

Fatty acid control of lipoprotein lipase: A link between energy metabolism and lipid transport

(heparin binding/hepatic lipase/lipid emulsion/plasma/triacylglycerols)

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ABSTRACT Lipoprotein lipase (LPL) catalyzes the flux-generating step in transport of fatty acids from lipoprotein triacylglycerols into tissues for use in metabolic reactions. *In vitro* studies have shown that fatty acids can bind to the enzyme and impede its other interactions. In this study we have searched for evidence of fatty acid control of LPL *in vivo* by rapid infusion of a triacylglycerol emulsion to healthy volunteers. During infusion the activity of LPL but not of hepatic lipase increased in plasma, but to different degrees in different individuals. The time course for the increase in LPL activity differed from that for triacylglycerols but followed the plasma levels of free fatty acids. This was true during infusions and when the emulsion was given as a bolus injection. In particular there were several instances when plasma triacylglycerol levels were very high but free fatty acids and LPL activity remained low. Model studies with bovine LPL showed that fatty acids displace the enzyme from heparin-agarose. We suggest that in situations when fatty acids are generated more rapidly by LPL than they are used by the local tissue, they cause dissociation of the enzyme from its binding to endothelial heparan sulfate and are themselves released into circulation.

Triacylglycerol (TG) transport is a major pathway in energy metabolism and handles more than 100 g of lipid per day in individuals on a typical Western diet. The TGs are unloaded from the lipoproteins through hydrolysis by lipoprotein lipase (LPL) at the vascular endothelium in extrahepatic tissues (for review, see refs. 1 and 2). It is generally assumed that the rate-limiting factor is the amount of LPL available at the endothelium (3). In support of this, studies in animals have shown a correlation between the activity of LPL in a tissue and its uptake of fatty acids from chylomicra (1, 2, 4). Inherent in this view is the assumption that the tissue can assimilate the fatty acids at the rate that the enzyme provides them. The possibility that fatty acid assimilation can be rate-limiting has been raised (5) but has received little attention. *In vitro* studies have, however, shown that LPL has a built-in mechanism for product control. The enzyme can bind fatty acids, which reduces its affinity for lipid droplets (6, 7) as well as for heparin-like polysaccharides (8) and abolishes the activation by apolipoprotein C-II (9). This suggests that accumulation of fatty acids at the endothelium might inhibit further lipolysis and disrupt the binding of LPL to heparan sulfate. Whether this mechanism ever comes into play *in vivo* is not known. To demonstrate it one would need a condition in which the clearing capacity was overloaded. In this study we have tried to create such a situation by rapid infusion of a lipid emulsion.

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MATERIALS AND METHODS

Study Protocol. The 12 male volunteers [age, 21–27 years; body mass index (body mass/height² expressed in kg/m²), 27.0 ± 1.1] had fasting plasma TG < 100 mg/dl, cholesterol < 180 mg/dl, phospholipid < 200 mg/dl, and glucose < 110 mg/dl. After a standard meal on the previous evening followed by an overnight fast, they were admitted to the unit at 0800. They were kept at rest and not allowed anything *per os* throughout the study period. One infusion line was inserted into a cubital vein of each arm and kept patent (unobstructed) by a slow infusion of normal saline. One line was used for lipid administration and the other for blood sampling. Some of the subjects received infusions of Intralipid (Kabi Nutrition, Stockholm) 10%, 20%, or 30% for 6 hr at rates providing either 0.1 or 0.3 g of TG per kg (body weight) per hr. Infusion rates were maintained by use of a peristaltic pump (Frese-nius, Bad Homburg, F.R.G.). Four subjects received a bolus injection of Intralipid 20% (0.1 g of TG per kg) in less than 20 sec. The interval between infusions in the same individual was always more than 1 week, usually several weeks.

Analyses. Blood samples (5 ml) were collected in EDTA and immediately put in ice water. Plasma was rapidly separated by centrifugation for 5 min at 1000 × g using a Beckman refrigerated centrifuge. Plasma lipids were determined by the following enzymatic methods: free fatty acid (FFA) by NEFA Quick BMY (Boehringer Mannheim), as soon as possible and in every case less than 1 hr after blood drawing, TG by using triglycerides without free glycerol (Boehringer Mannheim) within 24 hr of blood sampling, phospholipids by using Phospholipid Enzyme PAP 1600 (BioMérieux, Charbonnières-les-Bains, France). Data are presented as means ± SEM.

Samples to be assayed for FFA were chilled immediately and analyzed within 1 hr. To rule out the possibility that *in vitro* lipolysis might significantly contribute to the registered values, the following experiment was carried out. Two subjects were infused with Intralipid 20% at the high rate, which resulted in increases of the FFA concentration from about 0.3 to about 1.1 mmol/liter. Plasma samples were immediately added to 0.2 vol of 5 M NaCl at 56°C and then incubated at this temperature for 30 min. Studies with bovine LPL added to human plasma and with human LPL in post-heparin plasma showed that LPL activity was completely and irreversibly inhibited within a few minutes by this procedure. The concentration of FFA in plasma samples was 99.8 ± 6.5% (mean ± SD for six samples after 2, 4, and 6 hr of infusion in the two individuals) of the concentration in heated samples. We conclude, therefore, that the FFA values measured accurately reflect *in vivo* concentrations.

Abbreviations: FFA, free fatty acids; LPL, lipoprotein lipase; TG, triacylglycerol.

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This protocol was approved by the Ethical Committee for Medical Research of the Free University of Brussels, and all the study subjects signed informed consent forms prior to study.

Materials. Antiserum to bovine LPL was raised in a chicken (10). It inhibited LPL activity in human post-heparin plasma by more than 98% but did not cause significant inhibition of hepatic lipase. Antiserum to human hepatic lipase was raised in a goat (11). Immunoglobulins were isolated and dialyzed into isotonic phosphate-buffered saline. Apolipoprotein C-II was prepared as in ref. 12. The 10% emulsion of Intralipid contains 10 g of soybean TG and 1.2 g of egg yolk phospholipid per 100 ml. The 20% and 30% emulsions contain 20 and 30 g of TG, respectively, but the same amount of phospholipid, 1.2 g/100 ml. The 30% emulsion is not commercially available but was specially provided for research purposes. Bovine serum albumin (fraction V) and gum arabic were obtained from Sigma. Heparin-agarose was prepared as in ref. 13.

Lipase Assays. The assay conditions were the same as those used (14) to measure LPL (sonicated emulsion of [³H]oleic acid-labeled triolein in phosphatidylcholine) and hepatic lipase (sonicated emulsion of ³H-labeled triolein in gum arabic) in rat plasma (14), with the following modifications: In LPL assay purified apolipoprotein C-II instead of rat plasma was used as activator and sample volume was 15 μ l. All samples were preincubated (2 hr on ice) with 0.5 vol of antiserum to hepatic lipase. Three samples received 0.5 vol of antiserum to LPL and three samples received a corresponding nonimmune serum; LPL activity was calculated as the difference between these samples—i.e., as the activity inhibited by the anti-LPL serum. Tests showed that the amount of antiserum was sufficient to cause more than 98% inhibition of LPL in post-heparin plasma where the activity is much higher than those encountered here.

In both assays release of fatty acids was linear with time for at least 2 hr and with the volume of plasma. Tests with post-heparin plasma showed that the linear range extended well above the lipase activities encountered here. All determinations were done in triplicate. The coefficients of variation when samples from the same pool were measured were 3.7% and 5.3% for LPL and hepatic lipase, respectively. Lipase activities are expressed in milliunits, which correspond to 1 nmol of fatty acid released per min. The contribution of TG from the sample to total TGs in the assay for LPL was always less than 15%. Model experiments showed that this amount of unlabeled Intralipid in the assay caused 10–12% reduction of apparent activity. The same amount of Intralipid caused about 15% reduction of apparent activity in the assay for hepatic lipase.

Effect of Fatty Acids on the Interaction of LPL with Heparin. ¹²⁵I-labeled bovine LPL (5 μ g, about 65,000 cpm) was allowed to bind to 0.1 ml of heparin-agarose in test tubes. The total volume was 1.5 ml and the buffer was 0.1 M NaCl/10 mM Tris Cl, pH 7.4/bovine serum albumin (1 mg/ml). After 2 hr at 4°C the tubes were centrifuged and the supernatants were removed. The gels were then washed three times with buffer and the radioactivity was measured in a γ counter. About 85% of the added lipase remained with the gels. Then 1.5 ml of a mixture containing Intralipid and linoleate was added to each tube. The buffer was 75 mM Tris Cl, pH 7.4/75 mM NaCl/5 mM EDTA. The final concentration of TG was 5 mg/ml in all tubes and the concentration of linoleate was varied. In some experiments bovine serum albumin at 67 mg/ml was also included. After 60 min at 25°C, the tubes were centrifuged, the supernatants were decanted, and their radioactivity was measured. The same mixture (1.5 ml) was added to the gel, and after 10 min the tube was again centrifuged. The combined lipase radioactivity in the two

supernatants was calculated as percent of the amount initially bound to the gel.

RESULTS

Changes During Lipid Infusion. Five volunteers were infused for 6 hr with Intralipid 10% at a rate of 0.3 g of TG per kg (body weight) per hr. In all five subjects the TG levels increased progressively. In two (Fig. 1A, subjects WIL and DUB) LPL activity also increased dramatically, whereas in the other three (exemplified by subjects SHA and VAN in Fig. 1A) the increase in LPL was less marked. This was clearly not related to plasma TG levels that increased higher in subjects SHA and VAN than in WIL and DUB. In the latter two subjects, however, there were substantially greater increases in FFA levels, suggesting a relation between increases in plasma FFA concentration and in plasma LPL activity. In the next experiment four subjects were given Intralipid 30% (Fig. 1B). This differs from the 10% emulsion in that it contains less phospholipid for the same amount of TG. The increases in plasma TG tended to be lower with the 30% emulsion, but all four subjects showed large increases in plasma LPL activity and large increases in plasma FFA levels (Fig. 1B). In three subjects LPL activity increased to greater than 5 milliunits/ml; the highest value was 10.9 milliunits/ml. This corresponds to only a few percent of the LPL that can be released with heparin, typically 300 milliunits/ml in young males.

Infusions were also done at a lower rate [0.1 g of TG per kg (body weight) per hr], which corresponds approximately to basal energy requirements. Plasma TG initially increased 2- to 3-fold but then remained relatively constant during the rest of the infusion periods and increases in plasma FFA and in LPL activity were moderate (to 0.7–1 mM and to 0.8–2.2 milliunits/ml).

Fig. 2 shows LPL activities as a function of lipid concentration during and immediately after the infusion. For statistical analysis we used data at 6 hr—i.e., at the end of the infusion ($n = 9$). There was a strong correlation of LPL activities to FFA concentrations ($r = 0.90$; $P = 0.001$) but not to TG ($r = 0.02$) or phospholipid ($r = 0.11$) concentrations. Note that there was no instance where high levels of plasma LPL were not associated with substantial increases in plasma FFA concentrations, whereas there were several cases where a large increase in TG was associated with only a small increase in LPL activity.

Changes After Bolus Injection. Four subjects were injected with Intralipid 20% [0.1 g per kg (body weight) over 20 sec]. In all four subjects, plasma FFAs and LPL activity increased. The time curves for FFA and for LPL were similar but quite different from those for TG. Whereas plasma TG levels were highest at early times and then decreased continuously, FFA and LPL levels did not reach their maximal values until 20–40 min after the injection. Three of the four subjects showed modest increases in FFA levels and modest increases in LPL activity in plasma, as illustrated in Fig. 3A for one subject (VDW). The fourth subject (NSA, Fig. 3B) gave a more dramatic response. His FFA levels increased rapidly after the injection and reached somewhat higher levels than in the other three subjects (0.7 mM compared to about 0.5 mM). In this subject there was a pronounced increase in LPL activity that followed the same time course as the FFA levels (Fig. 3B).

Other Lipase Activities in Plasma. The activity of the hepatic heparin-releasable lipase varied by about 3-fold, but these variations were not correlated to any of the parameters measured.

Several lipases from pancreas are known to occur in plasma; the colipase-dependent lipase (15), the phospholipase (15), and the nonspecific lipase (16). In addition, there

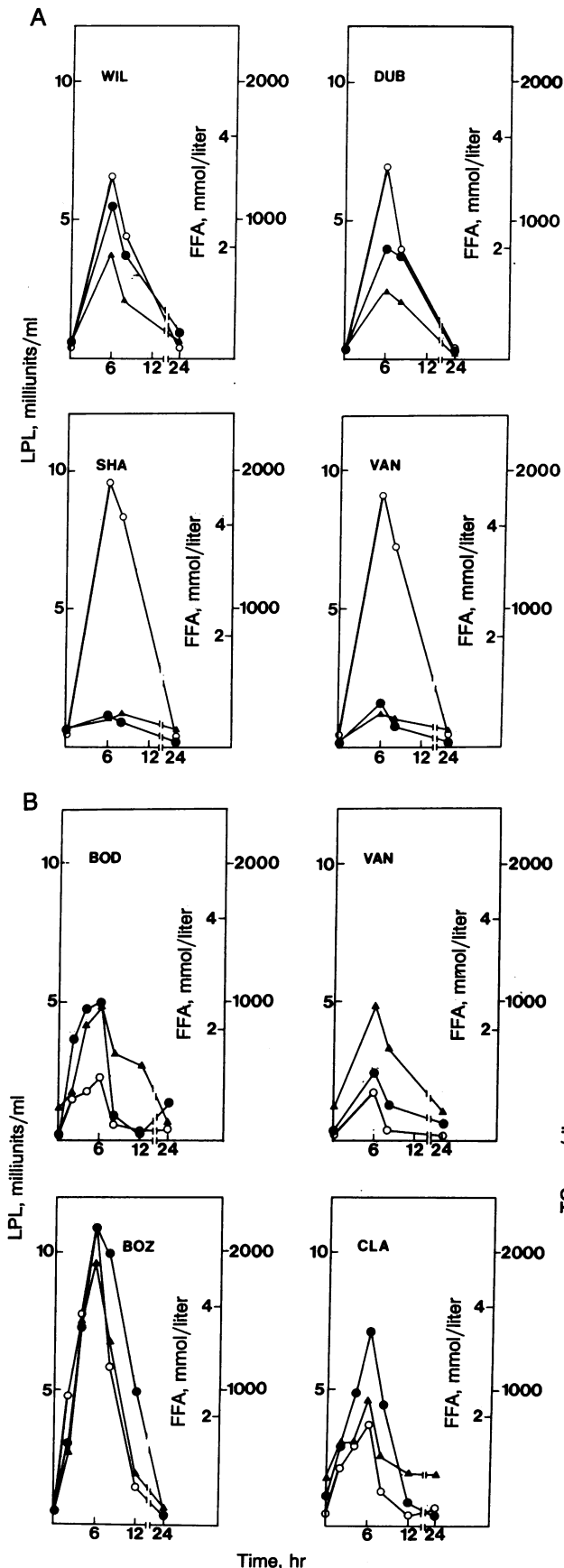


FIG. 1. Increases in plasma FFA (▲) and LPL (●) levels during infusion of Intralipid 10% (A) or 30% (B) at a high rate [0.3 g of TG per kg (body weight) per hr]. Measurements from four subjects at each concentration of Intralipid are shown. ●, LPL; ▲, FFA; ○, TG.

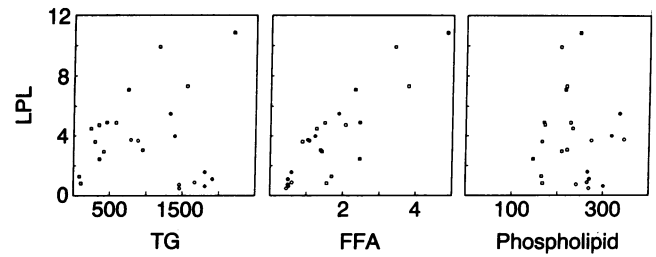


FIG. 2. Relation between the increases in LPL activity and in plasma lipid concentrations. Data from all individuals infused at the rate of 0.3 g of TG per kg (body weight) per hr are plotted for times between 2 and 8 hr after the start of the infusion—i.e., the time when plasma TG levels were markedly elevated. ○, 10% Intralipid; □, 30% Intralipid. Data points at 6 hr (i.e., at the end of the infusion) were used for statistical analysis and are indicated with solid symbols. There was a significant correlation between LPL and FFA ($r = 0.90$; $P = 0.001$) but not to TG and to phospholipid levels. Units: LPL, miliunits/ml; TG, mg/dl; FFA, mmol/liter; phospholipid, mg/dl.

are monoacylglycerol-hydrolyzing enzymes in plasma and in platelets (17). Each of these lipases has specific requirements for optimal activity and will express activity to an unknown extent in assays set up for another lipase. To obviate these ambiguities, we have determined LPL activity as the difference in activity registered with and without antiserum to LPL in an assay optimal for LPL. A low but significant lipase activity remained after the immunoinhibition, about 0.2 miliunits/ml. There were no statistically significant changes in this residual lipase activity during or after the infusion.

Effect of Fatty Acids on the Binding of LPL to Heparin. We have found (8) that interaction with fatty acids impedes the binding of LPL to heparin. To test if this effect was seen also in the presence of a lipid emulsion, the experiments in Fig. 4 were performed. LPL was allowed to bind to heparin-agarose. After washes, Intralipid with or without added linoleic acid in the presence or absence of albumin was added to the gels. Intralipid as such caused very little release of the labeled lipase, but the presence of fatty acids resulted in displacements of as much as 70% of the lipase. Albumin prevented the release. This suggests that *in vivo* it is not the presence of lipid emulsion that releases bound LPL but rather the FFAs that are produced upon hydrolysis of the emulsion.

DISCUSSION

In this study we have found that the LPL activity in plasma increases dramatically in some individuals given a lipid emulsion intravenously and that this occurs only when plasma FFA increases. Plasma lipase activity elevation during lipid infusion and after lipid-rich meals had been observed in humans (18, 19) and in animals (14, 20–22) and was generally assumed to be due to binding of LPL to the lipid particles. In our experiments there was no statistically significant correlation between TG levels and LPL activity in plasma, and there were several examples of high TG levels with low LPL activity. This indicates that high plasma TG levels as such were not the direct cause of the increase in LPL activity. In contrast, there was a strong correlation of LPL activity to increased FFA levels in plasma. This correlation held for individual time curves during infusion and after bolus injection of Intralipid. In studies with oral fat loads, we observe similar responses—i.e., increases of FFA and of LPL activity that coincide in time (unpublished results).

Studies with perfused tissues have shown that during normal LPL action most of the fatty acids are taken up locally (1, 2, 4). The same conclusion can be drawn from studies on the flux of radioactivity through the plasma FFA pool during clearing of fatty acid-labeled chylomicra (23) or very low

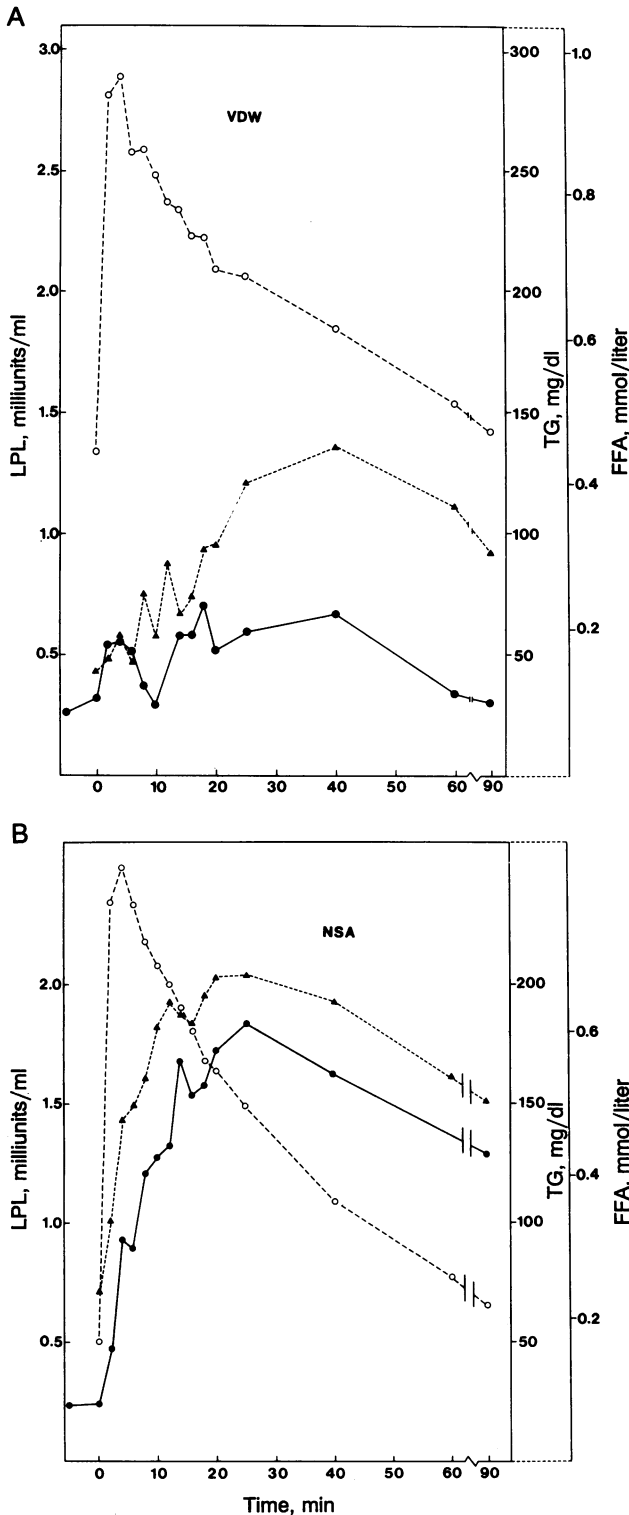


FIG. 3. Increases in plasma FFA and LPL levels after bolus injection of Intralipid 20% [0.1 g of TG per kg (body weight)] over 20 sec. Three of the four subjects studied showed modest increases in FFA and LPL levels; this is illustrated by the subject VDW (A). The fourth subject, NSA (B), showed a more pronounced increase in FFA and LPL that followed the same time course. ●, LPL; ○, TG; ▲, FFA.

density lipoprotein (24). In accord with this, there is only a moderate increase of plasma FFA during clearing of fat emulsions infused at slow or moderate rates (25). Heparin releases LPL into blood. In this situation hydrolysis of plasma TG occurs mainly in the circulation and the released fatty acids must pass the plasma FFA pool. When heparin is

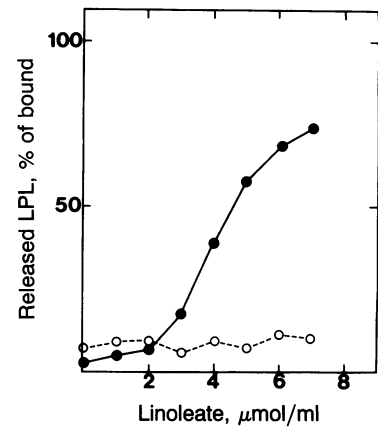


FIG. 4. Disruption of LPL binding to heparin-agarose by fatty acids in the presence of Intralipid. ¹²⁵I-labeled LPL was allowed to bind to heparin-agarose and, after washes, buffer containing Intralipid (5 mg of TG per ml) and the indicated amount of linoleic acid without (●) or with (○) bovine serum albumin at 67 mg/ml was added. The values plotted are the combined radioactivity in two supernatants, expressed as percent of the amount of lipase initially bound to the gel.

injected during infusion of Intralipid, plasma FFA levels increase to 2–3 mM (26, 27). This is similar to the high values observed here in some of the subjects, suggesting that in these individuals a large fraction of the fatty acids recirculated through plasma. Why would this happen? The subjects were fasted, hence their metabolism was not geared to lipid storage but rather to lipid mobilization and degradation. The high rate of lipid infusion was much above immediate energy requirement, and probably exceeded the local capacity of some tissues to absorb and utilize the fatty acids. This would result in accumulation of fatty acids at sites of lipolysis and spillage as FFA into the circulating blood. We propose that an additional effect is to displace LPL from these endothelial sites.

We demonstrate here that LPL is displaced from heparin-agarose by Intralipid/fatty acid mixtures, and Saxena *et al.* (28) have demonstrated that fatty acids displace LPL from cultured endothelial cells. Previous *in vitro* studies have demonstrated that FFAs abolish the lipolysis-promoting effects of apolipoprotein C-II (9) and impede binding of LPL to lipid droplets (6, 7). Clark and Quarfordt (29) have shown that FFAs disrupt the binding of apolipoprotein-E-supplemented TG emulsions to heparin-agarose. Thus, accumulation of FFA may regulate hydrolysis of lipoprotein TG by affecting several of the interactions involving the lipoprotein particle, the lipase, the activator protein, and the endothelial binding sites.

Chylomicra (and emulsion droplets) are large particles on a molecular scale and often contain millions of TG molecules. Yet they are rapidly delipidated by LPL *in vivo*, and calculations based on the turnover number of the enzyme show that many LPL molecules must act simultaneously on the particle (8). The interaction of soluble LPL with model lipoproteins is fairly tight (30). Hence, cooperative binding to several LPL molecules would hold the chylomicron firmly at endothelial-binding-lipolysis sites. It can be argued that to break these bonds the particle would either have to be reduced sufficiently in size or the affinity of its surface for LPL would have to decrease (e.g., by accumulation of fatty acids and/or other lipolysis products). In the present situation, FFAs were probably most important. In situations with unhindered fatty acid flow into the tissue, lysophospholipids and/or monoglycerides may be more important. It is thus possible that what we see in this study is an exaggeration of a normal mechanism in chylomicron metabolism.

In vitro studies have shown that albumin can reverse the effects of fatty acids on LPL (6–8, 31), and in the present experiments albumin abolished the ability of fatty acids to release LPL from heparin-agarose. It, therefore, appears unlikely that it was the circulating FFAs as such that caused the displacement of LPL from endothelial binding sites; more likely, most of the effect was exerted at endothelial sites of lipolysis, before the fatty acids were bound to albumin. On the other hand, Saxena *et al.* (28) have reported that fatty acids displace some LPL from endothelial cells even in the presence of albumin, indicating that the *in vivo* binding may be more sensitive to low concentrations of fatty acids than the binding to heparin-agarose studied here.

The hypothesis that overloading may bring product control of LPL action into play raises the question of what regulates the rate of TG clearing. Many studies have shown correlations of fasting plasma TG levels, of kinetically determined rates of TG turnover in very low density lipoproteins, and of the clearing of TG from plasma after test meals to LPL activity, as measured in tissue biopsies or in post-heparin plasma (for review, see ref. 3). On the other hand, there are cases where this correlation does not hold. The most dramatic examples are probably those patients with type V hyperlipoproteinemia, who have grossly elevated plasma TG despite normal or nearly normal tissue LPL activities (see ref. 3). Another well-studied case is the insulin-deficient rat, which has defective removal of very low density lipoprotein-TG from plasma with normal muscle and only moderately reduced adipose tissue LPL activity (32). In some of these cases the major rate-limiting factor in plasma TG turnover may be the capacity of the tissues to assimilate fatty acids rather than the capacity of the lipase to release them from the TG. In this respect it is interesting that Carlson and Walldius (5) have found an inverse relation between fasting plasma TG levels and the ability of adipose tissue to assimilate fatty acids *in vitro*.

Previous studies have demonstrated hormonal control of LPL synthesis and release from lipase-producing cells (for review, see ref. 2). These mechanisms determine the amount of LPL at the endothelium and hence the maximal capacity of the tissue to hydrolyze lipoprotein TG. Our study suggests that LPL is also subject to feedback control by its main product but that the mechanism is unusual in that the fatty acids may regulate not only the catalytic activity of the enzyme but also its distribution between endothelial sites.

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