Differential binding of triglyceride-rich lipoproteins to lipoprotein lipase

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Abstract In comparison to very low density lipoprotein (VLDL), chylomicrons are cleared quickly from plasma. However, small changes in fasting plasma VLDL concentration substantially delay postprandial chylomicron triglyceride clearance. We hypothesized that differential binding to lipoprotein lipase (LPL), the first step in the lipolytic pathway, might explain these otherwise paradoxical relationships. Competition binding assays of different lipoproteins were performed in a solid phase assay with purified bovine LPL at 4°C. The results showed that chylomicrons, VLDL, and low density lipoprotein (LDL) were able to inhibit specific binding of ^{125}I-labeled VLDL to the same extent (85.1% ± 13.1, 100% ± 6.8, 90.7% ± 23.2% inhibition, P = NS), but with markedly different efficiencies. The rank order of inhibition (K_i) was chylomicrons (0.27 ± 0.02 nM apoB) > VLDL (12.6 ± 3.11 nM apoB) > LDL (34.8 ± 11.1 nM apoB). By contrast, neither triglyceride (TG) liposomes, high density lipoprotein (HDL), nor LDL from patients with familial hypercholesterolemia were efficient at displacing the specific binding of ^{125}I-labeled VLDL to LPL (30%, 39%, and no displacement, respectively). Importantly, smaller hydrolyzed chylomicrons had less affinity than the larger chylomicrons (K_i = 2.34 ± 0.85 nM vs. 0.27 ± 0.02 nM apoB respectively, P < 0.01). This was also true for hydrolyzed VLDL, although to a lesser extent. Chylomicrons from patients with LPL deficiency and VLDL from hypertriglyceridemic subjects were also studied. ■ Taken together, our results indicate an inverse linear relationship between chylomicron size and K_i whereas none was present for VLDL. We hypothesize that the differences in binding affinity demonstrated in vitro when considered with the differences in particle number observed in vivo may largely explain the paradoxes we set out to study.—Xiang, S-Q., K. Cianflone, D. Kalant, and A. D. Sniderman. **Differential binding of triglyceride-rich lipoproteins to lipoprotein lipase.** *J. Lipid Res.* 1999. **40:** 1655–1662.

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Postprandial lipid metabolism involves the metabolism of all classes of lipoproteins, including remodeling of LDL and HDL substrates in plasma (1–3). It is important to appreciate that the absolute and relative concentrations of the substrate lipoproteins, particularly triglyceride-rich lipoproteins (TRL), are critical in modulating the direction of lipid transfer between lipoproteins (4). There are two major plasma TRL, chylomicrons, composed of dietary lipids from the intestinal mucosal cells, and the smaller very low density lipoproteins (VLDL) derived from the liver. The first step in catabolism of TRL is hydrolysis of the particle core triglycerides by lipoprotein lipase (LPL). Brunzell and colleagues (5) were the first to emphasize the inverse relationship between fasting plasma triglycerides and the rate of chylomicron clearance and suggested that this was due to competition between VLDL and chylomicrons for available LPL on the capillary endothelium. There is general agreement with the hypothesis that both chylomicrons and VLDL share the same saturable lipolytic pathway (6–8). It has been shown that VLDL accumulates in human plasma after fat injection due to delayed lipolysis of the VLDL particles because of a failure to compete efficiently with chylomicrons for the sites of LPL (8). Interestingly, the increase in number of VLDL particles postprandially is far greater than that of chylomicron particles. Several independent studies have shown that 80% of the increase in particle number was accounted for by apolipoprotein (apo) B-100-containing particles, not apoB-48 and was confined to large VLDL particles (9–11). Thus, even in the postprandial phase, VLDL particle number is much greater than that of chylomicrons. On the other hand, in vivo, chylomicron TG clearance is much faster, 50-fold faster in fact, than VLDL TG clearance (6). Two questions immediately arise. First, given the difference in particle number how can chylomicrons compete so effectively with VLDL for binding to LPL, and second, given the differences in their clearance rates, how can relatively small differences in VLDL concentration produce the large differences in chylomicron clearance which have been observed in vivo? Our hypothesis was that the differ-

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ential binding to LPL of TRL might answer these questions. Therefore, in this study, we performed competition binding experiments of lipoprotein particles to LPL in a solid phase assay in the absence of lipolysis and compared the binding affinities of apoB-containing lipoproteins, particle for particle, to LPL. We have also examined regulatory factors on substrate-LPL binding and the interrelationship of apoB-containing particles in TG hydrolysis.

MATERIALS AND METHODS

Preparation of lipoproteins

All lipoproteins were isolated from fresh normal human plasma by the standard sequential flotation method (12). Briefly, chylomicrons ($S_f > 400$) were isolated from plasma by ultracentrifugation in a Beckman Type 50 rotor at 30,000 rpm for 32 min at 5°C. VLDL (S_f 60–400) were isolated at 40,000 rpm for 122 min and LDL (S_f 2–12) were isolated from the infranatant, after removal of VLDL and IDL, at 40,000 rpm for 24.4 h. HDL (d 1.063–1.21 g/mL) were isolated at 50,000 rpm for 48 h. Each fraction was harvested by aspiration from the top of the tube. All lipoproteins were concentrated by ultracentrifugation and dialyzed against 0.15 m NaCl containing 0.01% EDTA. Chylomicrons were also isolated by ultracentrifugation from two homozygous patients with LPL deficiency; VLDL from one patient with hypertriglyceridemia and LDL from two patients with familial hypercholesterolemia.

Hydrolyzed lipoprotein remnants were prepared in vitro by using purified LPL (Sigma Chemical Co., St. Louis, MO) as described by Chajek-Shaul et al. (13) and re-isolated by ultracentrifugation to remove the LPL as described by Huff et al. (14). TG phospholipid emulsion containing no apolipoproteins was obtained from Sigma Chemical Co.

Triglyceride was determined with an enzymatic colorimetric kit (Boehringer Mannheim) while apoB was measured by ELISA immunoassay (15). Protein was measured by the procedure of the Lowry et al. (16), with BSA (fatty acid-free bovine serum albumin) as standard. Molar concentrations of chylomicrons, VLDL, and LDL were calculated based on apoB concentration where there is one apoB molecule per lipoprotein particle (17, 18). HDL molar concentration was calculated as described previously (19) where the total mass of the particle was calculated from the protein concentration assuming protein constitutes 41% of the particle. The molecular mass of an HDL particle is 3.6×10^5 Da (19).

Iodination and binding assay

VLDL was iodinated using Iodogen (Pierce Chemicals, Rockford, IL). The average specific activity was 1500 cpm/ng. More than 90% of the radioactivity was precipitated by 10% trichloroacetic acid, indicating that the radiolabel was protein-associated. Of the lipoprotein-associated label, 20% was extractable by chloroform–methanol 2:1 (v/v) containing primarily labeled cholesteryl ester, triglyceride, cholesterol, and free fatty acid (equally distributed). The protein-associated radiolabel was primarily present in apoB as well as apoC and apoE based on autoradiography after gel electrophoresis on 4–20% SDS-PAGE. The iodinated VLDL was kept at 4°C and used within 3 weeks.

Solid phase plate binding assays were performed as described by Williams et al. (20). Microtiter wells were coated overnight at 4°C with 100 μL of purified bovine LPL (1 μg/mL) in 50 mm Tris, 150 mm NaCl, pH 7.4 (TBS) containing 5 mm Ca^{2+}. After removing the unbound LPL, the wells were blocked with 3% BSA in TBS, 5 mM Ca^{2+} for 1 h at room temperature. Five μg/mL ^{125}I-labeled VLDL was added to each well along with increasing concentrations

of unlabeled ligands in TBS containing 5 mm Ca^{2+}, 3% BSA (fatty acid free) in a final volume of 100 μL. After overnight incubation at 4°C, the wells were washed with 0.3% BSA in TBS. Two hundred μL of 0.1 N NaOH was added, and 150-μL aliquots were counted to determine the amount of bound VLDL.

Data were analyzed using iterative four parameter logistic function analysis (Sigma Plot, Jandel Scientific, San Rafael, CA) to calculate the following parameters: *i*) top plateau of the competition curve defined as binding in the absence of competitor; *ii*) bottom plateau of the competition curve defined as non-specific binding in the presence of excess competitor. The difference between *i*) and *ii*) is defined as the specific component of binding while IC_{50} is defined as the concentration of competitor required to compete out 50% of the specific binding. The equilibrium dissociation constant (K_i) was calculated from IC_{50} using the equation of Cheng and Prusoff (21). On no occasion did a 2-site rather than 1-site binding model yield a significantly better fit. All results are presented as average ± standard error of the mean (SEM). Statistical differences were analyzed by *t*-test or ANOVA where significance was taken as $P < 0.05$ and NS = not significant.

RESULTS

Specific binding of VLDL to LPL

Our first objective was to investigate the specific binding of VLDL to lipoprotein lipase (LPL) and to analyze the affinity of association (K_i). Kinetics of LPL–lipoprotein interaction in solution might fail to assess some factors regulating LPL actions in normal physiology. In order to compare the initial binding affinity of different classes of lipoproteins to LPL and to assess the role of some factors in the binding, the present studies were conducted by coating LPL on microtiter plates. The association of VLDL to LPL was determined on the basis of VLDL apoB concentration in order to determine particle number as each apoB lipoprotein (chylomicrons, VLDL, LDL) contains only one molecule of apoB (17, 18). All experiments were conducted at 4°C in order to examine only enzyme–substrate interaction in the absence of active lipolysis, and to exclude the possible effects of catalytic products and the changing of the size and composition of lipoprotein particles on the binding to LPL.

Homologous competition binding assays were performed with ^{125}I-labeled VLDL and increasing concentrations of unlabeled VLDL. Microtiter wells were coated with purified LPL (1 μg/mL) and blocked with 3% BSA. ^{125}I-labeled VLDL (5 μg/mL) was added to each well along with increasing concentrations of the same native VLDL. Unlabeled VLDL displaced bound ^{125}I-labeled VLDL in a concentration-dependent manner indicating that VLDL can bind specifically to an apparently single binding site of LPL with high affinity (**Fig. 1**) with average K_i of 12.6 ± 3.11 nm apoB (n = 9, **Table 1**). In the absence of LPL (plates coated with BSA), there was only non-specific association of ^{125}I-labeled VLDL and no competition with unlabeled VLDL (Fig. 1).

Effect of NaCl, Ca^{2+} and FFA on the binding

NaCl, Ca^{2+}, and FFA have been shown to influence LPL activity (22–26). We therefore tested the effects of these factors on LPL–substrate interaction in the absence of active lipolysis. Results showed that the specific binding of VLDL to
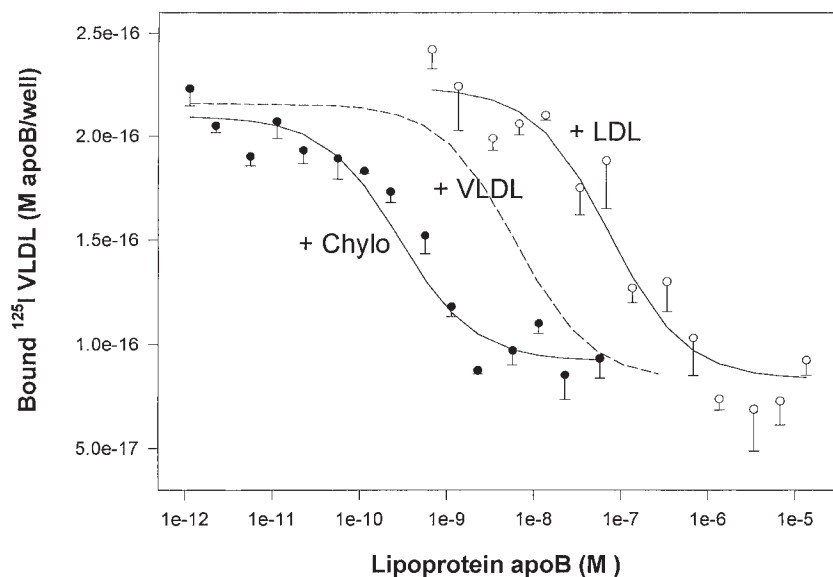
Fig. 1. The binding of VLDL to lipoprotein lipase. Microtiter wells were coated with purified bovine LPL (●) (1 μg/mL) or 3% BSA (○), and 5 μg/mL ^{125}I-labeled VLDL was added to each well along with increasing concentrations of native VLDL (5.4×10^{-11} to 2.7×10^{-7} m) in a buffer containing 5 mm Ca^{2+} and 3% fatty acid-free BSA. After overnight incubation at 4°C, the wells were washed, 200 μl of 0.1 N NaOH was added, and 150-μl aliquots were counted to determine the amount of bound ^{125}I-labeled VLDL. Each data point is the average of triplicate values. The curves represent the best fit of the data to a single class of binding sites by iterative 4 parameter logistic function analysis.

LPL was reduced (41.5% ± 9.5 inhibition) in the presence of 1 m NaCl without any effect on non-specific binding (**Fig. 2**). To assess the effect of divalent Ca^{2+} dependence, we performed the same binding with or without 5 mm Ca^{2+}. As shown in Fig. 2, there was no specific binding of ^{125}I-labeled VLDL to LPL in the absence of Ca^{2+} in the buffer. Thus full LPL enzymatic activity is dependent on initial substrate binding, which is Ca^{2+}-dependent and can be affected by the ionic strength of the buffer. Finally, we also tested the effect of free fatty acid on VLDL–LPL interaction. In contrast to the inhibitory effect that FFA have on LPL activity (22) and on LPL–endothelial cell interaction (24), FFA did not appear to have any effect on the VLDL–LPL interaction even when the molar ratio of oleic acid was as high as 6:1 (Fig. 2, insert). However, most LPL were released from endothelial cells at this ratio of oleic acid/BSA (24).

Displacement of ^{125}I-labeled VLDL binding

To assess the relative affinities of different lipoprotein classes to LPL, the ability of chylomicrons, VLDL, LDL, and HDL to inhibit the specific binding of ^{125}I-labeled VLDL to LPL coated on microtiter wells was evaluated in heterologous competition binding experiments. Results demonstrated that all apoB-containing lipoproteins displaced ^{125}I-labeled VLDL from an apparently single binding site with high affinity (**Fig. 3**). In all cases chylomicrons, VLDL, and LDL were able to inhibit specific binding of ^{125}I-labeled VLDL to the same extent (85.1% ± 13.1, 100% ± 6.8, 90.7% ± 23.2 inhibition, PNS), although with different efficiencies. In fact, chylomicrons, particle for particle, had a much greater affinity for LPL and were more efficient than native VLDL at displacing ^{125}I-labeled VLDL (47-fold greater competition based on K_i, i.e., K_iVLDL/$K_i$chylomicrons). As noted above, on no occasion did a 2-site rather than a 1-site binding model yield a significantly better fit. There was therefore no evidence for steric hindrance in our system. On the other hand, the analytic methods may not be sufficiently precise to entirely rule out this possibility. Our data also indicated that LDL was less efficient than VLDL at binding to LPL. The rank order of inhibition (K_i) was chylomicrons (0.27 ± 0.02 nm apoB) > VLDL (12.6 ± 3.11 nm apoB) > LDL (34.8 ± 11.1 nm apoB) (Table 1). By contrast, HDL was not efficient at displacing the specific binding of ^{125}I-labeled VLDL to LPL even at high molar concentrations (2×10^{-3} m) and the inhibition potency of HDL was only 39% of VLDL ($P < 0.01$, Table 1). Of interest, LDL from two patients with familial hypercholesterolemia, in contrast to LDL from normal subjects, did not significantly compete with ^{125}I-labeled VLDL for binding to LPL.

Effect of size on the binding

It is still not clear which component of the lipoprotein particle surface is responsible for the binding of lipoproteins to LPL. Previous studies have suggested that larger particles are more favorable for hydrolysis by LPL (27–29). To assess the effect of lipoprotein particle size on the binding to LPL, both chylomicrons and VLDL were hydrolyzed in vitro and then reisolated under conditions that dissociated LPL from the particle as demonstrated by Huff et al. (14). This resulted in a reduction of the amount of triglyceride per particle based on apoB (moles TG/mole apoB) where chylomicron = 1.2×10^5 vs. 0.3×10^5 for hydrolyzed chylomicrons (a 4-fold difference).

TABLE 1. Efficiency and potency of lipoprotein binding to LPL

Lipoprotein	K_i	% Inhibition	n
	n$_M$ apoB	%	
Chylomicrons	0.27 ± 0.02[a]	85.1 ± 13.1	6
VLDL	12.6 ± 3.11	100	9
LDL	34.8 ± 11.1[a]	90.7 ± 23.2	3
HDL	170 ± 110[a]	39 ± 3.6[a]	3

Results are presented as the average ± SEM for the indicated number of experiments for the displacement of ^{125}I-labeled VLDL binding to LPL, where [a] $P < 0.01$ vs. VLDL. Specific binding was calculated using four parameter logistic function analysis (see Methods for details) and competition by VLDL set as 100% inhibition.
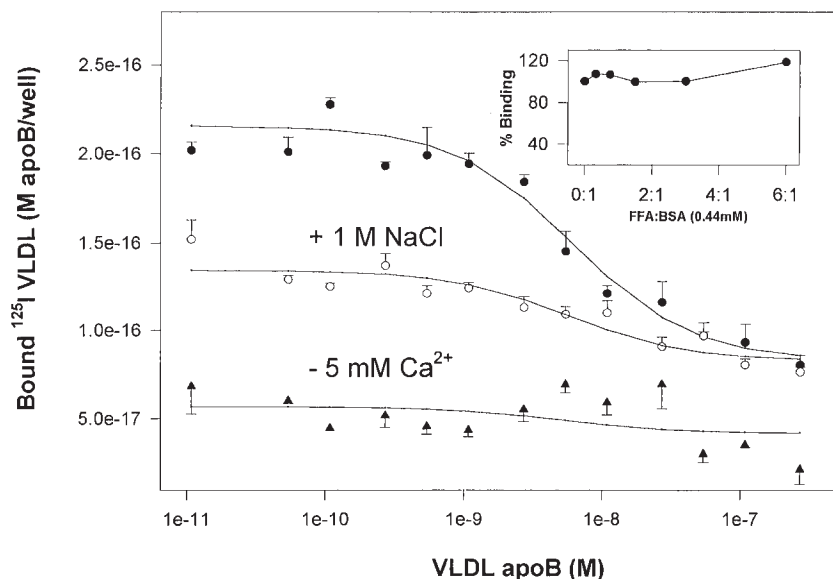
Fig. 2. Effect of concentration of NaCl, Ca^{2+}, and free fatty acids on competition binding of VLDL to LPL. Competition binding was performed as described in Fig. 1 with ^{125}I-labeled VLDL (●), in the presence of 1 m NaCl (○) or absence of 5 mm Ca^{2+} (▲). Each data point is an average of triplicate values. Effect of increasing oleic acid/BSA molar ratio on the binding of VLDL to LPL: competition binding was performed as described in Fig. 1; 5 μg/mL ^{125}I-labeled VLDL was added with 0.44 mm BSA and oleic acid at various concentrations producing the oleic acid/BSA molar ratios indicated. Each data point is an average of triplicate values. The curves represent the best fit of the data to a single class of binding sites by iterative 4 parameter logistic function analysis.

Similarly, VLDL composition changed from 1.68×10^4 moles TG/mole apoB to 1.1×10^4 moles TG/mole apoB for hydrolyzed VLDL (a 50% reduction). In both cases, however, the maximal inhibition of specific binding of ^{125}I-labeled VLDL was comparable: 85.1% ± 13.1 vs. 103.9% ± 25 for chylomicrons and 100% ± 6.8 vs. 98.9% ± 32.9 for VLDL (native vs. hydrolyzed, respectively). However, as shown in **Fig. 4**, hydrolyzed chylomicrons were about 10 times less efficient at competition than native chylomicrons (K_i = 2.34 ± 0.85 nm apoB vs. 0.27 ± 0.02 nm apoB, respectively, n = 4, $P < 0.01$), although the hydrolyzed chylomicrons were still more efficient than native VLDL. With hydrolyzed VLDL, the small change in TG/apoB content only marginally altered the K_i (K_i = 14.9 ± 4.4 nm apoB for hydrolyzed VLDL vs. 12.6 ± 3.1 nm apoB for VLDL PNS).

Additional studies to examine the effect of size of the triglyceride-rich lipoproteins on their affinity to LPL were carried out. The data above plus data from chylomicrons isolated from two patients with homozygous LPL deficiency and VLDL from a patient with hypertriglyceridemia are shown in **Fig. 5**. Note that the K_i for VLDL of any size was greater than the K_i for chylomicrons of any size. On the other hand, as chylomicron size decreased there was a linear increase in K_i whereas this was not the case for VLDL in which no apparent relation between size and K_i was evident.

Effect of TG emulsion on the binding

The lipoprotein particle surface is composed of apolipoproteins as well as lipids, mainly phospholipids. Both components appear to be able to bind to LPL in solution (22, 30–32). In order to determine the participation of lipids alone in the solid-phase LPL interaction, we tested the capacity of a TG–phospholipid emulsion on ^{125}I-labeled VLDL competition. As shown in **Fig. 6**, even with much higher concentra-
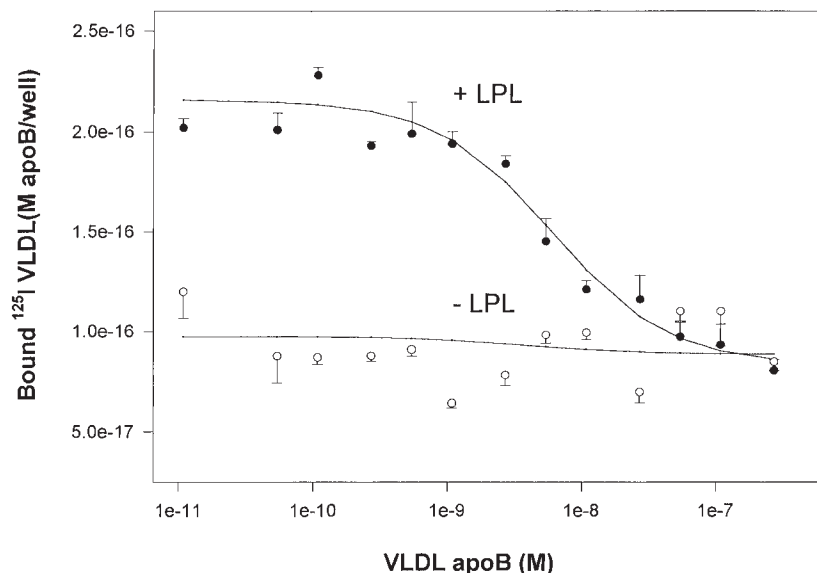
Fig. 3. Heterologous competition binding of chylomicrons, VLDL and LDL. Competition binding was performed as described in Fig. 1. The binding of ^{125}I-labeled VLDL to LPL was competed by increasing concentrations of native chylomicrons (Chylo) (●), or LDL (○) based on apoB concentration. Each data point is the average of triplicate values. A binding curve for VLDL homologous binding (Fig. 1) is also shown (- - - -). The curves represent the best fit of the data to a single class of binding sites calculated by iterative 4 parameter logistic function analysis.
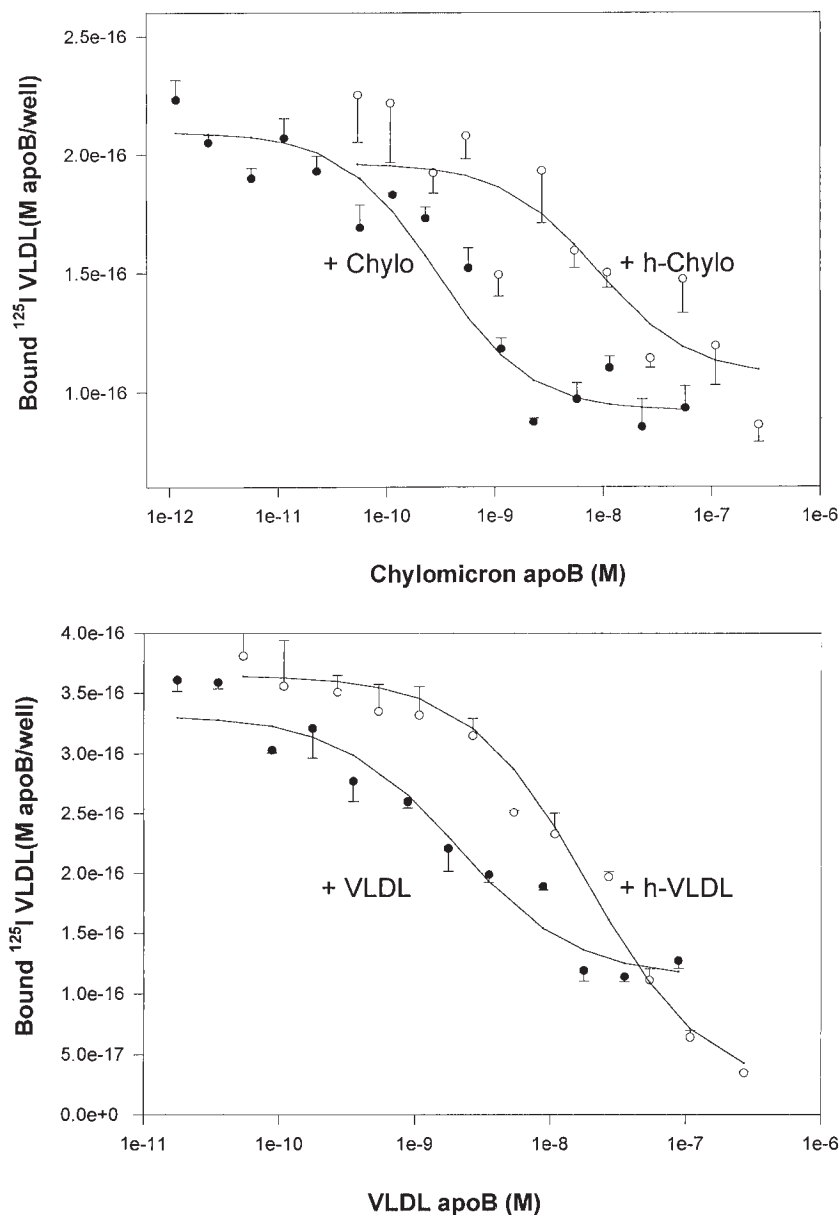
Fig. 4. Competition binding of native and hydrolyzed chylomicrons and VLDL to LPL. Competition binding was performed as described in Fig. 1 with competition of native chylomicrons (Chylo) (●) and hydrolyzed chylomicrons (h-Chylo) (○) (upper panel) or native VLDL and hydrolyzed VLDL (h-VLDL) (○) (lower panel). Each data point is the average of triplicate values. The curves represent the best fit of the data to a single class of binding sites by iterative 4 parameter logistic function analysis.

tions of triglyceride (relative to native VLDL triglyceride), the inhibition potency of the triglyceride–phospholipid emulsion achieved maximal inhibition at concentrations 83-fold higher than an equivalent concentration of native VLDL and was relatively low (maximum 30% displacement).

DISCUSSION

As early as the 1970s, Brunzell et al. (5) observed an inverse relationship between fasting TG and the rate of chylomicron clearance and proposed that chylomicrons and VLDL share a common saturable lipolytic pathway at the capillary endothelial surface. Data from later studies by Grundy and Mok (6) were in accordance with this hypothesis. However, they also emphasized the great difference in rates of TG clearance between chylomicrons and VLDL (6). The clearance rates for chylomicron TG were ex-

tremely rapid ($t_{1/2}$ = 4.5 min), even in hyperlipidemic subjects ($t_{1/2}$ = 23 min), as compared to clearance of VLDL TG ($t_{1/2}$ = 264 min). In agreement with this are the results of recent studies using the arterio-venous difference technique across subcutaneous abdominal adipose tissue which have shown the removal of TG from chylomicrons to be 10 times greater than that from VLDL after a mixed meal (33). In addition, it is known that VLDL particle number (based on apoB concentration) is much higher than that of chylomicrons in the postprandial state (the ratio is about 20:1) and that the increase in VLDL particle number in the postprandial state is greater than the increase in chylomicron particle number (34, 35).

Thus, a series of unresolved paradoxes seem to underlie the Brunzell hypothesis. If VLDL compete so effectively with chylomicrons for a common saturable lipolytic pathway, they presumably must have equal binding affinities to LPL. But if so, why are chylomicrons cleared so much
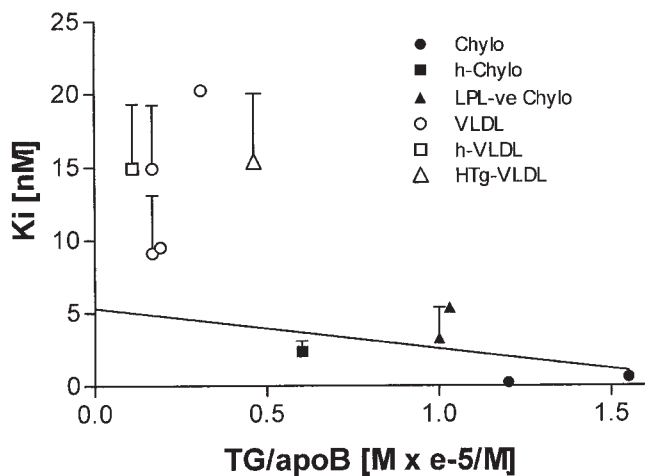
Fig. 5. The relation between the size of the triglyceride-rich lipoproteins and their binding affinity to LPL. Competition binding was performed as described in Fig. 1. Native chylomicrons (●), chylomicrons from patients with LPL deficiency (▲), hydrolyzed chylomicrons (■), native VLDL (○), VLDL from hypertriglycerdemic subjects (△) and hydrolyzed VLDL (□) were all studied. Each point is the average of 2–6 competition binding experiments. Size is expressed as TG/apoB molar ratio and binding affinity as K_i (nm apoB). An inverse linear relationship between K_i and size was evident for chylomicrons but not for VLDL.

faster than VLDL given that chylomicron particle number is so much smaller than VLDL particle number (8, 34, 35)? On the other hand, if VLDL do not compete effectively with chylomicrons, why do even small increases in fasting VLDL produce such obvious changes in postprandial chylomicron clearance?

Many studies have shown that chylomicrons and VLDL are metabolized in two steps (36). The first involves the lipoprotein catalysis by LPL anchored on the endothelial cell surface. A complex series of events occurs which includes core TG hydrolysis stimulated by interaction with apoC-II and the exchange of neutral lipids and particle surface components (phospholipids, cholesterol, and certain apoproteins), all of which lead to the formation of chylomicron and VLDL remnants and the massive and abrupt release of fatty acids. In the second step, chylomicron remnants and large VLDL remnant particles are taken up into hepatocytes by receptor-dependent endocytosis, while some VLDL remnants are further modified to form LDL.

However, before these catalytic processes can begin, the triglyceride-rich lipoprotein particles must first bind to LPL on the capillary wall. Although lipoproteins can bind to heparin sulfate proteoglycans on cell surfaces through apoB and apoE (37, 38), it has been demonstrated that LPL can dramatically enhance the binding of apoB-containing lipoproteins to endothelial cells (39). Therefore, our hypothesis was that differences in the binding affinity of the two triglyceride-rich lipoproteins to LPL might explain their different clearances in vivo. To the best of our knowledge, this hypothesis has not been put forward before but our data do lend it support.

Much remains to be learned about the specific determinants of the binding between LPL and the triglyceride-rich lipoproteins. Of importance, the binding site of LPL to the triglyceride-rich lipoproteins appears to be different from its catalytic site (39) and is presumably also different from the site on LPL that anchors it to endothelial cells. Our results demonstrate a clear inverse relationship between chylomicron size and K_i such that larger chylomicrons have a higher apparent affinity to LPL than do smaller ones. Moreover, overall, VLDL particles have substantially higher values for K_i than do chylomicrons. However there is no predictable relationship between VLDL size and K_i as there is for chylomicrons.

The specific basis for the difference in binding affinity between chylomicrons and VLDL and LPL remains to be determined. An effect of size cannot be entirely excluded but on the basis of our results seems unlikely to be the sole explanation as no relationship between K_i and VLDL
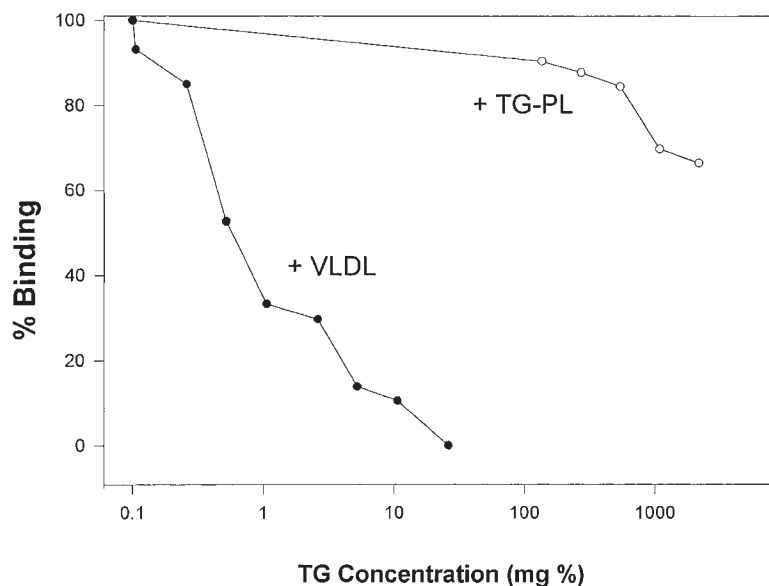
Fig. 6. Competition binding of TG-phospholipid emulsion. Competition binding of ^{125}I-labeled VLDL to LPL was competed by native VLDL (●) or increasing concentrations of TG-phospholipid emulsion (TG-PL) (○) expressed as triglyceride concentration. Each point is the average of triplicate values.

size was evident and the values for VLDL were very different from those obtained for chylomicrons. Certainly, chylomicrons and VLDL differ in many respects other than size and one of these properties might well be a more critical determinant of binding affinity. In these studies, no positive evidence for steric hindrance was obtained (40) but our analytic methods are not sufficiently robust that the possibility should be dismissed. In any case, our assay was designed to mimic the physiologic state in which VLDL are already present and interacting with LPL before chylomicrons appear on the scene and therefore it is not unreasonable to extrapolate our apparent in vitro K_i to the in vivo situation.

Recently Sungshin et al. (30), Sivaram et al. (41), and Choi et al. (42) in a series of experiments using different techniques, reported that LDL apoB binds to LPL through an amino-terminal fragment of apoB, and that apoE does not appear to be a major determinant of LPL binding to lipoproteins. Data from binding experiments by Carrero et al. (22), using lipoprotein co-precipitation after incubating lipoproteins with LPL in solution at 37°C, are in accordance with the above studies. However, in none of these studies were chylomicrons tested.

More recently, an elegant study by Lookene, Savonen, and Olivecrona (39) using a sensitive surface plasmon resonance technique demonstrated that LPL plays a major role in efficient binding of chylomicrons, VLDL, and LDL to heparin sulfate-covered surfaces. In this study, the calculated binding affinities did not differ between chylomicrons and VLDL. However, the estimate of particle number was not based on directly measured apoB. As the authors pointed out, because the experiments were conducted at 37°C, the size and compositions of lipoproteins were in a state of flux, therefore, the constants generated were the total of both native and hydrolyzed lipoproteins.

The present studies represent, therefore, the first effort to compare the binding affinities of different classes of lipoproteins to LPL in solid phase at 4°C, particle for particle. The results showed that the estimated K_i of both chylomicrons and VLDL were within the normal range of postprandial plasma concentrations (0.77 nm for chylomicrons and 49 nm for VLDL) (31, 32). However, the binding affinity of chylomicrons to LPL was almost 50-fold higher than VLDL.

These data suggest that the known differences in LPL-induced lipolysis in chylomicrons and VLDL (27) might be a function of their differences in binding affinity to LPL. More importantly, they suggest a solution to the paradoxes that follow from the hypothesis of Brunzell et al. (5). As just noted, particle for particle, chylomicrons bind to LPL much more avidly than to VLDL. On the other hand, in vivo, VLDL particle number is always 20-fold or more greater than chylomicron particle number. Therefore, given the difference in binding affinities which have been demonstrated in this study and the differences in particle number observed in vivo in the postprandial state, there should be a relatively equal chance of either a chylo-

micron or a VLDL particle binding to a newly unoccupied LPL binding site. This quantitative relationship would then explain how even modest increases in VLDL particle number could produce marked delays in chylomicron TG clearance.

How then can the fact that, even in hypertriglyceridemics, chylomicron TG clearance is always so much more rapid than VLDL TG clearance (6) be explained? The paradox disappears if we consider the pool size of the two lipoprotein classes in terms of their relative particle numbers. As just noted, the relative binding affinities of chylomicrons and VLDL documented in this study indicate that in vivo, there will be an equal chance of either a chylomicron or VLDL particle binding to an unoccupied LPL molecule. However, even though there is an equal likelihood of binding of either type of particle, binding of one or the other will produce a vastly different effect on unbound particle pool size.

That is, because there are always at least twenty times more VLDL than chylomicron particles, binding of a chylomicron particle will proportionately reduce the unbound chylomicron particle pool size much more than binding of a VLDL particle will reduce the unbound VLDL particle pool size. As the half life of each lipoprotein will depend on the rate of turnover of particles within the pool, the quantitative differences in binding affinity demonstrated in this in vitro study taken together with the quantitative differences in particle number in vivo appear to resolve the pathophysiologic paradoxes this study was intended to address. At the same time, however, we must not lose sight of other processes which may importantly affect the plasma half-lives of the triglyceride-rich lipoproteins in plasma such as the possibility of differential hepatic remnant removal rates for chylomicrons as opposed to VLDL remnants and the fact that our data were obtained in a non-physiologic in vitro model. Nevertheless, the data obtained in this study suggest that further understanding of the determinants of the binding of the triglyceride-rich lipoproteins to LPL may provide fruitful insights into the determinants of the rate of clearance from plasma of these biologically critical particles.

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