British Journal of Nutrition (2001), **85**, 575–582
DOI: 10.1079/BJN2000328

The differential hepatic uptake of chylomicron remnants of different fatty acid composition is not mediated by hepatic lipase

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(Received 29 June 2000 – Revised 20 October 2000 – Accepted 21 November 2000)

The hypothesis that hepatic lipase mediates the differential hepatic uptake of chylomicron remnants of different fatty acid composition, demonstrated in previous work from our laboratory, was tested by investigating the effect of antibodies to the enzyme on the uptake of remnants enriched with saturated or *n*-3 polyunsaturated fatty acids by the perfused rat liver. After perfusion of rat livers with polyclonal antibodies to rat hepatic lipase raised in rabbits or with rabbit non-immune serum for 15 min, [^{3}H]oleate-labelled chylomicron remnants, derived from chylomicrons of rats given a bolus of either palm (rich in saturated fatty acids) oil or fish (rich in *n*-3 polyunsaturated fatty acids) oil, were added. The disappearance of radioactivity from the perfusate during 120 min and its recovery in the liver at the end of the experiments were then measured. Although the rabbit anti-rat hepatic lipase antiserum was shown to inhibit hepatic lipase activity by up to 90 %, and to bind extensively to hepatic sinusoidal surfaces when added to the perfusate, radioactivity from remnants of chylomicrons from rats given a bolus of fish oil as compared with palm oil disappeared from the perfusate and appeared in the liver more rapidly in the presence both the antiserum and the non-immune serum, and the differences between the uptake of the two types of remnants were similar. We conclude, therefore, that differential interaction with hepatic lipase is not responsible for the differences in the rate of removal of chylomicron remnants of different fatty acid composition from the blood.

Dietary fat: Chylomicron remnants: Hepatic lipase: Perfused rat liver

Current evidence suggests that chylomicron remnants, which carry fat and cholesterol of dietary origin from the intestine to the liver, contribute to the development of atherosclerosis and related cardiovascular diseases in man (Patsch *et al.* 1992; Mamo, 1995). Triacylglycerol-rich remnant lipoproteins are taken up by human monocyte–macrophages *in vitro* (van Lenten *et al.* 1985), and are able to convert them to foam cells (Goldstein *et al.* 1980; Mahley *et al.* 1980). More recently, work in our laboratory and others have shown that chylomicron remnants are taken up by the arterial wall (Grieve *et al.* 1998*a*,*b*; Proctor & Mamo, 1998), and this has been associated with endothelial dysfunction (Grieve *et al.* 1998*a*,*b*, 2000). Since the liver is mainly responsible for the removal of chylomicron remnants from the blood (Redgrave, 1983), the factors regulating their hepatic uptake determine the length of time the cardiovascular system is exposed to the particles, and thus play an important part in the development of atherosclerosis.

The type of fat in the diet is known to influence plasma lipid levels and consequently, the risk of atherosclerosis development. Early studies showed that saturated fatty acids increase plasma cholesterol concentrations in comparison with *n*-6 polyunsaturated fatty acids, and later work has established that monounsaturated and *n*-3 polyunsaturated fatty acids are also hypolipidaemic (Mattson & Grundy, 1985; Truswell, 1985; Harris, 1989). Previous work in our laboratory has shown that the fatty acid composition of chylomicron remnants is determined by the fat consumed in the diet (Lambert *et al.* 1996), and we have further demonstrated that the hepatic uptake and processing of the lipoproteins is influenced by the type of dietary fat from which they were derived (Bravo *et al.* 1995; Lambert *et al.* 1995). Cholesterol carried in chylomicron remnants was taken up from the blood by the liver more rapidly when the particles were enriched with *n*-3 polyunsaturated as compared with saturated fatty acids, and this pattern was consistent both in studies in the rat *in vivo* (Bravo *et al.* 1995), and with the isolated perfused rat liver (Lambert *et al.* 1995). These findings clearly indicate that changes in the fatty acid composition of chylomicron remnants alter their uptake by the liver.

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The mechanism(s) mediating the differential hepatic uptake of chylomicron remnants derived from different dietary fats are not known. One possible explanation is that the different types of chylomicron remnants interact differentially with hepatic lipase, an enzyme located on the surface of liver cells (Breedveld *et al.* 1997). There is good evidence to suggest that hepatic lipase promotes an essential step in the hepatic uptake of chylomicron remnants. Triacylglycerol-rich remnants accumulate in the blood in heritable hepatic lipase deficiency (Connelly *et al.* 1990); the rate of uptake of the lipoproteins is inhibited both by administration of antibodies to the enzyme and by its removal by heparin release (Daggy & Bensadoun, 1986; Sultan *et al.* 1990; Shafi *et al.* 1994), and stimulated by prior treatment of the particles with hepatic lipase *in vitro* (Boresztajn *et al.* 1988; Crawford & Borensztajn, 1999). Furthermore, studies in our laboratory have demonstrated that hepatic lipase hydrolyses chylomicron remnants derived from olive oil (enriched in monounsaturated fatty acids) less rapidly than those derived from corn oil (enriched in *n*-6 polyunsaturated fatty acids) *in vitro* (Botham *et al.* 1995), and this is consistent with the slower clearance of olive oil as compared with soyabean oil (rich in *n*-6 polyunsaturated fatty acids) emulsions in human subjects *in vivo*, a difference which has been shown to correlate to hepatic lipase activity (Brouwer *et al.* 1993). Brasaemle *et al.* (1993) have found that metabolism of chylomicron remnants by the enzyme increases the exposure of apolipoprotein E on the surface of the particles, suggesting that the catalytic activity of hepatic lipase is involved in the mechanism by which it promotes remnant uptake. Other studies, however, have indicated that the enzyme protein may also act as a ligand for binding of the particles to the surface of the liver cells (Diard *et al.* 1994; Amar *et al.* 1998). It has been proposed, therefore, that hepatic lipase together with apolipoprotein E, participates in the initial interaction between chylomicron remnants and liver cells, facilitating their subsequent endocytosis via the LDL receptor or the LDL receptor-related protein (Cooper, 1997; Rohlmann *et al.* 1998).

The aim of the present investigation was to determine whether hepatic lipase activity mediates the differential hepatic uptake and removal of chylomicron remnants originating from different dietary fats. Using the perfused rat liver as an experimental model, the hepatic uptake and removal of chylomicron remnants enriched in *n*-3 polyunsaturated or saturated fatty acids (derived from chylomicrons from rats given a bolus of fish or palm oil respectively), which were the types of remnants taken up the most and the least rapidly by the liver in our previous work (Bravo *et al.* 1995; Lambert *et al.* 1995), was compared in livers in which the hepatic lipase activity was inhibited by rabbit anti-rat hepatic lipase antiserum. If hepatic lipase mediates the differential uptake of remnants of different fatty acid composition, the rabbit anti-rat hepatic lipase antiserum would be expected to abolish the difference between the uptake of the two types of remnants by the perfused liver.

Materials and methods

Animals and materials

Male Wistar rats (350–400 g) and male New Zealand white rabbits (1·5–3·5 kg) were fed on a pelleted diet from Special Diet Services (Witham, Essex, UK) and housed at constant daylength (12 h) and temperature (25°C). Sodium pentobarbital, Lowry protein reagent, Freund's complete and incomplete adjuvant, heparin (from porcine intestinal mucosa), fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G and menhaden fish oil were obtained from Sigma Chemical Company (Poole, Dorset, UK). Palm oil was purchased from domestic suppliers. [^{3}H]Oleate and glycerol tri[9,10(*n*)-^{3}H]oleate were supplied by Amersham International (Amersham, Bucks., UK). All other chemicals were obtained from Merck (Dagenham, Essex, UK) or Fisher (Loughborough, Leics., UK).

Preparation of [^{3}H]oleate-labelled chylomicron remnants

Rats were fed a bolus of fish or palm oil (1 ml, containing α-tocopherol (4 mg/ml) as antioxidant) by stomach tube, and 1 h later, their thoracic ducts were cannulated using sodium pentobarbital (60 mg/kg) anaesthesia as described previously (Lambert *et al.* 1996). When the chyle was flowing satisfactorily, [^{3}H]oleate (18·5 MBq) neutralised with 0·1 M-KOH and emulsified with 10 mg sodium taurocholate was injected into the pyloric region of the stomach, and the abdominal incision was sutured. Chyle was collected for 15–18 h in the presence of ampicillin (0·1 mg/ml), layered under NaCl (9 g NaCl/l; 1·006 g/ml) in polyallomer tubes (6·5 ml) and ultracentrifuged for 6×10^5 ***g***·min in a fixed angle rotor at 12°C, and large chylomicrons (diameter >100 nm) were removed from the upper fraction (1·0–1·5 ml) after tube slicing. The large chylomicrons (containing 30–40 μmol triacylglycerol and 50 mg added glucose) were injected intravenously into anaesthetised post-absorptive rats previously hepatectomised by ligation of all the major vessels supplying the liver, and allowed to circulate for 45 min. Following this period, the animals were terminally exsanguinated and the serum was separated by centrifugation (3000 rpm, 15 min, 12°C). The serum was ultracentrifuged for 6×10^7 ***g***·min, the upper fraction (1·0–1·5 ml) was layered under NaCl (1·006 g/ml) and further purified by ultracentrifugation for $3{\cdot}2\times10^7$ ***g***·min at 12°C. Chylomicron remnants, relatively free of VLDL and intermediate-density lipoproteins, were isolated from the upper fraction (1·0–1·5 ml) using a Beckman tube slicer (Beckman Instruments (UK) Ltd., High Wycombe, Bucks., UK). Our earlier work has shown that chylomicron remnants prepared in this way are enriched in the fatty acids predominating in the oils used, and their fatty acid composition shows little variation from preparation to preparation (Lambert *et al.* 1996). Approximately 90 % of the radioactivity in the particles was associated with triacylglycerol, with the remainder divided between mono- and diacylglycerols, phospholipids and non-esterified fatty acids, and there were no significant differences in this distribution or in the content of cholesterol and triacylglycerol between the two different types of remnants used.

Purification of hepatic lipase

The methods for the surgical isolation of the rat liver have been described previously (Lambert *et al.* 1995). Hepatic lipase was released from rat livers (approximately ten livers) by perfusion for 5–7 min with Krebs bicarbonate buffer (45 ml; mM: NaCl 118, KCl 4·7, $CaCl_2$ 1·32, $MgSO_4$ 1·2, Na_2HCO_3 24, KH_2PO_4 1·2, glucose 13·9, and plasma amino acids (670 mg/l) containing heparin (40 U/ml). The pooled perfusates were passed down a HiTrap Heparin–Sepharose column (5 ml, Pharmacia, Upsala, Sweden) pre-equilibrated with veronal buffer (50 mM, pH 7·4) containing 0·15 M-NaCl. Weakly bound heparin-released proteins were removed from the column using veronal buffer NaCl (0·3 M), and hepatic lipase protein was then eluted with the same buffer containing 0·9 M-NaCl. The enzyme protein was dialysed against Tris HCl buffer (50 mM, pH 7·2) containing glycerol (300 ml/l) and purified by passage through a HiTrap Q-Sepharose column (pharmacia) pre-equilibrated with the dialysis buffer described earlier. After removal of the glycerol from the column using Tris HCl (50 mM, pH 7·2), the purified hepatic lipase was eluted by the stepwise addition of the same buffer containing 100 mM-α-D-methyl-mannoside and increasing concentrations of NaCl (80 mM, 0·2 M, and 0·5 M). The final preparation showed a purification of approximately 300-fold compared with the crude perfusate, and no bands were detected after SDS polyacrylamide electrophoresis, except in the region corresponding to the molecular mass of hepatic lipase (62 000 Da).

Preparation of rabbit anti-rat hepatic lipase antiserum and non-immune serum

Purified hepatic lipase was sonicated (3×5 s) with Freund's complete adjuvant (1:1) and injected (4×250 μl, approximately 9·6 μg protein/injection site) subcutaneously into a New Zealand white rabbit. Injections were repeated once per week for 3 weeks using the protein and Freund's incomplete adjuvant, and test blood samples were taken from the middle ear vein after a further 2 weeks and found to have anti-hepatic lipase activity. To remove the lipoproteins, antiserum raised against rat hepatic lipase or non-immune sera was layered under a KBr solution (density 1·210 g/ml) in polyallomer tubes (6·5 ml) and ultracentrifuged (40 000 rpm, 12°C) for 20–22 h. The upper fraction from each tube containing lipoproteins (density<1·210 g/ml) was removed and the remaining lipoprotein-deficient serum was dialysed against NaCl (9 g/l) for 5–6 h.

Assay of lipase activity

Hepatic lipase activity was measured by the release of [^{3}H]oleate from triolein in the presence of 1 M-NaCl, to inhibit lipoprotein lipase activity, bovine serum albumin (25 g/l, fatty acid free) and Tris HCl (0·2 M, pH 8·4). Lipoprotein lipase activity was measured in the absence of NaCl using the same conditions. To test the rabbit anti-rat hepatic lipase antiserum, hepatic lipase or lipoprotein lipase was pre-incubated with the antiserum (0–25 μl) for 15 min at 37°C, and the assay was started by the addition of an emulsified glycerol tri[9,10(*n*)-^{3}H]oleate substrate (1·5 mg triolein mass/tube). After 2 h, the reaction was stopped by the addition of 50 μl, 1 M-NaOH and 1·5 ml chloroform–methanol–toluene (2:2·4:1, by vol.), and the [^{3}H]oleate released into the aqueous medium was determined by a liquid scintillation counting. Enzyme activity is expressed as nmol triolein hydrolysed/h.

Liver perfusions

Rat livers were isolated and perfused with whole rat blood as previously described (Lambert *et al.*, 1995). The liver was perfused with 52 ml recirculating defibrinated blood perfusate (30–35 % packed cell volume) and 6 ml lipoprotein deficient rabbit anti-rat hepatic lipase antiserum or non-immune serum was added and allowed to circulate for 15 min. [^{3}H]Oleate-labelled chylomicron remnants (37–74 KBq in 5 ml) were then added to the perfusate and samples (2 ml) were taken after 10, 20, 40, 60, 90 and 120 min. After 120 min the experiment was terminated, the liver was flushed free of residual blood perfusate with Krebs bicabonate buffer containing bovine serum albumin (10 g/l), and the lipids were extracted with chloroform–methanol (2:1, v/v, twenty volumes).

Immunocytochemistry studies

Rat livers were perfused with 35 ml Krebs bicarbonate buffer and 6 ml rabbit anti-rat hepatic lipase antiserum or non-immune serum continuously gassed with O_2–CO_2 (95:5, v/v) for 15 min. At the end of the perfusion, livers were flushed with the same buffer (50 ml) containing bovine serum albumin (10 g/l), cut into small cubes and rapidly frozen. Liver sections (10 μm) were prepared using a cryostat and incubated with 0·1 M-PBS (pH 7·2) containing goat serum (100 ml/l) for 40 min, and then with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G (1:40 dilution) for 40 min. Sections were washed four times with PBS and examined using a fluorescence microscope.

Other methods

To obtain plasma containing lipoprotein lipase, male Wistar rats were functionally hepatectomised by ligation of all the major vessels supplying the liver (to exclude hepatic lipase from the preparation), and 100 μl saline (9 g NaCl/l) containing heparin (100 U/kg body weight) was injected into the ileolumbar vein. After 5 min, the blood was withdrawn via the abdominal aorta and allowed to clot and centrifuged (3000 ***g***, 15 min) to remove the red blood cells.

Lipid extracts were prepared from liver and serum samples using chloroform–methanol (2:1, v/v, twenty volumes), and these were partitioned with 0·4 volumes 0·03 M-HCl. Portions of the chloroform phase were dried in scintillation vials using a stream of air and the radioactivity was determined by liquid scintillation counting using a toluene-based scintillant (18 ml; 3 g 2,5-diphenyloxazole/l, 0·25 g 1,4-bis-(4-methyl-5-phenyloxazol-2-yl) benzene/l.

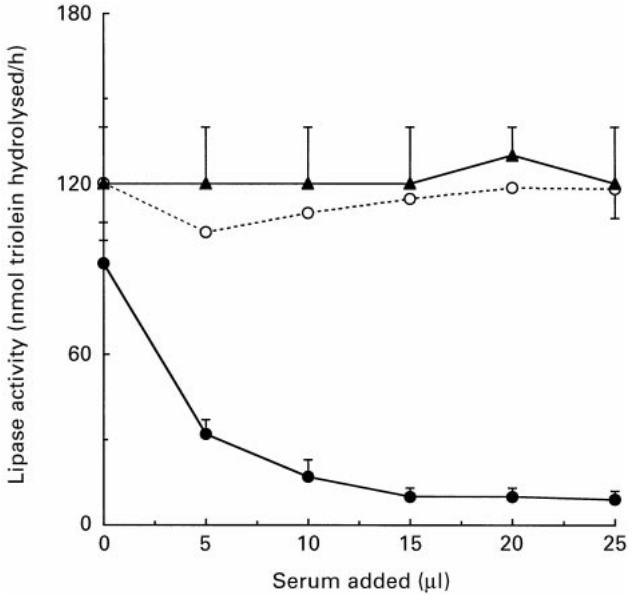

Fig. 1. The activity of hepatic lipase or lipoprotein lipase in the presence of rabbit anti-rat hepatic lipase antiserum or non-immune rabbit serum in rat liver. Enzyme activities were assayed as described on p. 577. —○—, Hepatic lipase + non immune rabbit serum; —●—, hepatic lipase + rabbit anti-rat hepatic lipase antiserum; —▲—, lipoprotein lipase + anti-rat hepatic lipase antiserum. Values are means from six experiments with standard errors of the means shown by vertical bars.

The total cholesterol and triacylglycerol content of the chylomicron remnants were determined using commercially available kits from Boehringer Mannheim (Mannheim, Germany). Statistical significance was determined using ANOVA.

Results

Characterisation of the rabbit anti-rat hepatic lipase antiserum

The activities of hepatic lipase and lipoprotein lipase in the presence of rabbit anti-rat hepatic lipase antiserum and non-immune rabbit serum are shown in Fig. 1. Hepatic lipase activity in the absence of the antiserum (92 (SEM 8) nmol triolein hydrolysed/h) was markedly inhibited in its presence. The addition of 5 μl caused a 65 % reduction in activity, and maximal inhibition (89·1 (SEM 3·2) %) was reached with 15–25 μl. In contrast, lipoprotein lipase activity (120·0 (SEM 20·0) nmol triolein/h) was not affected by the antiserum. Furthermore, hepatic lipase activity was unaffected by similar volumes of non-immune rabbit serum.

The amount of hepatic lipase activity released from one rat liver was markedly reduced (75–94 %) by 2–6 ml antiserum in six experiments (data not reported), and larger volumes did not increase the inhibition further. Thus, 6 ml rabbit anti-rat hepatic lipase antiserum inhibited more than 90 % of the heparin-releasable hepatic lipase activity from one liver, and this is likely to represent most of the extracellular surface-located enzyme. This volume, therefore, was used in all subsequent liver perfusion experiments.

Immunocytochemistry was used to compare interactions of rabbit anti-rat hepatic lipase antiserum and non-immune serum with the liver in the conditions used in our experiments. After perfusion of the liver with the antiserum or non immune serum for 15 min, sections of the tissue were incubated with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G to visualise antibody bound to the surface of the cells. Considerable fluorescence was observed on sinusoidal surfaces in the liver in all sections examined following perfusions with rabbit anti-rat hepatic lipase antiserum (Fig. 2(a)), but no significant fluorescence was seen with rabbit non-immune serum in any areas of the liver (Fig. 2(b)). These results show that perfusion of rat livers with the rabbit anti-rat hepatic lipase antiserum results in widespread binding of antibodies to the enzyme on the surface of liver cells.

Effects of rabbit anti-rat hepatic lipase antiserum on the uptake by the perfused rat liver of [^{3}H]oleate-labelled chylomicron remnants from chylomicrons from rats given a bolus of fish or palm oil

The removal of the two types of [^{3}H]oleate-labelled chylomicron remnants by perfused rat livers treated with rabbit anti-rat hepatic lipase antiserum or rabbit non-immune serum is shown in Fig. 3. In the presence of the non-immune serum (Fig. 3(a)), remnants from chylomicrons from rats given a bolus of fish oil as compared with palm oil were removed from the perfusate more rapidly, as expected from our previous work (Lambert *et al.* 1995). However, in the presence of the rabbit anti-rat hepatic lipase antiserum the values obtained for the disappearance of both types of remnants from the perfusate were very similar to those seen with non-immune serum, and an almost identical pattern of more rapid removal of the remnants enriched in the fatty acids found in fish oil was observed (Fig. 3(b)).

In the presence of either rabbit anti-rat antiserum or non-immune serum, significantly more [^{3}H] radioactivity was found in livers from rats perfused with remnants from chylomicrons from the rats given fish oil rather than palm oil (Fig. 4), and these values are consistent with the differential removal of the different types of remnants from the perfusate.

Discussion

Previous studies in our laboratory have shown that the fatty acid composition of chylomicron remnants influences the rate of their uptake by the perfused rat liver (Lambert *et al.* 1995), and that the pattern of the differences mimics that observed in the rat *in vivo* (Bravo *et al.* 1995). We have also demonstrated that the various types of remnants are similar in all other respects, including the size of the particles and their lipid and apolipoprotein content (Botham *et al.* 1997; Lambert *et al.* 1996). Thus, the differential rates of removal from the blood of remnants derived from different dietary fats can only be explained by the differences in their fatty acid composition, and furthermore, hepatic processes must be involved in the mechanism of the effects.

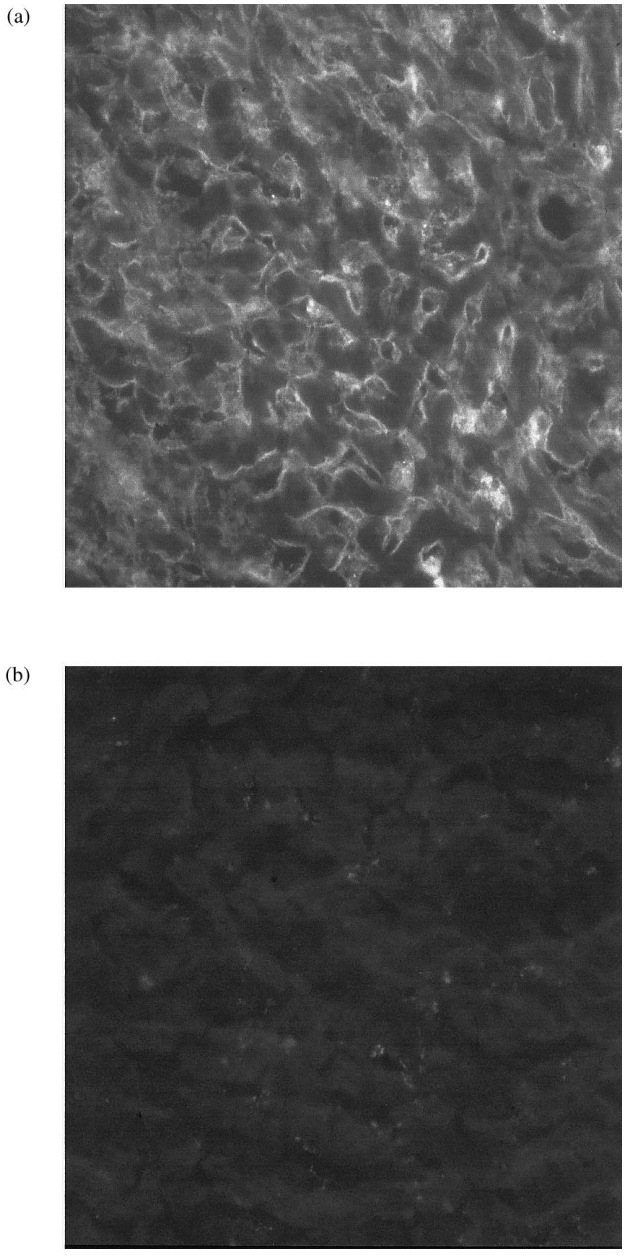

Fig. 2. Photographs (× 20 magnification) showing typical liver sections from rat livers perfused with rabbit anti-rat hepatic lipase antiserum (a) or non-immune serum (b) (6 ml). For details of procedures, see p. 577. The sections were prepared and incubated with goat serum (100 ml/l) followed by fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G (1:40 dilution) and viewed using a fluorescence microscope.

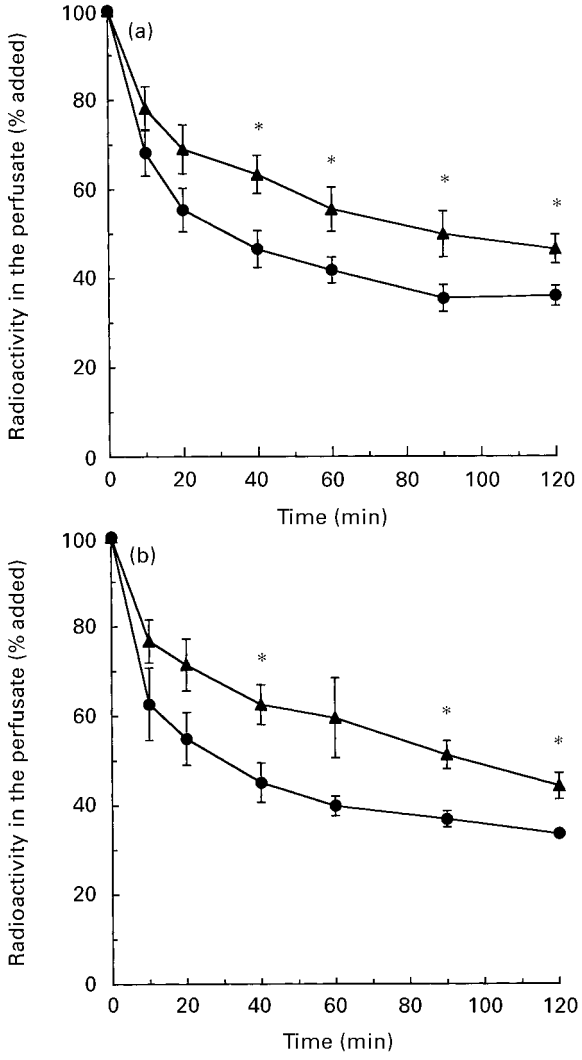

Fig. 3. The effect of rabbit anti-rat hepatic lipase antiserum on the removal of chylomicron remnants derived from chylomicrons from rats given a bolus of palm oil (—▲—) or fish oil (—●—) from the perfusate by the perfused rat liver. Livers were perfused with chylomicron remnants in the presence of non-immune rabbit serum (a) or an equal volume of rabbit anti-rat hepatic lipase antiserum (b), and the radioactivity remaining in the perfusate was determined at the times indicated. Values are expressed as a percentage of the amount of radioactivity added to the perfusate. Values are means from four (antiserum) or five (non-immune serum) experiments with standard errors of the means shown by vertical bars. Mean values were significantly different between the two different types of remnants: *$P<0{\cdot}05$.

In the liver, chylomicron remnants first interact with hepatic lipase, which modifies the lipid content of the particles through its catalytic activity (Brasaemle *et al.* 1993; Botham *et al.* 1995; Fan & Watanabe, 1998), and may also aid in their binding to the surface of hepatocytes by acting as a ligand (Diard *et al.* 1994; Amar *et al.* 1998; Chappell & Medh, 1998), and they are subsequently endocytosed by receptor-mediated pathways involving either the LDL receptor or the LDL receptor-related protein (Cooper, 1997; Rohlmann *et al.* 1998). Differential interaction of chylomicron remnants of different fatty acid composition with either hepatic lipase, or the receptors responsible for their internalisation by liver cells, therefore, are the two major mechanisms by which the differential rates of uptake of the various types of particles by the liver may be modulated, and we have investigated the former possibility in the present work.

Polyclonal antibodies to hepatic lipase have been used in a number investigations of the role of the enzyme in

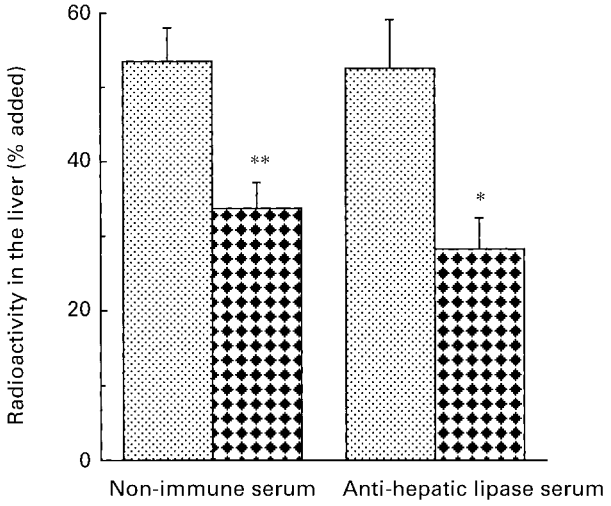

Fig. 4. The effect of anti-rat hepatic lipase antiserum on the uptake of chylomicron remnants derived from chylomicrons of rats given a bolus of palm oil ▪ or fish oil ▨ by the perfused rat liver. Livers were perfused with chylomicron remnants in the presence of non-immune rabbit serum or an equal volume of rabbit anti-rat hepatic lipase antiserum, and the radioactivity found in the liver after 120 min was determined. Values are expressed as a percentage of the amount of radioactivity added to the perfusate. Values are means from four (antiserum) or five (non-immune serum) experiments with standard errors of the means shown by vertical bars. Mean values were significantly different between the two different types of remnants: $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$.

chylomicron remnant uptake by the liver (Daggy & Bensadoun, 1986; Sultan *et al.* 1990; Shafi *et al.* 1994), but their effects on the uptake of remnants of different fatty acid composition have not been studied previously. In the current work, rat hepatic lipase obtained by perfusion of rat livers with heparin was purified by heparin–Sepharose chromatography and used to raise polyclonal antibodies in rabbits. The rabbit anti-rat hepatic lipase antiserum was shown to strongly inhibit hepatic lipase activity to a maximum of 90 % (achieved with 15 μl antiserum) (Fig. 1). Furthermore, there was no detectable cross-reactivity with lipoprotein lipase, and enzyme which belongs to the same gene family as hepatic lipase, and with which it shares extensive amino acid sequence homology and a similar three-dimensional structure (Kirchgessner *et al.* 1989; Derewenda & Cambillau, 1991). In addition, we demonstrated that >90 % heparin-releasable hepatic lipase activity from one rat liver was inhibited by the volume of antiserum (6 ml) used in our experiments (Fig. 2). Immunocytochemical studies also indicate that after perfusion of the rat liver with the rabbit anti-rat hepatic lipase antiserum, antibodies to the enzyme were widely distributed in the tissue, and accessed and specifically adhered to the sinusoidal cell surfaces (Fig. 2), which are known to be the sites where hepatic lipase is located (Breedveld *et al.* 1997). These results indicate that the catalytic activity of hepatic lipase in the perfused liver in our experiments was substantially inhibited by the antiserum used, and that the antibody protein was bound to the enzyme at cell surfaces throughout the tissue, thus blocking its possible function as a ligand for remnant binding prior to internalisation by hepatocytes. Thus, if the hypothesis that hepatic lipase mediates the differential hepatic uptake of chylomicron remnants of different fatty acid composition is correct, exposure of the perfused liver to the rabbit anti-rat hepatic lipase antiserum would be expected to reduce or abolish the difference in the rate of uptake of particles derived from fish or palm oil observed in our earlier work (Lambert *et al.* 1995.)

When non-immune rabbit serum was added to the perfusate, the removal of [^{3}H]triacylglycerol-labelled chylomicron remnants was slower when the lipoproteins were derived from chylomicrons from rats given a bolus of palm oil as compared with fish oil (Fig. 3(a)), and the pattern observed was very similar to that found in our previous experiments (Lambert *et al.* 1995). Moreover, perfusions with rabbit anti-hepatic lipase antiserum showed an almost identical pattern (Fig. 3(b)), and measurement of the recovery of radioactivity in the liver after 120 min perfusion also showed no difference between perfusions with non-immune serum and anti-hepatic lipase antiserum (Fig. 4). Thus, the antibodies to hepatic lipase had no effect on the differential uptake of chylomicron remnants enriched in saturated or *n*-3 polyunsaturated fatty acids by the liver, indicating that differential interaction with the enzyme is not responsible for the variations in the rates of removal of remnants of different fatty acid composition from the blood (Bravo *et al.* 1995; Lambert *et al.* 1995).

Unlike Shafi *et al.* (1994), we did not observe inhibition of the uptake of the same type of chylomicron remnants in the presence and absence of antibodies to hepatic lipase. However, the aim of the present work was to investigate the part played by hepatic lipase in the differential uptake of chylomicron remnants of different fatty acid composition, and the experiments were not optimised for the study of the general role of the enzyme in remnant uptake. The studies of Shafi *et al.* (1994) with the perfused rat liver and Sultan *et al.* (1990) in the rat *in vivo* demonstrate that antibodies to hepatic lipase retard remnant clearance at very early time points (<15 min). In our study, however, remnant uptake was followed over 120 min in order to mimic the conditions of our earlier work (Lambert *et al.* 1995) more closely, and only one time point at <15 min was included. Our results, therefore, do not necessarily conflict with earlier work suggesting a role for hepatic lipase in hepatic remnant uptake, but rather show specifically that the enzyme does not mediate the differential uptake of remnants of different fatty acid composition.

The hepatic uptake of chylomicron remnants is believed to occur in two stages, an initial sequestration of the particles onto cell surface heparan sulfate proteoglycans which is facilitated by hepatic lipase and also by lipoprotein lipase (Ji *et al.* 1994, 1995; Cooper, 1997; Chappell & Medh, 1998), followed by receptor-mediated endocytosis via either the LDL receptor or the LDL receptor-related protein (Cooper, 1997; Chappell & Medh, 1998). Preliminary experiments in our laboratory with isolated rat hepatocytes have shown that the initial binding step is unaffected by the fatty acid composition of chylomicron remnants, but that the rate of internalisation of particles enriched with saturated, monounsaturated or *n*-3 or *n*-6 polyunsaturated fatty acids varies (Lambert *et al.*

1999), showing a pattern which is strikingly similar to that found in our earlier work on their uptake rates in the rat *in vivo* and in the perfused liver (Bravo *et al.* 1995; Lambert *et al.* 1995). Thus, remnants enriched in *n*-3 polyunsaturated fatty acids are internalised at the fastest rate, and those enriched in saturated fatty acids at the slowest, with those enriched in mono- or *n*-6 polyunsaturated fatty acids showing intermediate rates. Since the experiments reported here indicate that the differential rates of removal of chylomicron remnants of different fatty acid composition from the blood by the liver cannot be explained by differential interaction with hepatic lipase, these findings suggest that the major mechanism involved in this effect is differential rates of endocytosis by the LDL receptor and/or the LDL receptor-related protein, and this is currently under further investigation in our laboratory.

Acknowledgements

This work was supported by a grant from the Wellcome Trust (grant no. 048022/Z/96).

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