Triacylglycerol and Phospholipid Hydrolysis in Human Plasma Lipoproteins: Role of Lipoprotein and Hepatic Lipase†

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ABSTRACT: To explore the interactions of triacylglycerol and phospholipid hydrolysis in lipoprotein conversions and remodeling, we compared the activities of lipoprotein and hepatic lipases on human VLDL, IDL, LDL, and HDL_2. Triacylglycerol and phospholipid hydrolysis by each enzyme were measured concomitantly in each lipoprotein class by measuring hydrolysis of [^{14}C]triolein and [^{3}H]dipalmitoylphosphatidylcholine incorporated into each lipoprotein by lipid transfer processes. Hepatic lipase was 2–3 times more efficient than lipoprotein lipase at hydrolyzing phospholipid both in absolute terms and in relation to triacylglycerol hydrolysis in all lipoproteins. The relationship between phospholipid hydrolysis and triacylglycerol hydrolysis was generally linear until half of particle triacylglycerol was hydrolyzed. For either enzyme acting on a single lipoprotein fraction, the degree of phosphohydrolysis closely correlated with triacylglycerol hydrolysis and was largely independent of the kinetics of hydrolysis, suggesting that triacylglycerol removed from a lipoprotein core is an important determinant of phospholipid removal via hydrolysis by the lipase. Phospholipid hydrolysis relative to triacylglycerol hydrolysis was most efficient in VLDL followed in descending order by IDL, HDL, and LDL. Even with hepatic lipase, phospholipid hydrolysis could not deplete VLDL and IDL of sufficient phospholipid molecules to account for the loss of surface phospholipid that accompanies triacylglycerol hydrolysis and decreasing core volume as LDL is formed (or for conversion of HDL_2 to HDL_3). Thus, shedding of whole phospholipid molecules, presumably in liposomal-like particles, must be a major mechanism for losing excess surface lipid as large lipoprotein particles are converted to smaller particles. Also, this shedding phenomenon, like phospholipid hydrolysis, is closely related to the hydrolysis of lipoprotein triacylglycerol.

Lipases are major regulators of the metabolism of human plasma lipoproteins. Triacylglycerol hydrolysis has long been recognized as essential for the catabolism of the triacylglycerol-rich lipoprotein particles, chylomicrons, and very low density lipoproteins (VLDL)[1] to smaller particles. Formation of remnant particles and conversion of VLDL to LDL are dependent upon removal of triacylglycerol core molecules by lipolytic pathways as larger particles are converted to smaller particles such as chylomicron remnants and LDL (Eisenberg & Levy, 1975; Havel, 1978). More recently, it has been recognized that the lipases also hydrolyze triacylglycerols in the cholesteryl ester rich lipoproteins, LDL and HDL, and are important in particle remodeling after transfer of triacylglycerol into these particles in exchange for cholesteryl ester (Musliner et al., 1979; Deckelbaum et al., 1982, 1986; Barter, 1990). Concomitant with triacylglycerol hydrolysis in lipoprotein particles, surface phospholipids are removed as the surface area required to cover a shrinking particle core diminishes. Phospholipid can be either shed as intact molecules from these particles as surface remnants or removed after hydrolysis of phosphatidylcholine to lysophosphatidylcholine with binding of the latter to albumin (Chajek & Eisenberg, 1978). The relative contributions of these two processes to particle remodeling are not well defined.

Two lipases, lipoprotein lipase and hepatic lipase, mediate lipolysis in plasma lipoproteins. These enzymes are structurally related and likely have similar active sites (Jackson, 1983; Garfinkel & Schotz, 1987; Olivecrona & Bengtsson-Olivecrona, 1990). Analysis of their cDNA suggests that they are derived from a common ancestral gene (Kirchgesser et al., 1989). Genetic deficiencies of either enzyme result in abnormal composition and structure of all plasma lipoproteins and derangements in lipoprotein triacylglycerol metabolism (Hayden et al., 1991). Both enzymes hydrolyze triacylglycerol and phospholipid with the same positional specificity. They are presumed to have similar (or the same) active sites. But these two enzymes have important differences. Lipoprotein lipase requires the presence of an activator protein, apoprotein C-II, to express maximal activity (Smith & Pownall, 1984). Such an activator is not required by hepatic lipase. While lipoprotein lipase is though to prefer large triacylglycerol-rich lipoproteins as substrate particles, hepatic lipase has a higher affinity for smaller and denser particles, i.e., HDL (Jackson, 1983; Bengtsson & Olivecrona, 1980). It has been repeatedly suggested that hepatic lipase is a more potent phospholipase than lipoprotein lipase is (Ehnholm et al., 1975; Van Tol et al., 1980; Jackson, 1983), but the basis for this has not been explored in any detail.

Little attention has been focused on a direct comparison of triacylglycerol and phospholipid hydrolysis by the two enzymes in different lipoprotein subclasses. We demonstrate herein that when the activities of the two enzymes on a single substrate are compared, substantial differences are observed between

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[1] Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; CETP, cholesteryl ester transfer protein.

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them and between lipoprotein classes. Our results help delineate the contribution of each of these two enzymes in regulating phospholipid removal during lipoprotein conversion and remodeling processes.

MATERIALS AND METHODS

Lipoproteins. Blood was obtained in plastic syringes containing disodium EDTA (1 mg/mL) from fasting normal and hypertriglyceridemic (type IV phenotype) donors. Plasma was immediately separated at 4 °C by low-speed centrifugation. VLDL (d < 1.006 g/mL), IDL (d = 1.006–1.019 g/mL), and LDL (d = 1.019–1.063 g/mL) were then isolated by sequential salt density ultracentrifugation in a Beckman Ti-50 rotor at 48 000 rpm for 18 h at 4 °C as previously detailed (Hatch & Lees, 1968). HDL_2 (d = 1.063–1.125 g/mL) was similarly isolated but after 48 h ultracentrifugation. Each lipoprotein fraction was washed once at its highest density by repeated ultracentrifugation and then dialyzed at 4 °C in the dark for 24 h against saline (0.15 M NaCl, 1 mM EDTA, pH 8.5; 100:1 v/v) with a minimum of three changes of dialysate. All lipoproteins were then overlayered with N_2 and stored in the dark at 4 °C.

Radiolabeling of Lipoproteins. Lipoproteins were radiolabeled both in the triacylglycerol and phospholipid moieties. For radiolabeling with triacylglycerol, an individual lipoprotein was incubated with a triacylglycerol–phospholipid emulsion, 10% Intralipid, containing glycerol tri[1-^{14}C]oleate, with a specific radioactivity of 5 μCi/mL, kindly prepared and provided by KabiVitrum, Stockholm, Sweden. All incubations were performed with ^{14}C-Intralipid that had been previously washed to remove excess phospholipid liposomal-like particles present with the emulsion (Granot et al., 1985), in the presence of d > 1.21 g/mL lipoprotein-poor plasma (as a source of neutral lipid transfer activity) or partially purified cholesteryl ester transfer protein (CETP) (Deckelbaum et al., 1986). Prior to incubation the d > 1.21 g/mL plasma fraction was heated to 56 °C for 30 min to inactivate lecithin:cholesterol acyltransferase activity. Generally, incubations were carried out under conditions in which a lipoprotein (total of 2–4 mg of protein) was incubated with a 5-fold excess of emulsion triacylglycerol (emulsion triacylglycerol:lipoprotein protein = 5:1) in the presence of d > 1.21 g/mL plasma (55–70 mg/mL) or semipurified CETP for 37 °C for 7 h in an incubation volume of 5 mL containing 0.15 NaCl, 1 mM EDTA, pH 8.5. This resulted in the uptake of radiolabeled [^{14}C]triolein by the lipoprotein via neutral lipid transfer processes (Deckelbaum et al., 1982, 1986). To separate the emulsion from lipoprotein, the incubation mixture was then centrifuged twice, 30 min each time, in a SW50.1 swinging bucket rotor at 50 000 rpm.

To label the lipoproteins with radioactive phospholipid, the above mixture was then incubated in 5-mL Erlenmyer flasks that had been precoated (and dried) with 1,2-dipalmitoyl-1-phosphatidyl[*N-methyl*-^{3}H]choline (Amersham Corp.) originally dissolved in hexane. This second incubation was performed under N_2 for 2.5–4 h at 42 °C (i.e., above the crystal–liquid crystal transition temperature of dipalmitoylphosphatidylcholine). Doubly radiolabeled VLDL and IDL were then reisolated at d < 1.006 mg/mL and 1.006–1.019 g/mL by salt density ultracentrifugation as detailed above. To reisolate doubly radiolabeled LDL and HDL_2, zonal ultracentrifugation was used as previously detailed (Patsch et al., 1974; Deckelbaum et al., 1982, 1986). Briefly, the entire incubation was centrifuged in a Ti-14 zonal rotor at 14 °C at 42 000 rpm in NaBr gradients spanning d = 1.00–1.30 g/mL for LDL and d = 1.00–1.40 g/mL for HDL_2, for exactly 140 min (LDL) or 22 h (HDL_2) respectively, with the rotor effluent monitored continuously at 280 nm. The effluent fractions containing LDL or HDL_2 were then concentrated by vacuum dialysis and dialyzed as above.

Analyses. Lipoprotein lipid and protein composition was assayed as follows: total phospholipid content was determined using the method of Bartlett (1959). Total and free cholesterol was assayed using the Test-Combination Cholesterol kit (Boehringer Mannheim GmbH, Mannheim, West Germany), and triacylglycerols were assayed by the Rapidchem enzymatic triacylglycerols reagent kit (Macomb Biotechnology, Inc., Mt. Clemens, MI). Protein was measured by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

Lipases and Enzyme Activity Assay. Bovine lipoprotein lipase (LPL) was isolated from skim milk by heparin–Sepharose affinity chromatography using methods detailed elsewhere (Bengtsson-Olivecrona & Olivecrona, 1985). The purified enzyme was stored frozen (–70 °C) in 10 mM Tris-HCl, 5 mM deoxycholate, and 1.0 mM linoleate, at pH 8.5 at a concentration of ≈0.3 mg/mL. The activity of purified LPL was 300–400 units/mg of protein (1 unit = 1 μmol of fatty acid released from triglyceride/min at 25 °C). To facilitate pipeting of small volumes, the stock lipase solution was diluted 1:10–20 in 0.19 NaCl, pH 8.5, immediately before incubations. Hepatic lipase was prepared from human post-heparin plasma by chromatography on heparin–Sepharose and N-desulfated actylated heparin–Sepharose (Zaidan et al., 1985). Because hepatic lipase was not obtained as a homogeneous purified protein, activity was calculated per milliliter of enzyme solution, i.e., about 11 units/mL (25 °C).

Apoprotein C-II. Apoprotein C-II was isolated after ethanol/ether delipidation of human VLDL followed by chromatography of the pooled C-apoproteins on DEAE-cellulose in 6 M urea (Jackson & Holdsworth, 1986). Fractions containing apoprotein C-II were pooled, desalted by dialysis against 0.005 M NH_4HCO_3, and then lyophilized.

Incubations of Lipoproteins with Lipases. Incubations were carried out in 0.2 M Tris-HCl buffer, pH 8.4, containing 0.08 M NaCl, 2 IU of heparin/mL (Evans Medical Ltd., Liverpool), and 4% albumin (bovine, fatty acid free, Sigma). Generally 20–100 μg of VLDL and IDL triacylglycerol and 7–20 μg of LDL and 10–20 μg of HDL triacylglycerol were incubated in volumes of 100–250 μL. Incubations were generally at 37 °C for 30 min. Each single experiment was performed in duplicate except in a few cases where insufficient doubly radiolabeled lipoprotein was available. In incubations with LDL and HDL_2 and lipoprotein lipase, to ensure that the lack of enzyme activator would not be a limiting factor, apoprotein C-II was added in amounts shown to produce maximal triacylglycerol hydrolysis rates in pilot experiments. Hydrolysis was terminated by placing the entire incubation in 2:1 (v/v), chloroform:methanol, and the lipid extract was then separated by thin-layer chromatography using plastic silica coated sheets. The sheets were first run almost to the top of the plate in hexane:diethyl ether:acetic acid (70:30:1 v/v/v) to separate radiolabeled triacylglycerol from free fatty acid. The same plate was then dried and placed in chloroform:methanol:water:acetic acid (65:25:4:1 v/v/v/v), allowing the solvent front to run up to about half the vertical distance of the plate, thus separating radiolabeled phosphatidylcholine from lysophosphatidylcholine. After staining of standard lipids in adjacent lanes with I_2, the areas containing radiolabeled triacylglycerol, free fatty acids, lysophosphatidylcholine, and phosphatidylcholine were cut out and placed in scintillation vials and counted. No internal recovery standards were

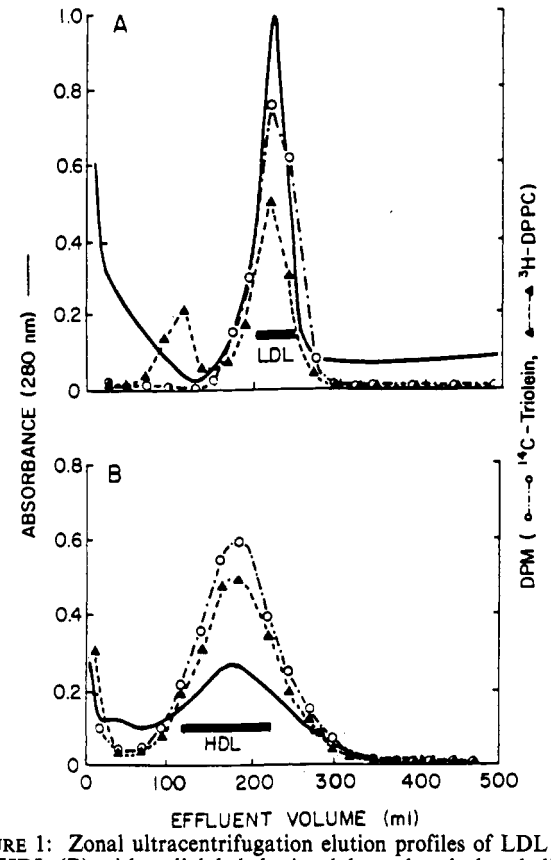

FIGURE 1: Zonal ultracentrifugation elution profiles of LDL (A) and HDL (B) with radiolabeled triacylglycerol and phospholipid. Lipoproteins were incubated with lipid emulsions containing [^{14}C]-triolein (and then in tubes coated with [^{3}H]dipalmitoylphosphatidylcholine ([^{3}H]DPPC) as detailed in Materials and Methods. The elution profiles of LDL and HDL as measured by absorbance at 280 nm (—) are compared to those of [^{14}C]triolein (O) and [^{3}H]-dipalmitoylphosphatidylcholine (DPPC; ▲). The horizontal thick bars represent the fractions of LDL and HDL collected for the hydrolysis experiments.

required because in each case percent lipolysis was calculated as

$$\frac{[^{14}\text{C}]\text{free fatty acid}}{[^{14}\text{C}]\text{free fatty acid} + [^{14}\text{C}]\text{triacylglycerol}}$$

or

$$\frac{[^{3}\text{H}]\text{lysophosphatidylcholine}}{[^{3}\text{H}]\text{lysophosphatidylcholine} + [^{3}\text{H}]\text{phosphatidylcholine}}$$

RESULTS

To determine if the radiolabeled lipids had similar distributions in lipoproteins, lipoprotein elution profiles were compared with those of [^{3}H]phospholipid and [^{14}C]triolein over the elution range of the zonal rotor effluent. As shown for a typical experiment in Figure 1, both radiolabeled triolein and phospholipid coeluted with lipoprotein with very similar distribution profiles, confirming that almost all the radiolabeling was in the particles themselves. The small phospholipid radioactivity peak (presumably representing phospholipid liposomes) preceding the LDL peak and the small amount of triacylglycerol and phospholipid radioactivity eluting before the HDL peak were not included in the lipoprotein fractions used for the experiments (Figure 1).

Incubations to incorporate radiolabeled triacylglycerol and phospholipid were designed so as to minimally modify lipoprotein composition but at the same time incorporate sufficient radioactivity to accurately determine lipolysis by measuring hydrolysis of the radiolabeled lipids. To achieve this, some enrichment of lipoproteins with triacylglycerol occurred with concomitant depletion of some cholesteryl esters, but with little change in relative protein and phospholipid contents (Table I).

To determine if the release of radiolabeled free fatty acid represented total triacylglycerol hydrolysis and if production of radiolabeled lysolecithin represented total phospholipolysis, free fatty acid and lysophosphatidylcholine mass production were compared to hydrolysis results obtained by measuring the radiolabel. Figure 2 demonstrates that hydrolysis of [^{14}C]-triolein and [^{3}H]phosphatidylcholine was similar to that of hydrolysis of total lipoprotein triacylglycerol and phosphatidylcholine. Thus, hydrolysis of the radiolabeled lipids provided an accurate and sensitive assay to measure hydrolysis of both triacylglycerol and phospholipid.

We compared, under a variety of conditions, the ability of the lipases to hydrolyze both triacylglycerols and phospholipids in different lipoproteins. Hepatic lipase was consistently more efficient at hydrolyzing phospholipids as a function of triacylglycerol hydrolysis both in experiments when the amount of enzyme was varied at a constant incubation time and in experiments when the amount of enzyme was fixed and lipolysis was assayed as a function of incubation time. For example, in Figure 3, hydrolysis of triacylglycerols and phospholipids by lipoprotein lipase and hepatic lipase are compared for VLDL and HDL_2 over increasing incubation times. In the top part of the figure (panels A and B), we show a general finding in our experiments: the ability to achieve higher levels of triacylglycerol hydrolysis in VLDL with lipoprotein lipase as compared with hepatic lipase. Conversely, in HDL_2 (panels C and D), lipoprotein lipase did not reach the degree of triacylglycerol hydrolysis which was obtained with hepatic lipase even at a high concentration of enzyme.

Independent of the maximal triacylglycerol hydrolysis reached, more phospholipid was hydrolyzed relative to triacylglycerol with hepatic lipase than with lipoprotein lipase in all lipoproteins. Figure 4 illustrates this for VLDL, IDL, and HDL_2. (Data for LDL were similar but are not depicted.) Also, in VLDL, the percent of phospholipid hydrolysis for any degree of triacylglycerol hydrolysis was higher than in HDL_2, with either enzyme. For a single lipoprotein class, the relationship between triacylglycerol and phospholipid hydrolysis was similar for data obtained by either time curves or enzyme curves. Note that this relationship was generally linear until $^{1}/_{2}$ (50%) of the particle triacylglycerols were hydrolyzed but phospholipid hydrolysis then tended to increase more steeply after $^{3}/_{4}$ of the particle triacylglycerol had been removed. This was most evident with hepatic lipase.

For either enzyme acting on a single lipoprotein fraction, the degree of phosphohydrolysis closely correlated with triacylglycerol hydrolysis, and this was largely independent of the kinetics of hydrolysis. As illustrated for VLDL in Figure 5, for both enzymes this relationship between phospholipid hydrolysis and triacylglycerol hydrolysis is maintained independent of whether enzyme concentration, incubation time, or substrate concentration was the changing variable. These results suggest that triacylglycerol removal from a lipoprotein core will be an important determinant of phospholipid removal via hydrolysis by the lipases.

By showing a comparison of the relationship of phospholipid hydrolysis to triacylglycerol hydrolysis between different lipoprotein classes, Figure 6 shows that with either enzyme this ratio was greatest with VLDL, followed in descending

Table I: Composition Analysis of Lipoproteins before and after Radiolabeling with [^{14}C]Triolein and [^{3}H]Dipalmitoylphosphatidylcholine

	protein	triacylglycerol	free cholesterol	cholesteryl ester	phospholipid
VLDL(3)[a]	13.4 ± 3.1[b]	58.3 ± 6.5	4.2 ± 0.6	7.5 ± 3.5	16.7 ± 4.6
M-VLDL(3)[a]	14.5 ± 4.4	60.7 ± 7.1	3.3 ± 1.0	4.2 ± 1.9	17.3 ± 0.6
IDL(2)	17.0 ± 3.5	44.5 ± 6.1	8.4 ± 2.8	10.3 ± 3.2	19.9 ± 1.2
M-IDL(2)	15.4 ± 1.3	54.1 ± 1.7	2.4 ± 0.9	7.3 ± 2.1	20.9 ± 0.9
LDL(2)	20.9 ± 0.1	7.2 ± 0.6	8.3 ± 0.2	41.0 ± 2.4	22.7 ± 1.9
M-LDL(3)	23.8 ± 1.6	15.4 ± 0.5	7.9 ± 0.2	30.4 ± 0.8	22.5 ± 1.0
HDL_2(3)	35.3 ± 5.3	5.0 ± 1.5	4.3 ± 1.1	21.1 ± 0.9	34.4 ± 5.4
M-HDL_2(4)	35.6 ± 3.7	14.3 ± 4.2	2.3 ± 0.6	13.9 ± 2.5	33.6 ± 3.9

[a] VLDL, IDL, LDL, and HDL_2 are lipoproteins isolated from human plasma; and M-VLDL, M-IDL, M-LDL, and M-HDL_2 are the reisolated modified lipoproteins after enrichment with [^{14}C]triolein and [^{3}H]diphosphatidylcholine using procedures as outlined in Materials and Methods. The numbers in parentheses indicate the number of separate lipoprotein preparations from different donors and the number of separate double radiolabeled preparations used for the experiments described herein. [b] Composition is expressed as relative weight composition (percentage of total lipoprotein mass). Results are means ± SD.

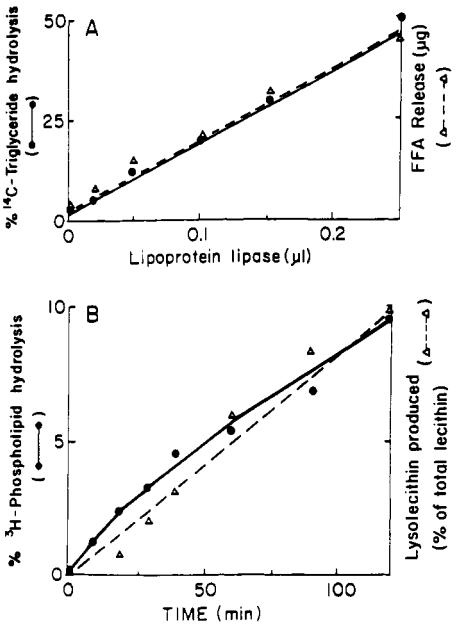

FIGURE 2: Percent of hydrolysis of VLDL triacylglycerol (A) and phosphatidylcholine (B) determined by radioisotopic as compared to mass assay. VLDL (100 μg of triaclyglycerol) was incubated (A) with varying amounts of lipoprotein lipase for a fixed time (30 min) and (B) with a fixed amount of lipase (0.1 μL) for increasing incubation times. The percent of hydrolysis of triacylglycerol and phospholipid was calculated from isotopic assays of [^{14}C]oleic acid and [^{3}H]lysophosphatidylcholine (●) produced, or by mass assays (Δ) of free fatty acid as determined after Dole's extraction (Dole, 1956) and Ho's method (Ho, 1970) and of lysophosphatidylcholine after thin-layer chromatography of individual phospholipid classes (see Materials and Methods) and measurement by Bartlett's technique (Bartlett, 1959).

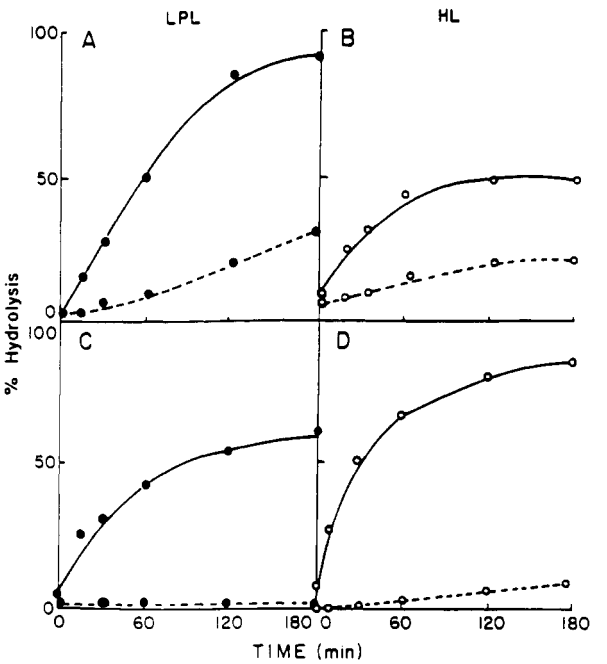

FIGURE 3: Percent of hydrolysis of triacylglycerols and phospholipid in VLDL and HDL_2 over time with lipoprotein and hepatic lipases. (Top) Incubations of VLDL (100 μg of triacylglycerol) with (A) LPL (lipoprotein lipase; 0.015 μL) and (B) HL (hepatic lipase; 2.5 μL). (Bottom) Incubations of HDL_2 (20 μg of triacylglycerol) with (C) lipoprotein lipase (0.6 μL) and (D) hepatic lipase (12 μL). Results with lipoprotein lipase are shown with closed circles (●), and those with hepatic lipase are shown with open circles (○). Triacylglycerol hydrolysis is shown with solid lines, and phospholipid hydrolysis is shown with broken lines.

order by IDL, HDL_2, and LDL.

Comparisons of the percent of lipolysis between lipoprotein classes will not accurately reflect the actual number of molecules hydrolyzed because of large differences in molecular weights and compositions among different lipoproteins. We, therefore, estimated the number of phospholipid molecules hydrolyzed for each 100 molecules of triacylglycerol hydrolyzed in each lipoprotein class (Table II). Under conditions where this relationship was still linear, i.e., 50% triacylglycerol hydrolysis, it was again apparent that for each lipoprotein class hepatic lipase hydrolyzed about 2–3-fold more phospholipid than did lipoprotein lipase. Expressed in terms of phospholipid molecules per triacylglycerol, however, large differences in phospholipolysis were not observed between VLDL, IDL, and LDL although, again, LDL tended to have the lowest phospholipid hydrolysis with either enzyme. HDL_2, however, loses substantially more phospholipid molecules during triacylglycerol hydrolysis than any other lipoprotein assayed.

We questioned whether the hydrolysis of lipoprotein phospholipid that was recorded in our in vitro experiments would be sufficient to account for the decreasing surface area needed to cover a shrinking particle core volume, with triacylglycerol hydrolysis. To explore this, we expressed the number of phospholipid molecules present in each lipoprotein class as a function of the number of triacylglycerol molecules in that particle. We then determined if the number of phospholipid molecules hydrolyzed in our actual experiments (as triacylglycerol hydrolysis proceeded) was sufficient to deplete surface phospholipid from the shrinking particle core to the same degree as it occurs in vivo. Figure 7 demonstrates that as VLDL is hydrolyzed toward LDL neither enzyme could hydrolyze, in vitro, nearly sufficient phospholipid molecules to account for the loss of surface phospholipids that accompany triacylglycerol hydrolysis and decreasing core volume in vivo. For example, in our in vitro experiments on

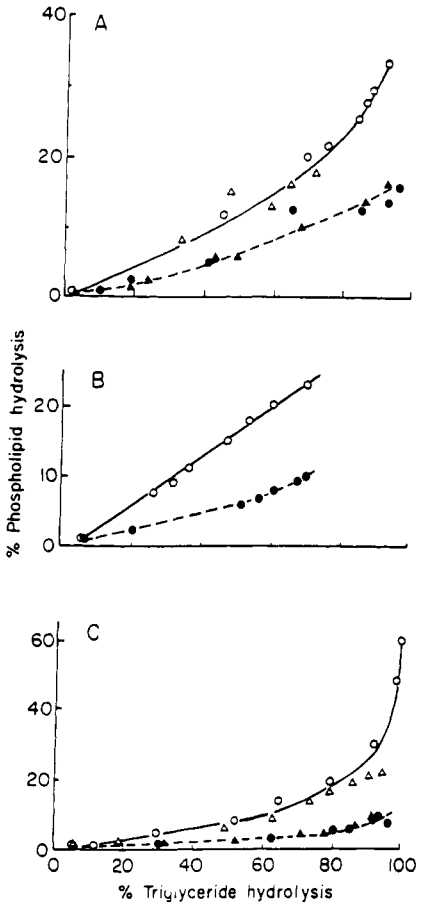

FIGURE 4: Phospholipid hydrolysis relative to triacylglycerol hydrolysis by lipoprotein (▲, ●) and hepatic (△, ○) lipases. Points shown are from different incubations of (A) VLDL (100–200 μg of triacylglycerol), (B) IDL (20 μg of triacylglycerol), and (C) HDL_2 (20 μg of triacylglycerol). Data obtained from experiments with increasing amounts of incubated enzyme are shown by triangles (▲, △). Incubations with fixed enzyme but increasing incubation times are shown by circles (●, ○) and demonstrate relationships very similar to those obtained by time curves.

VLDL, hydrolyzing even 50%–90% of the triacylglycerol molecules was accompanied by a degree of phospholipid lipolysis that would not result in a particle with a phospholipid:triacylglycerol ratio even close to that of IDL. Similarly, phospholipid hydrolysis of IDL in vitro would not deplete phospholipid to the levels found in LDL. In LDL, where under most circumstances triacylglycerol is only a minor core lipid, the lipases could remove the required small amount of phospholipid as the LDL core volume is reduced only minimally by triacylglycerol hydrolysis.

From the data points depicted in Figure 7, a linear relationship was found between log PL_n (number of phospholipid molecules) and log TG_n (number of triacylglycerol molecules) over the range between VLDL and the largest LDL. The slope of the straight line was calculated as 0.75, and the relationship is

$$PL_n = 3.35(TG_n)^{0.75}$$

If the particle volumes were entirely occupied by triacylglycerol and the surfaces were made up of only phospholipid, the exponent should be $^2/_3$. (Surface is proportional to volume$^{2/3}$.) The finding that the exponent is 0.75, not 0.67, could be due to phospholipid making up more of the surface in larger particles (relative to protein).

In HDL_2, hepatic lipase in vitro could reduce the number of phospholipids per particle to the average found in HDL_3,

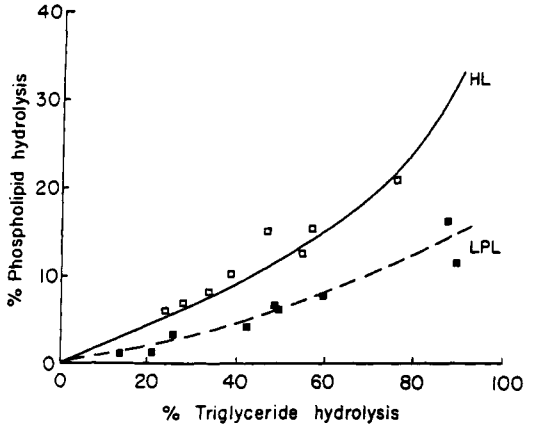

FIGURE 5: Comparison of results of phospholipid hydrolysis relative to triacylglycerol hydrolysis on data obtained on substrate curves as compared to data obtained on time or enzyme curves. The solid line for hepatic lipase (HL; —) and the dashed line for lipoprotein lipase (LPL; – –) show the relationship between phospholipid and triacylglycerol hydrolysis obtained with increasing enzyme concentration or increasing incubation time for VLDL. These lines are the same as shown in Figure 4A. □ represents the points obtained in experiments with a fixed enzyme concentration (0.015 μL of lipoprotein lipase, 4 μL of hepatic lipase) and a fixed incubation time (30 min for lipoprotein lipase, 20 min for hepatic lipase) with increasing amounts of VLDL triacylglycerol (20–300 μg).

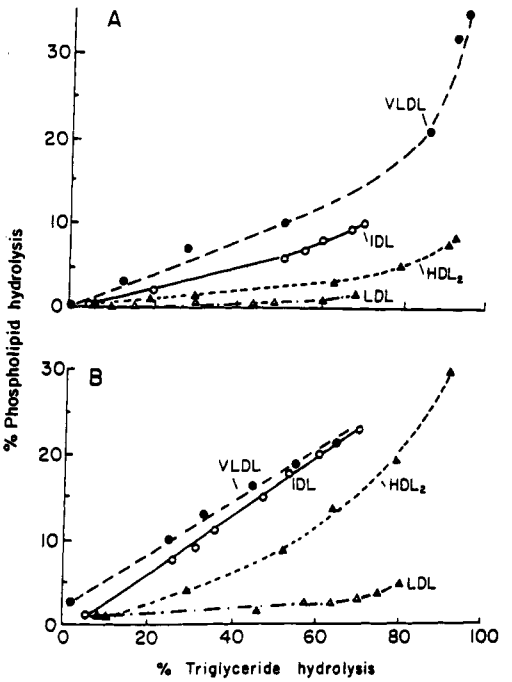

FIGURE 6: Phospholipid hydrolysis relative to triacylglycerol hydrolysis in different lipoproteins. (A) Lipoprotein lipase. (B) Hepatic lipase. Some of the data included in this figure are also shown in Figure 4.

but only at extreme levels of triacylglycerol hydrolysis (>95%) (Figure 7, inset). Previous work by us and others has suggested that HDL_2 can be converted to HDL_3 after neutral lipid exchange processes replace HDL_2 cholesteryl ester with triacylglycerol (Deckelbaum et al., 1986; Barter, 1990). Triacylglycerol can then be hydrolyzed by lipases to reduce core volume, resulting in the production of a smaller particle, presumably HDL_3. Results in Figure 7 show again that phospholipid hydrolysis, with hepatic lipase, will deplete sufficient phospholipid from HDL_2 to produce HDL_3, but only after >98% triacylglycerol hydrolysis, which reduces the number of triacylglycerol molecules to less than that found in HDL_3 in vivo. (However, in most experiments, such high

Table II: Number of Phospholipid Molecules Hydrolyzed for Every 100 Molecules of Triacylglycerol Hydrolyzed at 50% Triacylglycerol Hydrolysis in Different Lipoproteins[a]

	lipoprotein lipase	hepatic lipase
VLDL	4.4	8.5
IDL	4.1	13.0
LDL	2.7	6.6
HDL_2	10.0	46.7

[a] These data were calculated using the relative weight composition data for radiolabeled M-VLDL, M-IDL, M-LDL, and M-HDL_2 as presented in Table I. The molecular weights for VLDL, IDL, LDL, and HDL_2 were estimated as 15×10^6, 5×10^6, 2.5×10^6, and 3.6×10^5, respectively (Deckelbaum, 1987). Molecular weights for triacylglycerol and phospholipid were estimated as 850 and 750, respectively, and the degree of phospholipid hydrolysis at 50% triacylglycerol hydrolysis was obtained from the data shown in Figure 6.

degrees of triacylglycerol hydrolysis were not achieved in HDL_2 even with high enzyme concentrations and prolonged incubation times.)

DISCUSSION

During triacylglycerol hydrolysis, depletion of surface constituents accompanies reduction of core size in plasma lipoproteins. This process necessitates loss of molecules or groups of molecules either by shedding from the surface or, in the case of phospholipid, by phospholipid hydrolysis. Thus, as lipoproteins decrease in size, surface phospholipid can have 3 possible fates: (a) hydrolyzing to lysolecithin and transfer to albumin, (b) shedding as intact phospholipid molecules, and (c) remaining as part of the surface of the original particle. Our results in vitro strongly support the hypothesis that phospholipid hydrolysis alone, even in the presence of hepatic lipase, is insufficient to remove excess surface phospholipids from larger lipoprotein particles as they are converted to smaller particles, e.g., VLDL to IDL to LDL. Rather, shedding of whole phospholipid molecules, presumably in liposomal-like particles, must be a *major* mechanism for the loss of excess surface lipid as larger particles are converted to smaller particles.

With either lipase, VLDL and IDL demonstrated higher percentages of phospholipid hydrolysis compared to triacylglycerol hydrolysis than did LDL or HDL_2 (Figure 6). In terms of the actual number of molecules hydrolyzed, however, most phospholipid was hydrolyzed per triacylglycerol in HDL_2 (Table II). Likely, there are properties characteristic for each lipoprotein class which determine the relative rates of phospholipid and triacylglycerol hydrolysis as supported by the following results. First, similar ratios of phospholipid and triacylglycerol hydrolysis were recorded independent of whether experiments were performed with variations in enzyme or substrate concentrations, or with variations in incubation times, or under conditions of linear or nonlinear kinetics. Second, even under wide variability of incubation conditions, the relation between phospholipid and triacylglycerol remained almost linear until triacylglycerol hydrolysis exceeded 50%–60%. (On a log scale for triacylglycerol the relationship was linear all the way to >95%, even for HDL_2.) One possible link between triacylglycerol and phospholipid hydrolysis is the chemical composition of the core and the surface of the particles (Demel et al., 1984; Demel & Jackson, 1985), another is the relative reduction of core volume and surface area as the particle becomes smaller.

Are phospholipid and triacylglycerol hydrolysis coupled events? Probably not. Previous studies have established that both enzymes hydrolyze triacylglycerol efficiently in systems containing no phospholipid and phospholipid in systems containing no triacylglycerol (Bengtsson & Olivecrona, 1980). Our results, similar to those of Simard et al. (1989) show that in HDL_2, after triacylglycerol hydrolysis reaches 95%–99%, phospholipid hydrolysis can still continue. In a recent study, Rojas et al. (1991) found that both enzymes hydrolyzed phospholipids more rapidly than triacylglycerols in phospholipid liposomes containing 2.5 mol% triacylglycerol. This was in sharp contrast to their action on emulsion droplets, where triacylglycerol hydrolysis was several times faster than phospholipid hydrolysis. In this respect, all the lipoprotein fractions here behaved as emulsion droplets, in that triacylglycerol was always hydrolyzed more rapidly than phospholipid. The decisive property may be that lipoprotein particles have a core which acts as a reservoir of triacylglycerol molecules which can be recruited by the enzyme's active site for continuing hydrolysis while the enzyme remains adsorbed to the particle (Rojas et al., 1991).

Our results demonstrate directly that with all native lipoproteins hepatic lipase always hydrolyzes more phospholipid relative to triacylglycerol than lipoprotein lipase does. It is of interest that lipoprotein lipase has restricted activity against ester bonds involving fatty acids with double bonds close to the carbonyl, e.g., arachidonic acid, whereas hepatic lipase does not show this restriction, and also appears to have relatively higher activity against monoglycerides (Olivecrona et al., 1990). Hence, lipoprotein lipase may have somewhat more stringent substrate specificity than hepatic lipase has. The third member of the lipase family, pancreatic lipase, can also hydrolyze phospholipids (Verger, 1984). No direct comparison has been made, but available data suggest this activity may be low compared to that exerted by lipoprotein and hepatic lipases. It seems that there are subtle differences between the three lipases which modulate how they select substrate molecules at the lipid–water interface.

There are possible concerns when relating our in vitro results to what occurs in vivo. One is the labeling procedure, but our data show that hydrolysis of the introduced labeled lipids closely followed total hydrolysis, as monitored by mass assay. As well, our results are similar to those of other reports which used endogenous enrichment for radiolabeling VLDL (Chajek & Eisenberg, 1978; Eisenberg & Olivecrona, 1979) or HDL_2 (Simard et al., 1989). Another limitation is that in the plasma environment particles interact with each other and are acted on not only by lipases but also by lecithin:cholesterol acyltransferase and by neutral lipid transfer processes (Barter, 1990). These processes may affect lipolysis, and lipolysis may affect these processes (Tall et al., 1984; Barter, 1990). While free fatty acids have been suggested to stimulate CETP-mediated neutral lipid transfers, CETP itself has little if any effect on triglyceride-rich particle hydrolysis (Tall et al., 1984). The presence of higher concentrations of cholesteryl ester at the phospholipid–water interface in cholesteryl ester rich particles may decrease the surface triacylglycerol concentration available for lipase hydrolysis (Demel & Jackson, 1985) and contribute to the need for the higher amounts of lipase required to hydrolyze triacylglycerol in LDL and HDL, as compared to in VLDL and IDL. The purpose of the present study was not to explain all these interplays, but to better understand one of them, i.e., interactions between lipoprotein phospholipid and triacylglycerol hydrolysis.

During triacylglycerol hydrolysis, depletion of surface constituents accompanies reduction of core size in plasma lipoproteins (Chajek & Eisenberg, 1978). Our results show that phospholipid hydrolysis, even by hepatic lipase, is

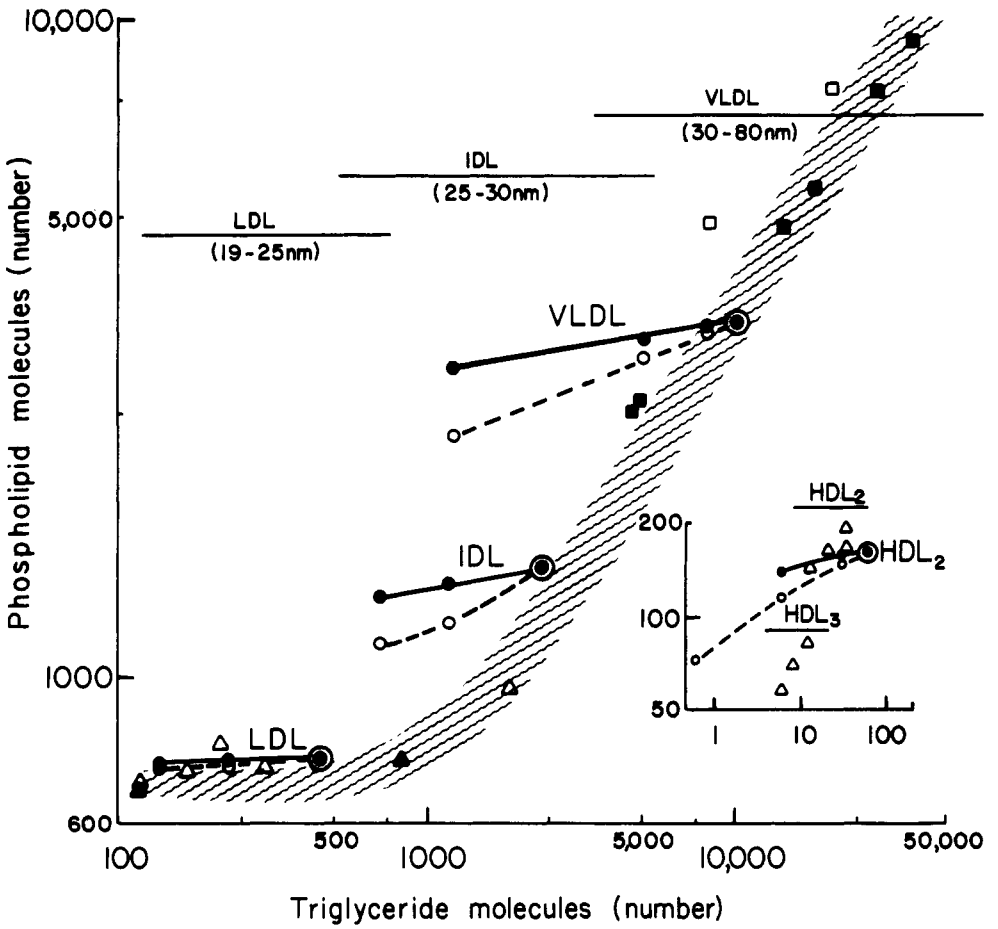

FIGURE 7: A comparison of the relationship of surface phospholipid molecules and core triacylglycerol molecules in human plasma VLDL, IDL, LDL, HDL_2, and HDL_3 in vivo and the ability of lipoprotein and hepatic lipases to remove phospholipid molecules by hydrolysis from VLDL, IDL, LDL, and HDL_2 in vitro. The number of phospholipid and triacylglycerol molecules in different normal lipoprotein classes were calculated from data published by Kichinskiene and Carlson (1982) (■), Packard et al. (1979) (□), Deckelbaum (1987) (△), and Lee and Alaupovic (1970) (▲) and by using molecular weights for triacylglycerol and phospholipid of 850 and 750, respectively. The broad hatched area represents, in vivo, a schematized relationship between the number of phospholipid molecules and triacylglycerol molecules in the different lipoprotein classes as VLDL undergoes lipolysis to IDL and then to LDL. The size ranges for human plasma VLDL, IDL, and LDL are shown by the horizontal lines near the top of the figure. The double circles (⊙) show the calculated number of initial phospholipid/triacylglycerol molecules for VLDL, IDL, and LDL used for the experiments herein and calculated from the compositional data in Table I for double radiolabeled lipoproteins. The number of phospholipid/triacylglycerol molecules remaining after 50% and 90% triacylglycerol hydrolysis is depicted by solid circles (●) for lipoprotein lipase and open circles (○) for hepatic lipase. The inset shows similar representations for HDL_2, but with one additional point depicting 98.5% triacylglycerol hydrolysis in HDL_2.

insufficient to remove surface phospholipids from larger lipoproteins as they are converted to smaller particles. As illustrated in Figure 7, phospholipid hydrolysis by lipoprotein lipase could account for less than $^1/_3$ of the required loss of phospholipid when VLDL is catabolized to IDL, and hydrolysis by hepatic lipase could account for only about $^1/_2$ of the phospholipid loss for IDL to convert to LDL. On the other hand, phospholipolysis could account for removal of a coordinate amount of phospholipid as the small amount of triacylglycerol in LDL is hydrolyzed by hepatic lipase. For a hypothetical conversion of HDL_2 to HDL_3, Figure 7 shows that hepatic lipase could remove enough phospholipid, but only after >98% hydrolysis of triacylglycerol molecules to less than that found in HDL_3 in vivo. Therefore, other mechanisms must also contribute to HDL_2 to HDL_3 conversions, e.g., loss of apoprotein AI (Deckelbaum et al., 1986; Barter, 1990).

Our results thus imply that shedding of unhydrolyzed surface phospholipid (and not phospholipolysis) is the key quantitative pathway during formation of human LDL from VLDL via IDL, and remnant particles from chylomicrons. This is in accord with previous observations where, in in vivo and in vitro experiments, surface remnants have been recovered in substantial quantity after VLDL (Chajek & Eisenberg, 1978; Eisenberg & Olivecrona, 1979) or chylomicron (Schaefer et al., 1982) lipolysis. Liposomal-like particles have been observed in animal and human plasma after intestinal absorption or intravenous infusion of triacylglycerol-rich particles in humans (Forte et al., 1979; Griffin et al., 1979). Normally, released surface remnants likely fuse with acceptor particles, e.g., HDL_3, so that large amounts of liposomal-like particles do not accumulate (Patsch et al., 1978). One may question what are the potential advantages of the relative sparing of phospholipid. One perspective is that the slow phospholipid hydrolysis may be a safety built into the system so that the enzymes, in particular lipoprotein lipase, can act on even the largest chylomicron particles without risk of denuding and destabilizing them. Another is that the shed phospholipid is an important source of material for the generation of HDL particles.

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