of conditions (Cryer, 1981; Quinn *et al.*, 1982; Hamosh & Hamosh, 1983) and are subject to hormonal control.

Although the enzyme exerts its physiological function at the capillary endothelium (Cryer, 1983), it can also be recovered from adipose tissue and cardiac muscle with isolated adipocytes (Rodbell, 1964; Cryer *et al.*, 1975) and cardiac muscle cells (Bagby *et al.*, 1977; Chohan & Cryer, 1978) respectively. Much evidence (Cryer, 1981, 1983) indicates, however, that the enzyme present at the endothelium does not originate there (Howard, 1977), but is synthesized and exported by the tissue parenchymal cells. Although the intracellular localization of lipoprotein lipase in cardiac muscle cells is consistent with is nature as a secretory protein (Chohan & Cryer, 1979), similar studies with adipose tissue and cells have yielded rather variable data (Wolf *et al.*, 1975; Vanhove *et al.*, 1978), which are in turn at variance with observations on differentiated adipocyte precursor (ob_{17}) cells (Vannier *et al.*, 1982, 1985). Furthermore, although Boyer & co-workers (Arnaud & Boyer, 1977; Arnaud *et al.*, 1979; Verine *et al.*, 1982) have demonstrated extracellular lipoprotein triacylglycerol breakdown by intact adipocytes and its modification by changes in nutritional status, the implied adipocyte cell-surface localization for at least a proportion of adipocyte lipoprotein lipase has not been supported by the cellular-fractionation studies carried out by others. It was the purpose of the present work to use both subcellular-fractionation procedures and immunoassay studies to re-investigate the subcellular localization of lipoprotein lipase in isolated adipocytes and to establish the relationship that this may have to how the adipocyte enzyme may be transferred to the endothelium.

MATERIALS AND METHODS

Animals, tissues and cells

Male rats of the MRC Hooded strain were used throughout. The animals (200–270 g body wt.) were maintained on modified diet 41B (Pilsbury and Co., Birmingham B5 7UG, U.K.). Where necessary the rats were deprived of food for either 24 or 48 h and were killed by cervical dislocation between 09:30 and 11:00 h. Immediately after death the epididymal fat-pads were removed, rinsed in Krebs–Ringer bicarbonate buffer, pH 7.4 at 37 °C (Cryer *et al.*, 1975), and used, without delay, for the isolation of adipocytes.

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Isolation of adipocytes

Cells were prepared by a modification of the procedure of Rodbell (1964) as described by Cunningham & Robinson (1969) and Cryer *et al.* (1975). To minimize the morphologically and immunologically detectable cell-surface damage (Al-Jafari *et al.*, 1986) produced by proteolytic action during the isolation procedure, crude bacterial collagenase (150 units/mg; from *Clostridium histolyticum*; Sigma Chemical Co., type II, code C2139) was used at relatively low concentrations (0.5 mg/ml) in the isolation medium. The yield of cells under these conditions was approx. 0.75 ml (0.8×10^6 cells) per two fat-bodies.

Determination of lipoprotein lipase activities

The lipoprotein lipase activity of adipocytes and subcellular fractions was determined by using acetone/ether-dried powders of the materials prepared by the procedure outlined by Borensztajn *et al.* (1970), with 2.5% (w/v) casein as carrier protein. Activities were determined as the rate of non-esterified fatty acid release from an apolipoprotein-C_{II}-activated triacylglycerol emulsion (Intralipid; Vitrum, Stockholm, Sweden) at 30 °C as described by Cryer & Jones (1978). The enzyme activity was characterized in all the preparations by the degree of inhibition observed when 0.6 M-NaCl was present and by the obligatory requirement for serum in the assay. Activities (expressed as μmol of fatty acid released from the triacylglyerol substrate at 30 °C in 1 h), and distributions are expressed as means ± S.E.M. Student's *t* test was used to assess the significance of the differences between means (Fisher & Yates, 1957).

Subcellular fractionation of adipocytes

The method used was a modification of that described by Belsham *et al.* (1980) and Tume *et al.* (1985). Typically, cells were suspended in an equal volume of 0.25 M-sucrose/10 mM-Tris/HCl (pH 7.4) containing 2 mM-EDTA and disrupted by agitation with a vortex mixer (3 × 30 s). The mixture was centrifuged (1000 *g*, 30 s) and the infranatant beneath the fat plug was removed by aspiration. The fat plug was then re-extracted with fresh extraction medium and the second infranatant so produced was pooled with the first. This defatted homogenate was centrifuged at 30000 $g_{av.}$ for 30 min at 4 °C. The supernatant fraction obtained was centrifuged at 60000 $g_{av.}$ for 90 min and the supernatant and pellet so produced were designated 'cytosol' and 'microsomal fractions' respectively. The particulate material from the initial centrifugation step at 30000 *g* was resuspended and applied to a self-forming gradient of Percoll, which was centrifuged at 10000 $g_{av.}$ for 15 min. After centrifugation, two well-defined bands were visible, one just below the liquid surface (the putative plasma-membrane fraction) and one close to or at the bottom of the tube (crude mixed mitochondrial fraction). Each of the particulate fractions was removed by aspiration and washed by resuspension and re-centrifugation.

The subcellular fractions were characterized by marker-enzyme analysis and transmission electron microscopy. The activities of 5'-nucleotidase (EC 3.1.3.5), lactate dehydrogenase (EC 1.1.1.27), succinate:cytochrome *c* oxidoreductase (EC 1.3.99.1) and NADPH: cytochrome *c* oxidoreductase (EC 1.6.99.1) were determined at 25 °C by the methods of Newby *et al.* (1975), Saggerson & Greenbaum (1969), Polakis *et al.* (1965) and Sottocasa *et al.* (1967) respectively. Protein was determined by the Hartree (1972) modification of the Lowry *et al.* (1951) method, with dry bovine serum albumin as standard. In fractions containing Percoll, due account was taken of the effect of this agent on the colorimetric determination of protein.

Samples for electron-microscopic observation were fixed, post fixed, dehydrated and embedded by using conventional procedures; 60–90 nm-thick sections were stained as described by Reynolds (1963) and viewed with a Philips 400T electron microscope at an accelerating voltage of 80 kV.

Immunodetection of cell-surface lipoprotein lipase

Antiserum to rat lipoprotein lipase was raised in either chickens or guinea pigs by using the immunization protocol described by Al-Jafari & Cryer (1985), with enzyme isolated from rat heart, by the methods described in Williams *et al.* (1983), as immunogen. The immunogen displayed a single major band of M_r 54600 ± 1590 (means ± S.E.M., $n = 4$) when analysed by gradient polyacrylamide-gel electrophoresis in the presence of SDS (Siemankowski & Dreizen, 1978) in the buffer system of Laemmli (1970). Parkin *et al.* (1982) have reported that rat adipose-tissue lipoprotein has an M_r of 56000. No band equivalent to antithrombin III (M_r 62000–65000; Miller-Anderson *et al.*, 1974) was present in the preparations used here as immunogen.

Purified lipoprotein lipase from bovine milk was prepared as described previously (Williams *et al.*, 1983).

The capacity of the antisera to inhibit lipoprotein lipase activity present in extracts of rat and mouse adipose and heart acetone/ether-dried powders and purified bovine milk lipoprotein lipase was assessed as described by Olivecrona & Bengtsson (1983) (see Al-Jafari & Cryer, 1985).

Adipocyte surface lipoprotein lipase was detected by using an indirect labelled-second-antibody cellular immunoassay. For this, samples of isolated adipocyte suspension (25–100 μl) were incubated for 30 min at 37 °C in the presence of appropriate dilutions (10 μl; see the Results and discussion section) of either chicken or guinea-pig anti-(rat lipoprotein lipase) serum. The cells were washed in Krebs–Ringer bicarbonate buffer (3 × 500 μl) before being reincubated in the presence (100 μl) of either ^{125}I-labelled rabbit anti-(chicken IgG) antibodies or ^{125}I-labelled sheep anti-(guinea-pig IgG) antibodies respectively (20000–40000 c.p.m./assay). After 30 min at 37 °C, the cells were washed free of unbound radioactivity with 3 × 500 μl of Krebs–Ringer bicarbonate buffer. Finally the cells were suspended in 200 μl of Krebs–Ringer bicarbonate buffer and mixed with 100 μl of dinonyl phthalate (BDH Chemicals). The inclusion of the oil allowed for the complete removal of the aqueous infranatant before the cell-bound radioactivity was determined. The specifically bound radioactivity was calculated by comparison of each experimental assay (in triplicate) with equivalent assays containing non-immune serum (in duplicate).

Rabbit anti-(chicken IgG) and sheep anti-(guinea-pig IgG) sera were prepared by the injection of either affinity-purified (Cryer *et al.*, 1984*a*) or commercial (Koch–Light) preparations of the IgGs into rabbits or sheep respectively. Immunoadsorbants consisting of either chicken or guinea-pig IgG coupled to the

diazonium salts of reprecipitated powdered cellulose (Miles *et al.*, 1974) were prepared as described by Gurevich *et al.* (1962). The respective anti-IgG antibodies were allowed to adsorb to these matrixes. The immunoadsorbant with bound antibody present was washed before iodination by the method described by Plaas *et al.* (1981). The immunoreactive iodine-labelled rabbit anti-(chicken IgG) or sheep anti-(guinea-pig IgG) antibodies were then eluted and stored bound to fresh immunoadsorbants, from which they were eluted and used under the conditions described by Plaas *et al.* (1981). The immunoreactivity of eluted antibody was checked periodically by assessing the reactivity it showed toward freshly washed immunoadsorbant (Plaas *et al.*, 1981).

RESULTS AND DISCUSSION

Characterization of subcellular fractions obtained from homogenates of rat epididymal adipocytes

Because of the synthesis by and secretion from adipocytes of lipoprotein lipase, it was the primary aim of the present investigations to study the subcellular distribution of the enzyme activity, particularly among the membraneous fractions of the isolated adipocyte.

Subcellular fractions were obtained by a slight modification of the differential-centrifugation procedure described by Belsham *et al.*, 1980 (see the Materials and methods section). This procedure was considered preferable to certain others (e.g. Avruch & Wallach, 1971; Vanhove *et al.*, 1978), mainly because it was rapid, thus minimizing any losses of lipoprotein lipase activity owing to the known lability of the enzyme. The subcellular fractions obtained were characterized by using both enzymic and morphological markers. Fig. 1 shows the distribution and enrichment of the enzyme markers.

The plasma membrane was the only fraction characterized by a high percentage recovery (mean 94%) and elevated relative specific activity of 5′-nucleotidase (Fig. 1*a*), a known ectoenzyme of the adipocyte exclusive to the plasma membrane (Newby *et al.*, 1975). This fraction also contained NADPH: cytochrome *c* oxidoreductase (Fig. 1*b*), but the enrichment by comparison with that in the putative microsomal fraction was low. The plasma-membrane fraction was free of lactate dehydrogenase (Fig. 1*c*) and had a low relative specific activity of succinate: cytochrome *c* oxidoreductase (Fig. 1*d*). The plasma-membrane fraction obtained here was therefore at least as pure as those described previously (McKeel & Jarett, 1970; Belsham *et al.*, 1980) and of greater purity than those obtained when whole adipose tissue was used as the starting material (Vanhove *et al.*, 1978; Tume *et al.*, 1985). Electron-micrographic analysis of the plasma-membrane fraction revealed the absence of mitochondria and showed the characteristic smooth vesicular morphology and double membrane boundaries associated with such fractions from this and other sources (McKeel & Jarett, 1970).

The mitochondrial fraction collected here was an operationally defined fraction produced as a by-product of the density-gradient technique for the isolation of adipocyte plasma membranes. The fraction was relatively free of 5′-nucleotidase (Fig. 1*a*) and showed a high enrichment of succinate: cytochrome *c* oxidoreductase (Fig. 1*d*). Morphologically the fraction was made up

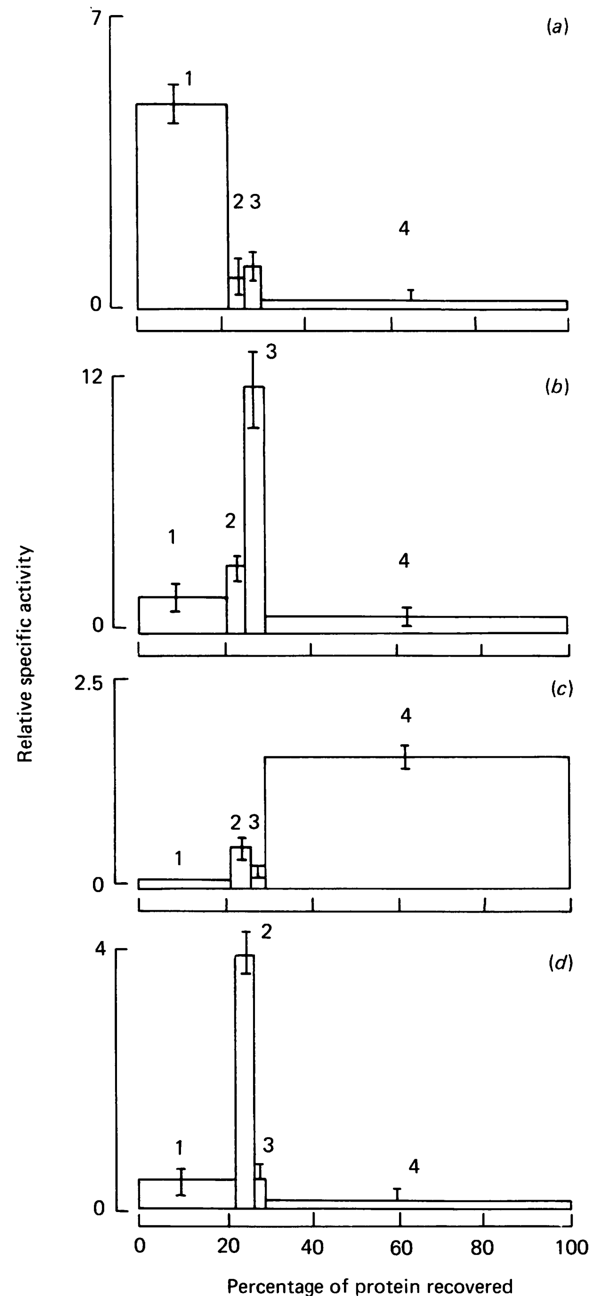

Fig. 1. Distribution of marker enzymes in subcellular fractions prepared from isolated rat adipocytes

Cell fractions from four independent fractionation experiments were assayed for the range of enzymes shown and for protein. The activities were related to that present in the defatted homogenate. (*a*) 5′-Nucleotidase, (*b*) NADPH: cytochrome *c* oxidoreductase (*c*) lactate dehydrogenase and (*d*) succinate: cytochrome *c* oxidoreductase are expressed as relative specific activities (percentage of enzyme activity in the fraction/percentage of protein in the fraction) and related to the protein distribution. The bars indicate S.E.M. The specific activities of the marker enzymes in the defatted homogenate were (*a*) 1690–3390, (*b*) 16.4–19.6, (*c*) 55.8–119.3 and (*d*) 12 nmol/min per μg of protein respectively. The total recovery of the enzyme activities was in general greater than 75%. There was no difference in the analysis of the fractions when material from either fed or 24 h-starved rats was used. The fractions labelled 1, 2, 3 and 4 were the plasma-membrane, mitochondrial, microsomal and cytosol respectively.

Table 1. Distribution of lipoprotein lipase (LPL) activity in fractions from homogenates of isolated adipocytes prepared from the white adipose tissue of fed rats

Acetone/diethyl ether-dried powders of cells and subcellular fractions of cells prepared as described in the text were assayed for lipoprotein lipase activity. In some of the experiments described, the marker-enzyme activities shown in Fig. 1 were also measured on samples of the fractions to check their identity. Lipoprotein lipase activities are expressed as μmol/h per ml of cells (units). Experimental series 1 represents a group of independent fractionations ($n = 4$) in which the activity present in the original cell suspension was measured, and this value used for comparison with the activities recovered in the fractions. Experimental series 2 represents a separate group of independent fractionations ($n = 5$) in which the activity present in the defatted homogenate was measured, and this value was used for comparison with the activities recovered in the fractions. The relative specific activities for lipoprotein lipase in the plasma-membrane, mitochondrial, microsomal and cytosol fractions were 8.5, 2, 4 and 0.31 for experimental series 1, and 3, 0.92, 4.5 and 0.24 for experimental series 2, respectively.

	Experimental series 1				Experimental series 2			
	LPL activity				LPL activity			
Fraction	(units/ fraction)	(units/ mg of protein)	Activity recovered (%)	Protein recovered (%)	(units/ fraction)	(units/ mg of protein)	Activity recovered (%)	Protein recovered (%)
Whole cells	4.6±1.4	0.35±0.12	–	–	–	–	–	–
Defatted homogenate	–	–	–	–	1.56±0.3	0.8±0.2	–	–
Plasma membrane	0.5±0.1	1.5±0.5	53	6.2	0.4±0.16	3.4±3.2	68	23
Mitochondria	0.08±0.05	0.29±0.25	8	4	0.03±0	0.28±0	6	5.6
Microsomal	0.12±0.02	1.4±0.6	12	3	0.05±0.01	3.1±2.6	9	2
Cytosol	0.27±0.11	0.03±0.005	27	86	0.09	0.07	17	69

Table 2. Distribution of lipoprotein lipase (LPL) activity in fractions from homogenates of isolated adipocytes prepared from the white adipose tissue of starved rats

The details were as described in the legend to Table 1, except that in this case each separate experimental series (1 and 2) was made up of four independent fractionations. In this case the relative specific activities for lipoprotein lipase in the plasma membrane, mitochondrial, microsomal and cytosol fractions were 5, 1, 14 and 0.13 for experimental series 1, and 3.2, 0.5, 5.5 and 0.14 for experimental series 2, respectively.

	Experimental series 1				Experimental series 2			
	LPL activity				LPL activity			
Fraction	(units/ fraction)	(units/ mg of protein)	Activity recovered (%)	Protein recovered (%)	(units/ fraction)	(units/ mg of protein)	Activity recovered (%)	Protein recovered (%)
Whole cells	2.5±0.7	0.3±0.12	–	–	–	–	–	–
Defatted homogenate	–	–	–	–	1.61±0.2	–	–	–
Plasma membrane	0.7±0.2	0.66±0.13	72	14	0.37±0.1	1.2±0.3	77	24
Mitochondria	0.02±0.01	0.17±0.08	3	3	0.01±0.003	0.1.+0.03	2	4
Microsomal	0.14±0.08	7.9±4.2	14	1	0.05±0.01	8.3±1.9	11	2
Cytosol	0.1±0.07	0.06±0.08	11	83	0.05±0.02	0.025±0.004	10	70

substantially of swollen but intact mitochondria, together with other ill-defined amorphous material.

The microsomal fraction had a relative specific activity of NADPH: cytochrome *c* oxidoreductase invariably in excess of 10. The fraction was relatively free of contamination from other marker enzymes, with a morphology rich in smooth vesicles and free of mitochondria. The cytosol fraction contained 97% of the lactate dehydrogenase activity present in the defatted homogenate, other marker enzymes characteristic of membraneous fractions being absent.

From these data it is clear that, despite the problems inherent in the isolation of subcellular fractions from adipocytes (i.e. damage to cells during isolation, entrapment of components by fat, extended time scale of conventional protocols), the current approach produced four well-characterized fractions, in satisfactory recovery, within 3 h of cell disruption. The requirements of the fractionation for the study of lipoprotein lipase distribution among the membraneous fractions of the adipocyte were therefore met.

Distribution of lipoprotein lipase activity in subcellular fractions prepared from homogenates of adipocytes obtained from the epididymal white adipose tissue of fed or 24 h-starved rats

Tables 1 and 2 show the distribution of lipoprotein lipase activity among the four major fractions obtained from adipocytes prepared from fed and 24 h-starved rats respectively. Because each experimental fractionation was started with slightly different volumes of adipocytes (mean approx. 8 ml), the activities shown have been expressed on the basis of that present in 1 ml of cells or in fractions derived from a nominal 1 ml of cells. In the 17 fractionations reported, of the 12.2 ± 1.5 mg of protein/ml present in whole cells only 3.0 ± 1.9 mg/ml was recovered in the defatted homogenate (approx. 25%). This relatively low recovery of protein and lipoprotein lipase activity (mean 31%) in the homogenate was despite the considerable improvement in both achieved by re-extracting the fat plug to produce a second infranatant (see the Materials and methods section). However, 65–86% of the protein present in the defatted homogenate was recovered in the fractions, as was 22–39% of the lipoprotein lipase activity. In fed animals (Table 1) the highest specific activity (units/mg of protein) of the enzyme was found in the plasma membrane. This was the case for both experimental series, i.e. whether the activity in whole cells or the defatted homogenate was used as the basis for comparison. This specific activity was significantly higher ($P < 0.005$–0.01) than the equivalent activity for 24 h-starved animals (Table 2). For both fed and 24 h-starved rats the mitochondrial and cytosol fractions showed low specific activities for lipoprotein lipase, with no evidence of any enrichment.

The specific activity of the enzyme in the microsomal fractions of adipocytes from fed rats was not significantly different from that seen in equivalent plasma-membrane fractions. For 24 h-starved animals, however, the specific activity of lipoprotein lipase in microsomes was 7–10 times higher ($P < 0.001$) than that found in the equivalent plasma-membrane fractions, and was also elevated ($P < 0.001$–0.005) relative to the microsomal activity found in adipocytes taken from fed animals.

When acetone/ether-dried powders of homogenates from isolated adipocytes or the fractions prepared from either fed or 24 h-starved animals were exposed to 0.6 M-NaCl during the assay of lipoprotein lipase, the activity in all cases was inhibited by more than 85%. Likewise in all cases the presence of a source of apolipoprotein C_{II} was obligatory for the expression of activity (results not shown).

These data show that the lipoprotein lipase activity of rat adipocyte subcellular fractions is substantially confined to microsomal fractions and plasma membranes, with the latter containing the largest proportion of recovered activity. A loosely bound membraneous fraction separated from the nuclei of cardiac tissue homogenates has previously been shown to have a high relative specific activity of both lipoprotein lipase and 5′-nucleotidase activity (Chohan & Cryer, 1979; Chohan, 1979). These and other data quoted by Chohan & Cryer (1979) intimated that lipoprotein lipase was also present in cardiac plasma membranes, although the fractionation of adipocytes by Vanhove *et al.* (1978) indicated a relatively minor role for this fraction. These latter authors, however, used much more extreme conditions for the isolation of cells. The present demonstration of adipocyte plasma membrane lipoprotein lipase, the activity of which was affected by nutritional status, was also compatible with the observations by Boyer and co-workers (Arnaud & Boyer, 1977; Arnaud *et al.*, 1979; Verine *et al.*, 1982), who have demonstrated the extracellular hydrolysis of lipoprotein triacyglycerol by isolated adipocytes *in vitro*. All of these data provide only circumstantial evidence, however, for lipoprotein lipase being a normal component of the isolated adipocyte plasma membrane. To obtain information of a more direct nature, we have used a labelled-second-antibody cellular immunoassay to probe the surface of the intact adipocyte.

Immunodetection of lipoprotein lipase at the surface of intact adipocytes

As described in the Materials and methods section, two types of anti-(rat lipoprotein lipase) serum were prepared. The characteristics of these antisera with regard to their capacity to inhibit enzyme of rat, mouse and bovine origins are shown in Fig. 2. The chicken anti-(rat lipoprotein lipase) serum chosen (Fig. 2*a*) showed an identical capacity to inhibit both rat cardiac and adipose-tissue activity. The serum inhibited the activity from mouse heart and adipose tissue relatively weakly and had no effect on the activity of enzyme from bovine milk. The guinea-pig anti-(rat lipoprotein lipase) serum demonstrated a somewhat weaker inhibitory activity against rat enzyme and a higher inhibitory capacity for mouse and bovine enzyme than did the chicken antiserum.

Initially the detection of anti-(lipoprotein lipase) binding to intact adipocytes was investigated with regard to the optimum volume of adipocyte suspension used in the assays and the optimum concentration of antiserum. With both antisera, optimal specific second-antibody binding was obtained at a dilution of 10^2 (10^3 final dilution in the assay) and with 50 and 70 μl of cell suspension for the chicken and guinea-pig sera respectively.

When cells from fed animals were prepared at increasing concentrations of collagenase, the anti-(lipoprotein lipase) binding after isolation decreased. Thus, relative to the binding observed with cells prepared

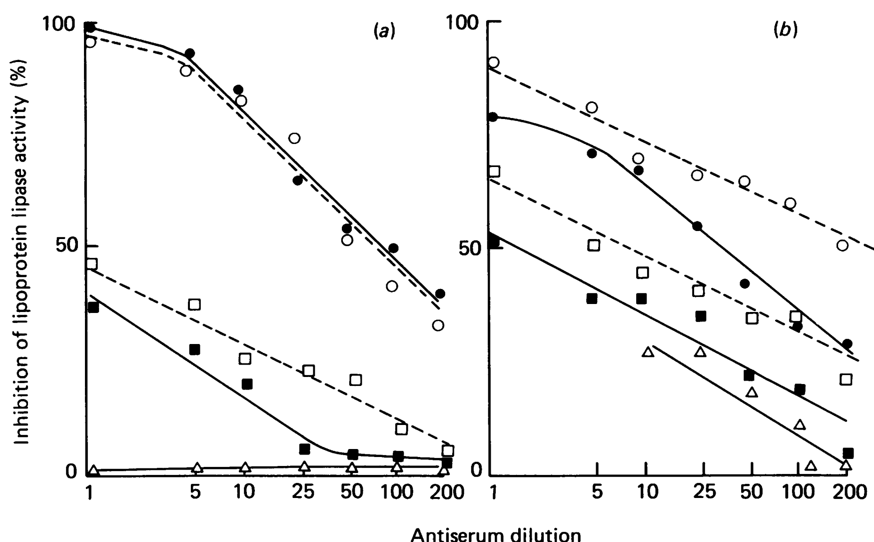

Fig. 2. Inhibitory effect of chicken or guinea-pig anti-(rat lipoprotein lipase) sera on the lipoprotein lipase activity of rat, mouse and bovine origins

Acetone/ether-dried powders from rat or mouse heart and adipose tissues, prepared as described in the Materials and methods section except that the carrier protein was omitted, were homogenized (10 mg/ml) in 25 mM-NH_4OH/4 mM-EDTA containing (per ml) 40 μg of heparin, 1 mg of bovine serum albumin, 4 mg of Triton X-100 and 0.4 mg of SDS, pH 8.1 (Olivecrona & Bengtsson, 1983). After standing for 30 min at 4 °C, the suspension was centrifuged at 20000 *g* for 45 min at 4 °C, and the supernatant was used as the enzyme source. Bovine milk lipoprotein lipase was purified as described in the Materials and methods section and diluted in the buffer described above to give preparations equal in activity to those of rat and mouse origins. Equal volumes (0.25 ml) of the enzyme solutions and the chicken (*a*) or guinea-pig (*b*) antiserum or antiserum diluted with non-immune serum were mixed and incubated at 4 °C for 16 h. The enzyme activity remaining was determined with apolipoprotein-C_{II}-activated Intralipid as the substrate as described in the Materials and methods section. No detectable loss of enzyme activity occurred during corresponding incubations with non-immune serum or ammonia buffer. The activities remaining were used to calculate the percentage inhibition. The data given are representative of three similar experiments in each of which enzyme from rat adipose tissue (●), rat heart (○), mouse adipose tissue (■), mouse heart (□) and bovine milk (△) was studied.

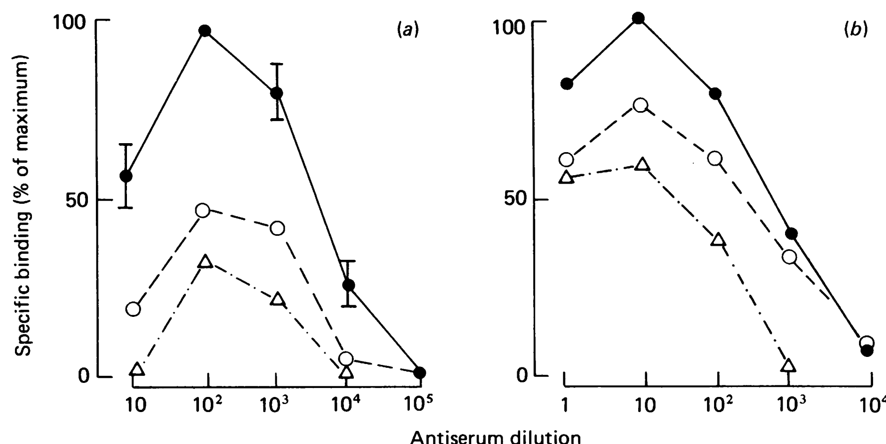

Fig. 3. Effect of nutritional status on anti-(lipoprotein lipase) antibody binding to isolated rat adipocytes

Rat epididymal adipocytes were prepared from fed, 24 h- or 48 h-starved rats as described in the Materials and methods section and studied in parallel. Samples (50 μl) of the cell suspensions were incubated with either chicken anti-(rat lipoprotein) serum (*a*) or guinea-pig anti-(rat lipoprotein) serum (*b*) at the dilutions shown. The binding of ^{125}I-labelled rabbit anti-(chicken IgG) antibody or ^{125}I-labelled sheep anti-(guinea-pig) IgG) antibody respectively was determined by using the indirect labelled-second-antibody cellular immunoassay described in the Materials and methods section. Non-immune chicken or guinea-pig serum was used appropriately in control cell incubations. Panel (*a*) shows binding of chicken anti-(lipoprotein lipase) antibodies to adipocytes from fed (●), 24 h- (○) and 48 h- (△) starved rats, with optimal binding to the cells of fed animals set at 100%. The data show the means ±S.E.M. for three independent experiments. Optimally with cells from fed animals 4.5±0.5% of the second-antibody radioactivity present in the assays (20000–40000 c.p.m.) became bound specifically to the 50 μl sample of cells. Panel (*b*) shows similar data derived from experiments in which guinea-pig anti-(rat lipoprotein lipase) serum was used. In these experiments 75 μl samples of cell suspension were used and optimally 5.9±0.2% of the available radioactivity became bound specifically. The mean of two independent experiments is shown. The variation on the mean between the two experiments was less than 10%.

with 0.5 mg of collagenase/ml, use of 1.0, 1.5 and 2.0 mg of collagenase/ml in the isolation caused the binding to decline to 70±10%, 50±5% and 46±2% ($n = 3$) respectively. Such a loss of surface reactivity is compatible with previous observations (Cunningham & Robinson, 1969; Davies & Robinson, 1973) which suggested that collagenase treatment of whole adipose tissue caused an irreversible loss of extra-adipocyte lipoprotein lipase activity.

Fig. 3 shows the effect of nutritional status on chicken and guinea-pig anti-(rat lipoprotein lipase) binding to intact adipocytes. Thus animals in each nutritional state were studied in parallel, and sample of cells were incubated (Fig. 3*a*) with chicken anti-rat lipoprotein lipase) serum and subsequently with ^{125}I-labelled rabbit anti-(chicken IgG) antibodies or (Fig. 3*b*) with guinea-pig anti-(rat lipoprotein lipase) serum and subsequently with ^{125}I-labelled sheep anti-(guinea-pig IgG) antibodies. Fig. 3(*a*) shows that there was a significant difference between the binding observed when the cells of each type were compared directly. Cells from fed animals showed the highest binding. The binding declined on starvation to approx. 50% of fed values at 24 h and to 30% of fed values of 48 h. Similar experiments carried out with the guinea-pig anti-(lipoprotein lipase) serum showed (Fig. 3*b*) that in this case also the binding to adipocytes decreased as starvation progressed. Thus, with this detection system, the cells of 24-h- and 48 h-starved animals demonstrated approx. 75 and 60% respectively of the binding seen with the cells of fed animals. Although the overall pattern was similar for each antiserum, the apparent resolution between the cells of each source was less clearly defined when the guinea-pig anti-(rat lipoprotein lipase) serum was used.

From these immunodetection experiments it is clear that at least a proportion of adipocyte lipoprotein lipase molecules are associated with and externally disposed on the plasma membrane of the cells. The same disposition for the enzyme found with isolated plasma-membrane fractions can also be intimated from two lines of evidence. Firstly, when freshly prepared adipocyte plasma membranes and acetone/ether-dried powder preparations of the membranes were assayed for lipoprotein lipase activity, the activities measured were the same in a series of eight parallel preparations. Thus the lipoprotein lipase activity of adipocyte plasma-membrane vesicles was apparently fully patent (see also Chohan & Cryer, 1979). Secondly, when optimal concentrations (40 μg of protein) of freshly prepared adipocyte plasma membranes were incubated with chicken anti-(rat lipoprotein lipase) serum under the conditions described for whole cells (the recovery step in this case being by sedimentation at 10000 $g_{av.}$ for 2 min), significant second-antibody binding was detected [2156±186 c.p.m./100 μg of protein ($n = 3$) optimally]. The binding was related to antiserum concentration in three identical experiments in a fashion which was identical in pattern with that seen with whole adipocytes (Fig. 3).

General comments

The functional significance of the lipoprotein lipase activity detected at the adipocyte plasma membrane is at present only a matter of speculation, but other experiments (Al-Jafari & Cryer, 1986) show that the size of this pool of enzyme molecules alters in a concerted fashion in response to specific extracellular effectors. It would be reasonable to propose that the lipoprotein lipase at this site represented a pool of enzyme molecules which either may continue, via some as yet unknown transport system, en route to the endothelium, or may be subject to re-uptake by the adipocyte. The adipocyte is known to have both an active fluid-phase endocytotic capacity (Gibbs & Lienhard, 1984) and the capacity to bind and degrade exogenously added lipoprotein lipase efficiently (Friedman *et al.*, 1982).

The possible routes whereby enzyme at this plasma-membrane location may become associated with the surface of endothelial cells require further investigation. Although the glycosaminoglycan heparin has been shown to be effective in causing the cellular release of lipoprotein lipase (Cryer *et al.*, 1984*b*) in a variety of systems, the endogenous release of the enzyme from isolated adipocytes is usually undetectable (e.g. Cryer *et al.*, 1975; Vannier *et al.*, 1985), and no evidence is available as yet to indicate that extracellular heparin has any role in stimulating lipoprotein lipase release into the extra-adipocyte space *in vivo*. The possibility that, in the absence of heparin, lipoprotein lipase is not transported to the endothelium in the extracellular fluid, arises when the adipocyte-plasma-membrane pool of lipoprotein lipase molecules described here is considered alongside the ultrastructural observations by Scow and co-workers (Blanchette-Mackie & Scow, 1981*a*,*b*). These latter authors have demonstrated not only membrane continuities between adjacent adipocytes but also the existence of extremely intimate cellular contacts between adipocytes and endothelial cells. The possibility that lipoprotein lipase may be transferred along such intracellular continuities, in a way similar to that postulated by Scow *et al.* (1980) for the fatty acid products of lipoprotein triacylglycerol breakdown at the endothelium, deserves consideration in future experimental approaches.

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