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Lipoprotein Lipase of Sheep and Rat Adipose Tissues

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Abstract

Lipoprotein lipase preparations were obtained by aqueous extraction of various tissues from sheep and rats. Preparations having high activity towards serum-activated triolein emulsions were obtained from actively growing sheep, provided that the tissue was maintained at 37°C throughout the extraction procedure. Activity of lipoprotein lipase from sheep adipose tissue, like that from the rat, was dependent upon the presence of serum in the reaction mixture, and was optimal at pH 8–9. Inhibition of sheep adipose tissue preparations by protamine sulfate (1 mg/ml) and 0·6 M NaCl was similar to that found for rat adipose tissue preparations. The affinity of the lipoprotein lipase preparation for the activated substrate from sheep and rat adipose tissue was, however, markedly different; sheep preparations being activated at much lower substrate concentrations (K_m 0·43 mM) than rat preparations (K_m >5 mM). These findings, confirmed with acetone–ether preparations of lipoprotein lipase, indicate that the enzyme from sheep adipose tissue has a greater potential to remove triacylglycerol from the plasma. This could result in a greater deposition of fat in the tissue.

Extra keywords: substrate saturation

Introduction

In ruminants the fatty acyl components of triacylglycerol in the adipocyte lipid stores may come from *de novo* synthesis within the adipocyte, or from microbial formation in the rumen or from the diet or from both of these sources. The uptake of triacylglycerol fatty acids by various tissues from circulating chylomicrons and very low density lipoproteins of the plasma is brought about via the action of lipoprotein lipase (triacylglycero-protein acylhydrolase, EC 3.1.1.34). Lipoprotein lipase hydrolyses the lipid at the lumenal surface of the capillary, before the fatty acyl components are transferred across the endothelial cells and into adipose tissue (and other extra hepatic tissues). As lipoprotein lipase is believed to be rate-limiting in this process (see Robinson 1970) its activity indicates the ability of a tissue to remove triacylglycerol from the plasma for storage or utilization.

Adipose tissue lipoprotein lipase of ruminants has been investigated by a number of workers. Their findings are summarized by Vernon (1981) who concluded that the enzyme is similar to that from other species. However, apart from the work of Chilliard *et al.* (1977) on omental tissue from the goat, most studies have investigated activities in various tissues in relation to nutritional or physiological states of animals (Rao *et al.* 1973; Haugebak *et al.* 1974; Cryer and Jones 1979). To our

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knowledge no studies have been made of the characteristics of the enzyme preparations from adipose tissue of sheep. Recently, we have found that incorporation of long-chain fatty acids, rather than *de novo* synthesis from acetate, could account for the major proportion of triacylglycerol accumulation in sheep adipose tissues (Thornton *et al.* 1982). The present work was therefore aimed at determining the properties of lipoprotein lipase from sheep adipose tissue and comparing them with the properties of similar preparations from the adipose tissue of rats.

Materials and Methods

Experimental Animals

The sheep were Border Leicester–Merino cross wethers weighing between 30 and 40 kg live weight. They were fed *ad libitum* a diet of pellets (Red Comb cattle feed lot pellets, 7230, Gillespie Bros. Pty. Ltd., Wacol, Qld) and lucerne chaff (1 : 1 w/w) and weighed each week to check their rate of weight gain. Sprague–Dawley rats (150–200 g live weight) were fed *ad libitum* a diet of formulated pellets (Expanded Rat and Mouse Cubes, Feed Supplies, Hemmant, Qld).

Slaughter and Tissue Sampling

Both the sheep and the rats were stunned prior to severing the large blood vessels of the neck. Immediately after slaughter samples of omental, perirenal, subcutaneous and brisket adipose tissue were removed from the sheep. The skeletal muscles M. semitendinosus, M. semimembranosus and M. soleus were also removed. Similarly samples of omental, and epididymal adipose tissue were removed from the rats. All tissue samples were placed in 0·73% (w/v) NaCl at 37°C and transferred to the laboratory for immediate extraction of lipoprotein lipase.

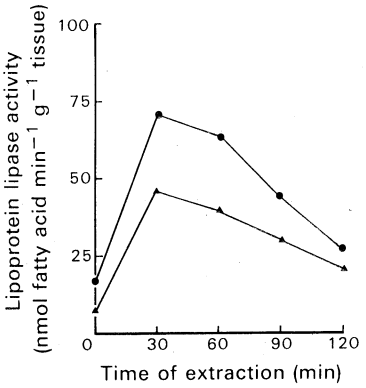

Fig. 1. Effect of extraction time at 37°C on lipoprotein lipase activity of the filtrate. ● Extract from sheep omental tissue. ▲ Extract from sheep brisket adipose tissue.

Lipoprotein Lipase Preparations

Aqueous homogenate method

In all, 1 g of adipose tissue was homogenized at 37°C in 4·5 ml Krebs–Ringer–bicarbonate, pH 7·4, 0·5 ml serum (from the same species) and 0·25 ml heparin (final concentration 50 μg/ml) using a Marsh–Snow homogenizer (Marsh and Snow 1950). Omission of heparin from the medium reduced the activity of the extract by more than 80%. The homogenate was generally incubated at 37°C for 60 min and then filtered (Whatman No. 1). The filtrate was used as a source of lipoprotein lipase and was stored in ice. In some experiments the homogenate was incubated for various times up to 120 min (Fig. 1). It can be seen that only a small amount of lipoprotein lipase activity was released into the filtrate by homogenization (0 min) but extraction of the homogenized tissue at 37°C for 30–60 min resulted in the maximum observed activity in the filtrate. Longer extraction times resulted in a rapid decline in activity (Cherkes and Gordon 1959). Fig. 1 also shows similar extraction characteristics for both omental and brisket adipose tissues even though they

represent extremes of adipose tissue types (that is, high fatty acid saturation compared with relatively high fatty acid unsaturation.

The triacylglycerol concentration of aqueous homogenate preparations of sheep omental tissue was low ($0 \cdot 17 \pm 0 \cdot 02$ mM, $n = 4$) and did not significantly effect the overall concentration of triacylglycerol in the reaction mixture.

Acetone–ether extraction method

Again, 1 g adipose tissue was cut with scissors and homogenized in 0·75 ml of 0·1 M Tris-HCl, pH 7·4, and 0·25 ml serum at 37°C using a Duall size 22 (Kontes, N. J.) all-glass tissue grinder (Tan *et al.* 1977). For rat preparations the homogenate was extracted four times with 40 ml ice-cold acetone and then twice with 40 ml ice-cold diethyl ether. For sheep preparations it was necessary to repeat the acetone extraction at least six times and the ether extraction four times in order to ensure a properly defatted sample. The defatted sample was dried under nitrogen and then homogenized in 7·0 ml ice-cold 0·05 M NH_4OH–NH_4Cl buffer, pH 8·1, containing 250 μg heparin. The suspension was left in ice for 30 min and then centrifuged at 1000 *g* for 20 min. The supernatant was removed and used as a source of lipoprotein lipase.

The aqueous homogenate and the acetone–ether preparations were generally used within 2 h of preparation. Occasionally they were frozen and stored in liquid nitrogen where they could be kept for several months without loss of activity.

Assay of Lipoprotein Lipase Activity

The determination of lipoprotein lipase activity was carried out essentially as described by Nilsson-Ehle and Schotz (1976). The standard reaction mixture (0·2 ml) contained 67 mM Tris-HCl, pH 8·1, 1·0% (w/v) bovine serum albumin, 8·3% (v/v) rat or sheep serum, 5·65 mM [^{3}H]triolein ($\approx$37 kBq ^{3}H) and an appropriate volume of enzyme preparation, generally 0·1 ml. Samples were incubated at 37°C for 30–90 min depending on the activity of the preparation. The reaction was linear for at least 90 min and in no experiment was more than 8% of the total triolein hydrolysed during the incubation. Omission of serum from the reaction mixture reduced the activity by at least 80%. The reaction was optimal at pH 8–9. All assays were performed in triplicate and tubes containing buffer instead of tissue preparation served as controls. The [^{3}H]oleic acid released during the reaction was isolated by solvent partition (Belfrage and Vaughan 1969) as modified by Nilsson-Ehle and Schotz (1976). The radioactivity of an aliquot of the upper phase was determined by liquid scintillation counting.

Reagents

Anhydrous emulsions of [^{3}H]triolein with glycerol and phosphatidylcholine were prepared as described by Nilsson-Ehle and Schotz (1976) and stored at 4°C until used. In one experiment a micellar solution of [^{3}H]triolein was prepared by subjecting 80 mg [^{3}H]triolein to sonic disruption with 2·5 mg Triton X-100 in 1·33 ml water. Tri-[9,10-^{3}H]oleylglycerol was purchased from Amersham Australia Pty. Ltd. Unlabelled triolein was purchased from Calbiochem-Behring Corp., California. Each batch of bovine serum albumin (fraction V, Sigma Chemical Co., Missouri), was tested for lipase activity. Only albumin free from lipase activity was used. Heparin (158 USP J–A units/mg) was purchased from Sigma Chemical Co., Missouri. Protamine sulfate (A grade) was purchased from Calbiochem-Behring Corp., California.

Serum

Blood was obtained from the jugular vein of sheep or by cardiac puncture of lightly anaesthetized rats. Serum was obtained from these samples by centrifugation (1800 *g* for 20 min). Triacylglycerol content of serum was determined by measuring the glycerol content following hydrolysis with ethanolic KOH (Eggstein and Kuhlman 1974).

Statistical Analyses

Results were compared using analyses of variance or *t*-tests.

Results

In order to ascertain that the triacylglycerol lipase activity present in these aqueous extracts resulted from lipoprotein lipase activity the reactions were performed in the presence of known inhibitors of lipoprotein lipase. Pre-incubation of the lipoprotein lipase preparations from both sheep and rat omental adipose tissue with protamine sulfate concentrations as low as 0·1 mg/ml for 15 min at 24°C caused a marked inhibition of activity (Fig. 2*a*). At 24°C the sheep preparation was not as affected as that from the rat, but when pre-incubation was done at 37°C more than 90% inhibition was observed at 0·2 mg/ml for each preparation. The presence of NaCl in the reaction mixture was almost equally effective in inhibiting both sheep and rat preparations (Fig. 2*b*).

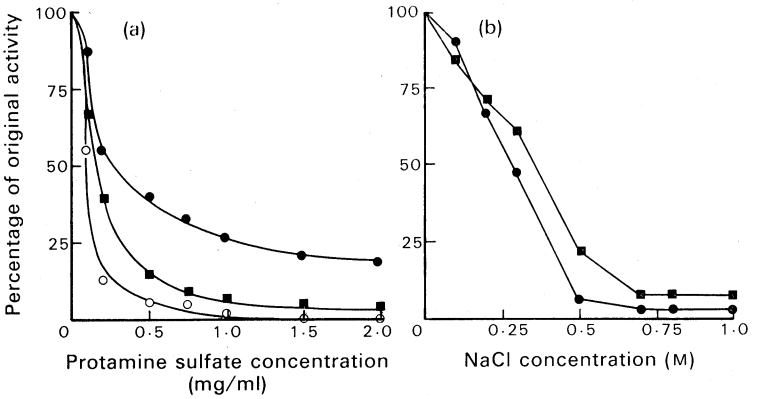

Fig. 2. Comparison of sheep and rat lipoprotein lipase from omental tissue. Inhibition by (*a*) protamine sulfate and (*b*) NaCl. Aqueous lipoprotein lipase extract was pre-incubated with inhibitor for 15 min at 24°C. ● Aqueous extract from sheep omental adipose tissue. ■ Extract from rat omental adipose tissue. ○ Extract from sheep omental adipose tissue pre-incubated with protamine sulfate for 15 min at 37°C.

Mean lipoprotein lipase activities (expressed as nmol oleic acid formed per minute per gram of tissue) of various tissues of the growing sheep (approx. 100 g/day) and of sheep fasted for 4 days are given in the following tabulation:

	No.	Omental	Perirenal	Subcutaneous	L.S.D.
Fed sheep	32	104·9	85·3	108·8	6·4
Fasted sheep	3	7·4	9·2	4·8	8·1

For a fed sheep activity was greater ($P < 0·05$) in omental and subcutaneous adipose tissues than in perirenal adipose tissue. Fasting sheep for 4 days caused a marked reduction in lipoprotein lipase activity of adipose tissue samples, which is in keeping with results reported for other species (Wing and Robinson 1968) and for sheep tissues by Haugebak *et al.* (1974). All adipose sites of fed sheep had considerably higher activity than that of the skeletal muscle preparations, the mean activities of M. semitendinosus, M. semimembranosus and M. soleus preparations of 11 sheep being 14·0, 10·1 and 6·3 respectively. The activities of all skeletal muscles tested were significantly different from each other (L.S.D. = 1·5, $P = 0·05$).

The affinity of the enzyme preparations for the substrate was investigated over the concentration range 0·1–6 mM triolein. Lipoprotein lipase preparations exhibited

Michaelis–Menten-type kinetics, maximum activity for the sheep adipose tissue preparations being observed at 2–3 mM triolein (Fig. 3*a*) and having an apparent K_m of 0·43 mM. The rat omental adipose tissue preparations approached maximum activity at triolein concentrations of 6 mM or higher (Fig. 3*a*). Preparations from rat adipose tissue showed considerable variability in affinity for triolein but the apparent K_m was always at least 10-fold greater than that calculated for sheep. Lipoprotein lipase preparations were also made from rat epididymal adipose tissue and these too exhibited a low affinity for triolein substrate (data not shown).

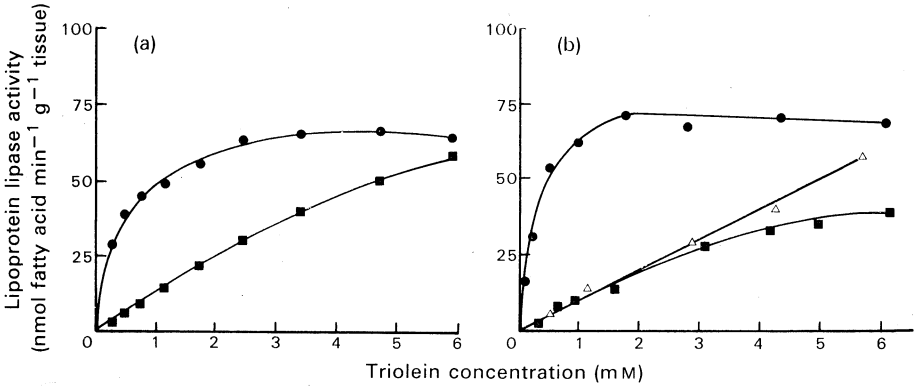

Fig. 3. Effect of substrate concentration on the activity of lipoprotein lipase prepared by (*a*) the aqueous homogenate method or (*b*) the acetone–ether extraction method. ● Extract from sheep omental adipose tissue. ■ Extract from rat omental tissue. △ Extract from rat omental tissue incubated with triolein–Triton X-100 as substrate.

In view of the observed differences in substrate affinity between the rat and sheep lipoprotein lipase preparations, acetone–ether preparations were made to check that the findings were not the result of the aqueous method of extraction. Generally, the acetone–ether preparations give higher activities than the aqueous preparations

Table 1. Kinetic properties of lipoprotein lipase preparations of sheep and rat omental adipose tissue

Values expressed are means ± s.e.m. and the number of preparations are shown in parentheses

Type of preparation	Sheep		Rat	
	Apparent K_m (mM)	V_{max} (nmol fatty acid min^{-1} g^{-1} tissue)	Apparent K_m (mM)	V_{max} (nmol fatty acid min^{-1} g^{-1} tissue)
Aqueous homogenate	0·43 ± 0·08	120 ± 14 (5)	5·2 ± 1·1	74 ± 17 (4)
Acetone–ether extraction	0·37 ± 0·08	148 ± 15 (6)	5·9 ± 1·7	258 ± 33 (9)

(Table 1), presumably because of the greater extraction of lipoprotein lipase from the fat-free tissue. However, the differences between the sheep and rat preparations with respect to substrate affinity were essentially as for the aqueous preparations (Fig. 3*b* and Table 1). When Triton X-100 was used in place of phosphatidylcholine

and glycerol for preparation of triolein substrate, the rat lipoprotein lipase preparation again exhibited low affinity for the triolein (Fig. 3*b*). That is, presentation of triolein either as an emulsion or as a micellar solution did not markedly alter the characteristics of the enzyme.

Serum triacylglycerol concentrations in sheep and rats fed *ad libitum* were $0 \cdot 38 \pm 0 \cdot 03$ mM and $1 \cdot 56 \pm 0 \cdot 12$ mM respectively.

Discussion

Various aqueous homogenate methods have been used for isolation of lipoprotein lipase (Borensztajn *et al.* 1970; Chilliard *et al.* 1977; Lithell and Boberg 1978; Cryer and Jones 1981). However, the resulting preparations generally exhibited a lower activity compared with preparations of acetone–ether extracts. With sheep adipose tissue, very low activity was recorded unless the tissue sample was maintained at 37°C throughout the extraction procedure. This was presumably because of the high saturated fatty acyl content which made the tissue extremely difficult to handle at lower temperatures (<35°C). However, provided this precaution is taken the aqueous extraction method offers certain advantages. Firstly, the method is straightforward and relatively fast and is therefore suitable for processing large numbers of tissue samples and, secondly, it overcomes the difficulty experienced in adequately defatting sheep adipose tissue when acetone–ether extractions are performed.

Unless sheep were actively growing only very low lipoprotein lipase activity was measured. Values for sheep perirenal and subcutaneous adipose lipoprotein lipase reported by Cryer and Jones (1979) are considerably lower than those reported here and possibly reflect the underfed state of the animals presented for slaughter at abattoirs.

Lipoprotein lipase extracts from aqueous homogenates of sheep adipose tissue exhibited characteristic properties. Any contribution to total triacylglycerol lipase activity from endogenous hormone-sensitive lipase is likely to be insignificant, since not only was lipolysis stimulated markedly by serum, but also was strongly inhibited by both protamine sulfate and NaCl. Further, lipase activity was extremely low in fasting animals. In all respects, other than for substrate affinity the properties of sheep preparations were essentially similar to those obtained for the rat in the present studies and to those reported previously for the rat (Hietanen and Greenwood 1977). The lower affinity of the rat lipoprotein lipase preparations for the triolein is in keeping with the substrate concentration data reported by Nilsson-Ehle and Schotz (1976) using a similar substrate preparation, where 5–6 mM triolein was required to saturate the enzyme. This is somewhat higher than that observed when using other substrate systems (Chilliard *et al.* 1977; Hietanen and Greenwood 1977). Benzonana and Desnuelle (1965) have shown that the physical parameters of the triacylglycerol substrate influence the value of K_m, and it is likely therefore that the lower affinities measured here are the result of the physical nature of the triacylglycerol–phosphatidylcholine particles. As, in the work reported here, substrate emulsions prepared in an identical manner were compared directly, it can be assumed that any differences in activities and affinities resulting from such emulsions would be minimal.

The uptake of plasma triacylglycerol appears to be controlled by two mechanisms (see Nilsson-Ehle *et al.* 1980). The first is active regulation where large amounts of lipoprotein lipase are synthesized in response to hormonal activity resulting from a shift from the fasting to the fed state. With sheep where there is essentially a continuous influx of lipids into the plasma, synthesis of lipoprotein lipase is probably maintained at a low but constant rate. The second is control by substrate concentration. Functionally active lipoprotein lipase at the capillary wall is unlikely to be saturated by the concentration of circulating triacylglycerol. Therefore its activity will depend on the triacylglycerol concentration. If a particular enzyme has a higher affinity for the substrate than another then it would show greater activity and therefore be responsible for a greater removal of triacylglycerol from the circulation.

Verger and de Haas (1976) have pointed out the difficulties in interpreting data obtained from systems where the substrate is not water-soluble. Any differences in enzyme affinity could reflect either differences in affinity for the substrate or the interface. Whatever the reason, it is clear that at low substrate concentrations (physiological) lipoprotein lipase from sheep has higher activity for hydrolysing triolein than does lipoprotein lipase from rats. Therefore, in the live sheep where the circulating triacylglycerol concentration is about 0·38 mM, lipoprotein lipase of adipose tissue would have the potential to operate at about 50% of its maximal activity. As the serum triacylglycerol content remains fairly constant in sheep it is apparent that its hydrolysis will remain high provided of course that active regulation ensures that tissue synthesis of lipoprotein lipase is continued. In contrast, even in the fed rat the serum triacylglycerol concentration is much less than the apparent K_m and is lower again in the animal fasted overnight. Sheep adipose tissue, has therefore, a greater potential for removing triacylglycerol from the plasma than does rat adipose tissue.

Acknowledgment

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