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Changes with starvation in the rat of the lipoprotein lipase activity and hydrolysis of triacylglycerols from triacylglycerol-rich lipoproteins in adipose tissue preparations

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Lipoprotein lipase activity was higher in fat-pad pieces than in isolated adipocytes from the same fed rats, whereas hydrolysis of triacylglycerols from triacylglycerol-rich lipoproteins was similar in the two preparations when incubated either in basal conditions or in the presence of heparin. In both preparations there was a similar release of lipoprotein lipase activity into the medium during basal incubation, enhanced by the presence of heparin. In fat-pad pieces, but not in isolated adipocytes, incubation with heparin produced a decrease in the lipoprotein lipase activity measured in the tissue preparation. In fat-pad pieces from 24h-starved rats, lipoprotein lipase activity was the same as in isolated adipocytes from the same animals and incubation with heparin did not affect the appearance of lipoprotein lipase in the medium or the utilization of triacylglycerols from triacylglycerol-rich lipoproteins. These results support the following conclusions. (1) The effectiveness of lipoprotein lipase in adipose tissue preparations *in vitro* depends more on its availability to the substrate than on its total activity. (2) Heparin acts on adipose tissue preparations from fed animals both by enhancing the release of pre-existing extracellular enzyme (which is absent in isolated adipocytes) and by enhancing the transfer outside the cells of the intracellular (and mainly undetectable) enzyme that is activated in the secretion process. (3) In adipose tissue from starved animals there is not only a decrease in the active extracellular form of lipoprotein lipase activity but also a reduction in the intracellular (and mainly undetectable) pool of the enzyme.

The activity of lipoprotein lipase in adipose tissue changes with the nutritional status and has been correlated with parallel alterations in the uptake of triacylglycerol fatty acids by the tissue (Bezman *et al.*, 1962; Garfinkel *et al.*, 1967; Austin & Nestel, 1968; Cryer *et al.*, 1976). Although the importance of these changes in lipoprotein lipase activity measured in isolated adipocytes has been emphasized (Cryer *et al.*, 1976) it has recently been shown that differences in the activity of the enzyme in adipocytes from fed and starved rats decreases when adipose tissue has been pre-treated with collagenase for adipocyte isolation (Vanhove *et al.*, 1978). This finding suggests that the enzyme inactivated by collagenase is extracellular and constitutes most of the augmented activity in fat-pads from fed animals as compared with starved ones. Heparin is known to release lipoprotein lipase activity into the medium from incubated adipose tissue pieces and isolated fat-cells (Hollenberg, 1959; Stewart & Schotz, 1974). The present work was performed to determine whether heparin affected the release of the enzyme differently in preparations from fed and 24h-starved rats. As we recently reported significant differences in the utilization of prelabelled triacylglycerol-rich lipoproteins *in vitro* by epididymal fat-pad pieces and isolated adipocytes from fed rats (Lasunción & Herrera, 1981), the present study was extended to measure the hydrolysis *in vitro* of prelabelled triacylglycerol-rich lipoproteins and the fatty acid uptake by fat-pad pieces and isolated adipocytes from fed and 24h-starved rats incubated in the presence and in the absence of heparin, to determine the physiological role of changes in lipoprotein lipase activity in adipose tissue with starvation.

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Materials and methods

Labelled triacylglycerol-rich lipoproteins from rats were obtained as previously described (Lasunción & Herrera, 1980) by intravenously injecting

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adult Wistar female rats (164–185g body wt.) with 60μCi of sodium [9,10(n)-^{3}H]palmitate (sp. radioactivity 230Ci/mol; The Radiochemical Centre, Amersham, Bucks., U.K.). Animals were killed 30min later and triacylglycerol-rich lipoproteins were purified from plasma by ultracentrifugation and dialysis (Lasunción & Herrera, 1980). The recovered lipoproteins contained both chylomicrons and very-low-density lipoproteins as determined by electron microscopy (Lasunción *et al.*, 1981). Portions of these triacylglycerol-rich lipoproteins were used for lipid extraction (Folch *et al.*, 1957; Dominguez & Herrera, 1976). With this procedure over 88% of ^{3}H present in these lipoproteins was in the esterified fatty acids. Other portions (0.5ml from 0.4ml of initial plasma) of these prelabelled lipoproteins were incubated in a final volume of 1.25ml of Krebs–Ringer bicarbonate buffer, pH7.4, with a suitable salt content (Umbreit *et al.*, 1964) in the presence of glucose (4mM), 0.8% bovine albumin purified by the method of Chen (1967) and serum (5μl/vial) from 24h-starved rat. The incubation vials also contained either 20–25mg of fat-pad pieces or isolated adipocytes always from the lumbar fat-pads of the same rat for each pair of incubations. Fed donor rats (160–180g) or 24h-starved female Wistar rats were killed by cervical fracture. Adipocytes were isolated by a modification (Bellido & Herrera, 1978) of the method of Rodbell (1964) in the presence of ovomucoid trypsin inhibitor (2mg/ml; Sigma Chemical Co., St. Louis, MO, U.S.A.) and crude collagenase (2mg/ml; Worthington Biochemical Corp., Freehold, NJ, U.S.A.). The amount of protein measured (Wang & Smith, 1975) in fat-pad pieces and isolated adipocytes in each vial was always similar (207 ± 16 or $159 \pm 29\mu$g of protein/vial respectively). Incubations were performed for 120min at 37°C in sealed siliconized glass vials gassed for 5min with O_2/CO_2 (19:1) in a Dubnoff shaking incubator at 100cycles/min. Incubations were terminated by centrifuging tissue preparations and media at 200rev./min for 2min at room temperature in plastic syringes sealed at the tip. Media were allowed to elute for processing while tissue preparations were thoroughly washed with plain Krebs–Ringer bicarbonate buffer. Tissue preparations and portions of the media were placed in chloroform/methanol (2:1, v/v) for lipid extraction (Folch *et al.*, 1957) and fractionation (Kerpel *et al.*, 1961; Dominguez & Herrera, 1976). In the fractions containing acylglycerols, phospholipids were eliminated with activated silicic acid in chloroform medium. Portions of lipid fractions were counted for radioactivity in a 2,5-diphenyloxazole/1,4-bis-(5-phenyloxazol-2-yl)benzene/xylene/Triton X-100-based scintillation cocktail. Radioactivity values were always adjusted to 1×10^4 d.p.m. for the ^{3}H contained in each vial before incubations. For lipoprotein lipase determinations, pieces of lumbar fat-pads (80–100mg, corresponding to $1366 \pm 30\mu$g of protein) or adipocytes ($1289 \pm 75\mu$g of protein), isolated from the same tissue as described above, were incubated in 5ml of Krebs–Ringer bicarbonate buffer, pH7.4, containing 4mM-glucose, 0.8% purified bovine albumin and serum from 24h-starved rats (20μl/vial). Incubations were performed as described above for 0, 30 or 120min, after which media and tissue preparations were separated by slow centrifugation, as also described above. Tissue preparations were homogenized in 2ml of 0.2M-Tris/HCl buffer, pH8.2, and this homogenate as well as portions of media were used to prepare acetone/diethyl ether powders in the presence of 50μl of heated (60°C for 10min) rat plasma (Henson & Schotz, 1975). The defatted preparations were dried under N_2 and dissolved in 1ml of cold aq. 0.05M-NH_3/NH_4Cl buffer, pH8.1. The lipoprotein lipase substrate emulsion (Nilsson-Ehle & Schotz, 1976; Corey & Zilversmit, 1977) was prepared by taking 69mg of trioleoylglycerol, 3.3mg of egg phosphatidylcholine and 25μCi of tri[1-^{14}C]oleoylglycerol to dryness under N_2. The dried lipids were emulsified in 5ml of glycerol by sonication in an MSE sonifier (set at 12μm for 1min; five times). The lipoprotein lipase activity assay was performed (Llobera *et al.*, 1979) by incubating 200μl of the aq. NH_3/NH_4Cl sample suspension for 30min at 37°C in the presence of 100μl of a reaction mixture containing 6% bovine serum albumin dissolved in 0.2M-Tris/HCl, pH8.2, made up in 0.15M-NaCl/substrate emulsion/heated (60°C for 10min) rat plasma (2:2:1, by vol.). Incubations were terminated by the addition of 3.5ml of methanol/chloroform/heptane (141/125/100, by vol.) and ^{14}C-labelled unesterified fatty acids were extracted from the upper phase for counting after the addition of 1ml of 0.1M-K_2CO_3/KBO_3, pH10.5 (Belfrage & Vaughan, 1969; Nilsson-Ehle *et al.*, 1972). Blank assay tubes without enzyme were always processed in parallel to make proper corrections. Enzyme activity was calculated as the amount of hydrolysed acylglycerols and expressed as pkat. Proteins were measured by the method of Wang & Smith (1975). Statistical analysis of the data was done by using Student's *t* test, *t* paired test and ANOVA (Snedecor, 1956).

Results

As shown in Fig. 1(*a*), there was greater lipoprotein lipase activity release to the medium during incubation by adipocytes from fed than from 24h-starved rats ($P < 0.01$, when all values were integrated). When the medium was supplemented with heparin (2.4i.u./ml), there was a significant increase in the lipoprotein lipase activity in the medium using adipocytes from fed but not from

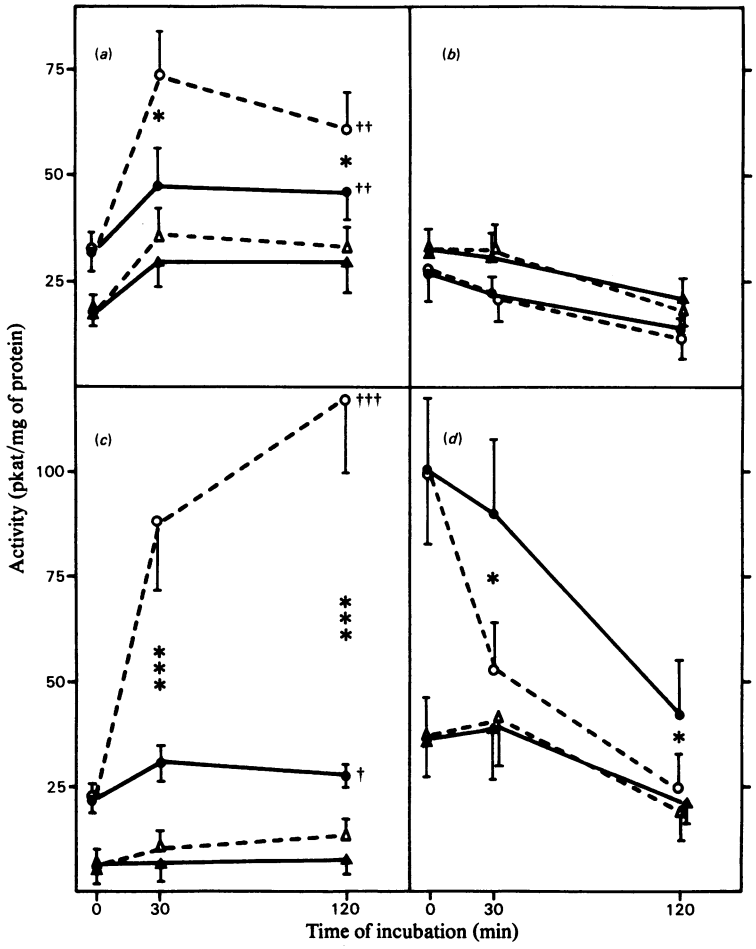

Fig. 1. *Lipoprotein lipase activity in the media and tissue preparations of isolated adipocytes (a and b) and fat-pad pieces (c and d) from lumbar adipose tissue of fed and 24 h-starved rats incubated in the absence or the presence of heparin.* Lipoprotein lipase activity was measured as indicated in the Materials and methods section in the media (*a*) or tissue preparation of isolated adipocytes (*b*) and the media (*c*) and tissue preparation of fat-pad pieces (*d*) from fed (●) or 24 h-starved rats (▲) incubated in the absence or in the presence of heparin (2.4 iu/ml) (○, fed; △, 24 h-starved rats). Statistical significances (by the *t* paired test) between values without or with heparin are shown by asterisks (*, $P < 0.05$; ***, $P < 0.001$), whereas those between values from fed and 24 h-starved animals were calculated by the ANOVA test for all the incubation times (††, $P < 0.01$; †††, $P < 0.001$).

24 h-starved animals (Fig. 1*a*). Lipoprotein lipase activity in the cells decreased with incubation time but there were no differences between values in fed versus 24 h-starved animals and no changes were produced with heparin in the incubation medium (Fig. 1*b*). To relate these changes in lipoprotein lipase activity to the actual capacity to hydrolyse triacylglycerol-rich lipoproteins, isolated adipocytes from both fed and 24 h-starved rats were incubated for 120 min in the presence of rat triacylglycerol-rich lipoproteins labelled with ^3H in their esterified fatty acids. The hydrolysis of triacylglycerols was estimated by the differences between those present at the end of incubation and those present at its onset. As shown in Table 1, the hydrolysis of [^3H]triacylglycerol was markedly greater when labelled lipoproteins were incubated in the presence of adipocytes from fed than from starved rats. In the presence of heparin, there was an increase in the hydrolysis of [^3H]triacylglycerols when incubations were made with adipocytes from fed animals, whereas heparin did not affect this parameter when adipocytes were from starved rats (Table 1). The uptake of ^3H-labelled fatty acids was measured as the appearance of ^3H-labelled lipids in the adipocytes at the end of incubation, and this value was also higher with adipocytes from fed than from starved rats, although the difference was only

Table 1. *Utilization of triacylglycerols from triacylglycerol-rich lipoproteins by isolated adipocytes pieces from fed and 24 h-starved rats*

Adipocytes isolated with collagenase and fat-pad pieces from lumbar adipose tissue of fed and 24 h-starved rats were incubated for 120 min in Krebs–Ringer bicarbonate buffer, pH 7.4, in the presence of glucose (4 mM). 0.8% bovine albumin, rat serum (5 μl/vial) and triacylglycerol-rich lipoproteins from rat labelled with ^{3}H in their esterified fatty acids. In half of the incubation vials from each animal the medium was also supplemented with heparin (2.4 i.u./ml). Values correspond to means ± S.E.M. of d.p.m. per 100 μg of tissue protein and were adjusted to 1×10^4 d.p.m. for the ^{3}H contained in each vial before the incubations. Statistical comparisons between values for heparin and basal values are shown by the *P* values, which correspond to the *t* paired test, those between starved and fed animals by asterisks indicating $P < 0.05$ and those between fat-pad pieces and their corresponding group in adipocytes by: †, $P < 0.05$; †††, $P < 0.001$. n = five to 11 rats/group. N.S., not significant.

Utilization of [^{3}H]triacylglycerol	Fed			Starved		
	Basal	Heparin	*P*	Basal	Heparin	*P*
Isolated adipocytes						
Hydrolysis	1449 ± 161	2495 ± 394	<0.001	657 ± 99*	722 ± 178*	N.S.
Uptake	959 ± 253	1045 ± 228	N.S.	441 ± 31	461 ± 46*	N.S.
Fat-pad pieces						
Hydrolysis	1240 ± 195	1700 ± 131	<0.05	360 ± 106*	511 ± 101*	N.S.
Uptake	308 ± 118†	399 ± 98†	N.S.	125 ± 22†††	152 ± 8*,†††	N.S.

significant when incubations were made in the presence of heparin (Table 1). As pretreatment with collagenase for the isolation of adipocytes may be responsible for some of the results described in other experimental conditions (Vanhove *et al.*, 1978), the study was also performed with fat-pad pieces from the same animals as the isolated adipocytes. As shown in Fig. 1(*c*), lipoprotein lipase activity present in the incubation medium of fat-pad pieces at different incubation times was also lower than in adipocytes from the same animals (Fig. 1*a*) ($P <$ 0.05 for the values from both fed and 24 h-starved animals, when all values were integrated). Medium lipoprotein lipase activity of fat-pad pieces was significantly higher in fed than in 24 h-starved rats, whereas the addition of heparin produced a marked increase in the release of lipoprotein lipase activity into the medium only in fat-pad pieces from fed rats and had no effect when they were from starved ones (Fig. 1*c*). Lipoprotein lipase activity at zero incubation time was significantly greater in fat-pad pieces from fed than from starved rats (Fig. 1*d*) ($P < 0.05$) but these differences disappeared with incubation, owing to the progressive decrease in activity detected during incubation in the pieces from fed rats, contrasting with no change in those from starved ones. The presence of heparin in the incubation medium enhanced the disappearance of lipoprotein lipase in the pieces from fed rats without affecting those from starved rats (Fig. 1*d*). Concerning [^{3}H]triacylglycerol-rich lipoprotein utilization (Table 1), fat-pad pieces from fed animals hydrolysed more acylglycerols than those from starved rats during 120 min of incubation *in vitro*, and this difference was even greater in the presence of heparin in the incubation medium as it increased hydrolysis with fat-pad pieces from fed but not with those from starved rats (Table 1). The uptake of ^{3}H-labelled fatty acids by fat-pad pieces was greater in those from fed than from starved animals, although this difference was only significant with heparin in the medium, in spite of the fact that heparin itself affected this parameter only slightly and not significantly (Table 1).

Discussion

The present study shows that when rat adipose tissue was incubated *in vitro*, lipoprotein lipase activity was lower in isolated adipocytes than in fat-pad pieces, whereas enzyme activity release into the medium was greater in isolated adipocytes. Although it is known that lipoprotein lipase activity is unstable under certain conditions (Cunninghan & Robinson, 1969; Stewart & Schotz, 1971) and the stability of the enzyme bound to the tissue may differ from that of the free enzyme (Davies & Robinson, 1973), those observed differences may be related to the greater hydrolysis and uptake of triacylglycerols from triacylglycerol-rich lipoproteins found when incubations were performed with isolated adipocytes rather than fat-pad pieces from the same animals. These findings suggest that hydrolysis of triacylglycerols *in vitro* from triacylglycerol-rich lipoproteins is a function of the lipoprotein lipase released into the medium and therefore accessible to its substrate, and that it is not a function of the amount present intracellularly. Lipoprotein lipase activity at the onset of incubation was higher in fat-pad pieces from fed than from starved animals, in agreement with other reports (Bezman *et al.*, 1962; Garfinkel *et al.*, 1967; Austin & Nestel, 1968; Cryer

et al., 1976), and corresponds to the greater release of the enzyme into the medium and the greater hydrolysis and uptake of triacylglycerols from triacylglycerol-rich lipoproteins in the fat-pad pieces from fed than from starved rats. Lipoprotein lipase activity was, however, the same in isolated adipocytes from fed and from starved rats, although the former produced greater hydrolysis of triacylglycerols from triacylglycerol-rich lipoproteins, corresponding again with a parallel change in the actual release of the enzyme into the medium.

In the presence of heparin, there was an enhanced release of lipoprotein lipase into the medium in both fat-pad pieces and isolated adipocytes from fed rats, in agreement with other reports (Hollenberg, 1959; Stewart & Schotz, 1974), corresponding to the increase in hydrolysis and utilization of triacylglycerols from triacylglycerol-rich lipoproteins seen in the present results. These heparin effects, enhancing both the secretion of enzyme and hydrolysis of triacylglycerols, were not directly correlated for fat-pad pieces and isolated cells from fed rats, suggesting either that differences exist in the stability of the enzyme in these preparations or that some other unknown factor affects the rate of hydrolysis of triacylglycerols from triacylglycerol-rich lipoprotein during the incubation. In the preparation of fat-pad pieces, the rise in lipoprotein lipase activity in the medium was followed by its decreased activity in the tissue, probably due to two effects produced by heparin: the release of pre-existing extracellular enzyme and the enhanced transfer outside the cells of the intracellular undetectable enzyme, which is only manifest in the secretion process. This latter action is substantiated by the data of isolated adipocytes in which increased lipoprotein lipase activity in the incubation medium, produced by heparin, was not counterbalanced by a decrease in intracellular enzyme activity, as also reported by others (Pokrajac *et al.*, 1967; Stewart & Schotz, 1971). In starvation, the active extracellular form of lipoprotein lipase is known to be markedly decreased (Nilsson-Ehle *et al.*, 1976). As our results showed that heparin does not affect the release of lipoprotein lipase activity into the medium in either fat-pad pieces or isolated adipocytes from starved animals, it is proposed that, with food deprivation, there is also a reduction in the intracellular undetectable pool of the enzyme.

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