Diacylglycerol is the preferred substrate in high density lipoproteins for human hepatic lipase

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Abstract The hydrolysis of HDL phospholipids (PL) and glycerides by hepatic lipase (HL) has been investigated in native and reconstituted HDL particles (Lp2A-I). Fasting, normolipidemic HDL exhibit total lipid hydrolytic rates of between 10 and 36 nM FA/h per μM PL. Of the total fatty acids liberated with HDL_3 only 1% are from triolein (TG), while 49% are from diolein (DG) and 50% are from PL. A spherical reconstituted particle containing 2 molecules of apoA-I, 120 molecules of PL, and 20 molecules of TG exhibits a total lipid hydrolytic rate of 18 nM FA/h per μM PL and 93% of the fatty acids liberated are from PL. Inclusion of 40 molecules of TG into the Lp2A-I particle doubles the rate of fatty acid hydrolysis by HL through a stimulation of TG hydrolysis. Further addition of 10 molecules of DG to the Lp2A-I complex has no effect on the overall rates of hydrolysis, but changes the substrate specificity, wherein 61% of the fatty acids are from DG and both TG and PL hydrolytic rates are significantly reduced. Increasing the amount of DG in the Lp2A-I particle further stimulates total lipid hydrolysis by raising DG hydrolytic rates at the expense of PL and TG hydrolysis. A particle containing 10 molecules of TG and 40 molecules DG yields the fastest lipid hydrolytic rate of 143 nM FA/h per μM PL, which constitutes 96% DG hydrolysis, 3% TG hydrolysis, and 1% PC hydrolysis. These data indicate that hepatic lipase acts primarily as a surface lipid lipase with HDL particles. DG is the preferred substrate of HL in HDL and the HDL-DG content regulates the hydrolysis of both PL and TG by HL.—**Coffill, C. R., T. A. Ramsamy, D. M. Hutt, J. R. Schultz, and D. L. Sparks.** Diacylglycerol is the preferred substrate in high density lipoproteins for human hepatic lipase. *J. Lipid Res.* 1997. **38:** 2224–2231.

Supplementary key words HDL remodeling • LpA-I • lipemia • lipolysis • lipase • glyceride

Hepatic lipase (HL) hydrolyzes the *sn*-1 fatty acyl ester bonds of *sn*-3 phospholipids as well as the *sn*-1 (*sn*-3) ester bonds of mono-, di-, and triacylglycerols (1–3). HL is bound to the surface of the sinusoidal endothelium of the liver and has been shown to have hydrolytic activity on circulating lipoproteins (4, 5). HL appears to be involved in the remodeling of high density lipoproteins (HDL) and is capable of converting triacylglycerol-enriched HDL_2 from postprandial plasma to smaller particles in the HDL_3 density range (6, 7). Studies have shown that the phospholipase activity of HL may also be important in the metabolism of cholesterol and may promote selective uptake of HDL cholesteryl esters by the liver (8, 9). HDL are a heterogeneous mixture of particles that vary in their lipid and apolipoprotein composition and also in their particle size and hydrated density (10). Typically, the lipid composition of HDL has been expressed in terms of cholesterol, phospholipid (PL), and triacylglycerol (TG), however, recent observations of Vieu et al. (11) have shown that the methods used to measure triacylglycerols have masked the contributions of diacylglycerols (DG) in this lipoprotein class. Their study showed that most of the glycerides in HDL are DG and that the DG:TG ratio in HDL can be up to 7:1. Because HDL lipid composition can have major effects on the structure and function of this lipoprotein (12–14), it is conceivable that DG may also affect HDL structure and the interactions between HL and HDL particles. It should be noted, however, that the heterogeneous nature of native lipoproteins can confound estimations of what may be regulating the activity or substrate specificity of an enzyme. As HL appears to be highly sensitive to the composition and structure of HDL (15), an unambiguous characterization of the catalytic regulation of HL requires the use of more homogeneous lipoprotein substrates with well-defined compositions and structures. In this investigation we show that homogeneous reconstituted HDL

Abbreviations: apoA-I, apolipoprotein A-I; CE, cholesteryl ester; DG, diacylglycerol; GGE, gradient gel electrophoresis; HDL, high density lipoproteins; HL, hepatic lipase; Lp2A-I, reconstituted particles containing 2 molecules of apoA-I; MGAT, monoacylglycerol acyltransferase; PC, phosphatidylcholine; PL, phospholipid; POPC, palmitoyl-oleoyl phosphatidylcholine; TG, triacylglycerol.

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(Lp2A-I) particles are good lipoprotein analogues to study HL-mediated lipolysis and that the DG content of Lp2A-I greatly affects the ability of human HL to hydrolyze HDL lipids.

EXPERIMENTAL PROCEDURES

Materials

Triolein (TG) and phospholipase C (Type I from *C. perfringens*) were purchased from Sigma Chemical Co. (St. Louis, MO) and 1,3-diolein (DG) was acquired from NuChek Prep, Inc. (Elysian, MN). Palmitoyloleoyl phosphatidylcholine (POPC) was obtained from Avanti Polar Lipids (Birmingham, AL). Tri[9,10-^{3}H(N)]olein was purchased from DuPont Canada Inc. (Mississauga, ON), while di[1-^{14}C]oleoyl phosphatidylcholine was obtained from Amersham Canada (Oakville, ON). All other reagents were analytical grade.

Methods

Isolation and labeling of HDL. Subfractions of HDL were obtained from plasma by density gradient ultracentrifugation as described by Guérin et al. (16). Incorporation of [^{3}H]triolein and [^{14}C]diolein (preparation described below) into HDL was accomplished by incubation of POPC vesicles containing the labeled lipids (17, 18). Phospholipid, tri- and diacylglycerol vesicles were prepared by drying 50 μg of POPC, 100,000 cpm of [^{3}H]triolein and 50,000 cpm of [^{14}C]diolein under N_2 addition of 200 μl of 10 mM phosphate-buffered saline (PBS), pH 7.2, and vortexing for 1 min. Vesicles were combined with HDL_2 or HDL_3 (2 mg of protein) and incubated with gentle shaking for 8 h at 37°C. Greater than 95% of the radioactive lipids were incorporated into the HDL particle after reisolation. HDL lipid mass values were determined as described below and the lipid specific activities were calculated.

Quantification of acylated glycerols. HDL lipids (per 1 mg protein) were extracted by the method of Bligh and Dyer after the addition of 12 μl of formic acid per ml of aqueous phase (11). Before extraction, internal standards of [^{14}C]diolein and [^{3}H]triolein (15,000 cpm each) were added to the tubes. The organic phase was removed, evaporated under nitrogen to dryness, and redissolved in 50 μl chloroform. Silica gel 60 plates and a solvent system of hexane–diethyl ether–acetic acid 105:45:1.5 (v/v) were used to separate the glycerol-containing neutral lipids. The following R_f values were obtained: MG = 0.01, 1,2-DG = 0.12, 1,3-DG = 0.14, and TG = 0.53. The spots corresponding to DG and TG were scraped into 1.5-ml Eppendorf tubes and 100 μl of isopropanol plus 500 μl of the enzyme solution from the Boehringer Mannheim TG kit (Cat. No. 701 882) were added. The tubes were incubated at room temperature for 1 h and then 200-μl aliquots of the supernatant were spectroscopically analyzed to determine glycerol content.

Preparation and characterization of reconstituted Lp2A-I complexes. Purified apoA-I was isolated from delipidated HDL by size exclusion chromatography and spherical reconstituted Lp2A-I were prepared by co-sonication of POPC, triolein, diolein, and apoA-I as previously described (18). POPC (3.2 mg) and other purified lipids in chloroform (see Table 1 for starting concentrations) were dried under nitrogen into a 12 × 75 mm test tube and 800 μl of phosphate/saline, pH 7.4, was added. The lipid–buffer solution was initially sonicated for 1 min in a 15°C water bath and under nitrogen using a Branson 450 sonicator with 1/8″ tapered microtip probe. The suspension was then incubated in a sealed tube for 30 min at 37°C and sonicated again for 5 min using a 95% duty cycle. ApoA-I (3 mg of a 1.8 mg protein/ml phosphate–saline solution, pH 7.4) was added to the lipid suspension and the protein–lipid mixture was sonicated for 4 × 1 min punctuated by 1-min cooling periods. All Lp2A-I complexes were filtered through a 0.22-μm syringe tip filter and reisolated by size exclusion chromatography on a Superose 6 column.

The size and homogeneity of apoA-I complexes were estimated by non-denaturing gradient gel electrophoresis on precast 8–25% acrylamide gels (Pharmacia Phastgel) after protein staining and densitometric scanning. Proteins were determined by the Lowry method as modified by Markwell et al. (19). Total glycerides, fatty acids, and phospholipids were determined enzymatically using Boehringer Mannheim kits.

Purification and assay of HL activity. Hepatic lipase was purified from post-heparin human plasma by heparin affinity chromatography as described by Ehnholm and Kuusi (20). Briefly, after post-heparin human plasma was collected from normolipidemic subjects, a 20% TG emulsion (LIPOSYN II, Abbott Laboratories, Ltd., Montréal, PQ) was added to the plasma and the resulting cakes of fat were harvested for delipidation. The resuspended, aqueous solution of crude hepatic lipase was loaded on to a heparin–Sepharose CL-6B column and eluted with a linear salt gradient of 0.15 M to 1.5 M NaCl in 5 mM sodium barbital, pH 7.4, 20% glycerol. Hepatic lipase activity was characterized using a standard particle containing a neutral lipid core (POPC:TG:apoA-I of 120:20:2, mol/mol/mol), quantified into units of enzyme activity (where 1 unit = 1 nmol fatty acid hydrolyzed/mg protein per h from a 120:20:2 POPC:TG:apoA-I particle) and was stored at −80°C until use.

To study the substrate specificity and catalytic regulation of hepatic lipase, reconstituted Lp2A-I particles

were prepared with [^{3}H]triolein and [^{14}C]diolein and then incubated with the purified enzyme. [^{14}C]diolein was isolated after a 4-h incubation at 37°C of 5 μCi di[1-^{14}C]oleoyl phosphatidylcholine, 1 ml of 0.2 units/ml phospholipase C in phosphate buffer (pH 7.3), 1 ml of 1% $CaCl_2$ and 1 ml of diethyl ether. The reaction was stopped with five drops of 0.1 M HCl and then the DG was extracted with 5 ml of chloroform - methanol 1:1. After centrifugation for 15 min at 2200 rpm, the lower phase was removed and dried under nitrogen. Preparative TLC, utilizing Silica Gel 60 plates and a solution of chloroform - acetone - methanol–acetic acid–water 60:80:20:20:10, was used to separate the DG from unreacted substrate.

Native and reconstituted Lp2A-I were characterized as substrates for purified HL using a standard assay system. Each enzyme assay contained the lipoprotein substrate, 214 units of purified HL, 3 mg of bovine serum albumin (essentially fatty acid-free), 1.5 mM $CaCl_2$, and 1 M NaCl in a 0.15 M Tris buffer (final vol = 500 μL). Incubations were carried out for 3 h at 37°C and were terminated by placing the tubes in an ice bath. The total amount of fatty acids released during the incubation was determined using an enzyme kit assay from Boehringer Mannheim. The phospholipid, tri- and diacylglycerol hydrolytic rates were determined by subtraction after measuring the quantity of radioactive fatty acids liberated from [^{3}H]triolein and [^{14}C]diolein during the incubation with HL using a liquid–liquid partitioning system (20). Under these conditions, >95% of the radioactive fatty acids were recovered in the supernatant aqueous phase at both low and high substrate concentrations (data not shown). Subtraction of the TG- and DG-hydrolytic values from the total yielded the phospholipid hydrolytic rate. Initial rates were estimated with minimal substrate conversion.

RESULTS

Characterization of native HDL as substrates of HL

Native HDL subfractions were isolated from normolipidemic subjects by sequential ultracentrifugation and then characterized as substrates for human HL. The relative total rates of lipid hydrolysis for the different HDL subfractions are similar to those described by others (4, 21); HDL_3 are the poorer HL substrates and exhibit total hydrolytic rates around 10 nM FA/h per μM PL, while HDL_2 particles are the better HL substrates and exhibit hydrolytic rates almost 4-fold greater. A fasting normolipidemic HDL_3 sample was incubated with [^{3}H]TG + [^{14}C]DG/POPC vesicles to incorporate

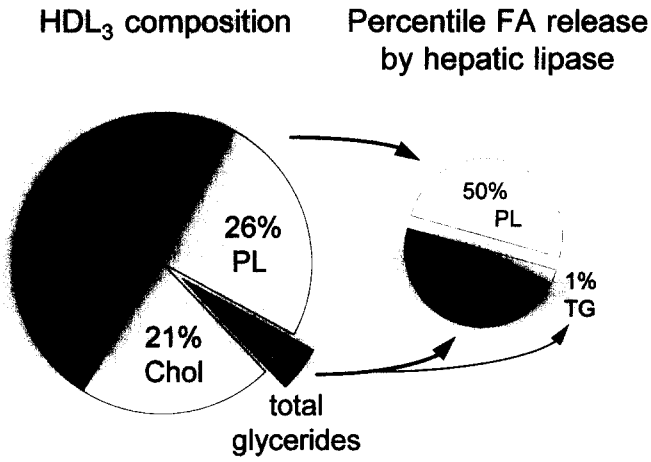

Fig. 1. Hydrolysis of polar and nonpolar lipids in native HDL_3 by HL. Normolipidemic HDL_3 was labeled with [^{3}H]triolein and [^{14}C]diolein as described in the text and then incubated with purified HL for 3 h. HDL_3 protein (Prot), total cholesterol (Chol), phospholipid (PL), and total glyceride composition (% by weight) are shown (left pie) relative to the percentage of fatty acids released from TG, DG, and PL by HL (right pie).

[^{3}H]triolein and [^{14}C]diolein. The amount of DG and TG in the HDL_3 subfraction was quantified by preparative TLC and shown to be approximately 2.2:1, DG:TG. Determinations of HL-catalyzed lipid hydrolytic rates for the HDL_3 sample showed that only 1% of the fatty acids liberated were from TG, the balance came from DG and PL hydrolysis (**Fig. 1**). Even though DG constituted only about 1% of the lipid weight of the HDL_3 particle, DG hydrolytic rates represented 49% of the total lipid hydrolysis. Hydrolytic rates for a fasting HDL_2 sample gave similar results, with 5% of fatty acids coming from TG and 30% coming from DG (data not shown).

Characterization of the size and composition of Lp2A-I particles

Spherical reconstituted HDL particles (Lp2A-I) were prepared to contain POPC, various amounts of tri- and diacylglycerol (triolein and diolein) and two molecules of apoA-I/particle (see **Table 1** for Lp2A-I compositions before and after particle reisolation). Particle size and homogeneity were determined from nondenaturing gradient gel electrophoresis; all Lp2A-I exhibited only one band on nondenaturing gels (**Fig. 2**) and ranged in size from 7.6 to 7.9 nm.

Effect of Lp2A-I triacylglycerol content on hepatic lipase activity

Figure 3 illustrates the rates of fatty acid liberation from both PC and TG in Lp2A-I containing various amounts of TG. Incubation of HL and an Lp2A-I containing 120:2, PC:apoA-I (mol:mol) shows that these

TABLE 1. Effect of Lp2A-I lipid composition on the diacylglycerol, triacylglycerol, and phospholipid hydrolytic activities of human hepatic lipase

Particle Composition[a] POPC:DG:TG:apoA-I (mol:mol:mol:mol)		Total Hydrolytic Rate[b]	Diacylglycerol Hydrolysis	Triacylglycerol Hydrolysis	Phospholipid Hydrolysis
Initial	Final				
			percentage of total hydrolysis (%)		
120:0:0:2	82:0:0:2	7	—	—	100
120:0:10:2	70:0:8:2	3	—	26	74
120:0:20:2	72:0:14:2	7	—	26	74
120:0:40:2	74:0:26:2	42	—	43	57
120:10:40:2	72:6:26:2	40	61	17	22
120:10:20:2	70:6:12:2	38	55	9	36
120:20:10:2	80:12:6:2	63	49	3	48
120:40:10:2	81:32:8:2	143	96	3	1
120:40:0:2	74:22:0:2	89	97	—	3

[a]LpA-I composition of phosphatidylcholine (POPC, ±10 mol), diacylglycerol (DG, ±3 mol), triacylglycerol (TG, ±3 mol), and apoA-I was determined after chromatographic reisolation as described in the text.

[b]Initial hydrolytic rates were determined from first-order kinetic analysis of the curves shown in Figs. 3 and 4. Values, given as nM FA/h per μM PL, are representative of three different preparations of Lp2A-I.

lipoproteins are good substrates for HL and that PC hydrolysis is the most rapid in Lp2A-I devoid of TG. Inclusion of 10 molecules of TG significantly reduces the phospholipase activity of HL, but is associated with a small amount of TG hydrolysis. Inclusion of 20 molecules of TG also reduces the phospholipase activity of HL, but to a lesser extent than with particles containing only 10 molecules of TG. Similarly, inclusion of twice as much TG into the Lp2A-I is also associated with an almost 2-fold increase in TG hydrolysis. The estimated rates of hydrolysis and the relative contribution of TG, DG, and PC hydrolytic rates for the data in Figs. 3 and 4 are presented in Table 1. Although enzyme saturation conditions exist in the absence of DG (Fig. 3), Lp2A-I containing DG are highly reactive with HL and give rise to hydrolysis curves that are almost linear over the sub-

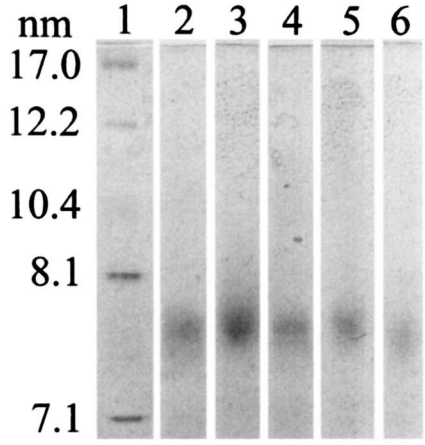

Fig. 2. Reconstituted spherical Lp2A-I subjected to electrophoresis in 8–25% nondenaturing gradient gels. Original gel profiles are shown for high molecular weight standards (lane 1) and five different reconstituted spherical Lp2A-I particles with the following POPC:DG:TG:apoA-I compositions; 120:0:0:2 (lane 2), 120:0:40:2 (lane 3), 120:40:0:2 (lane 4), 120:10:20:2 (lane 5), and 120:40:10:2 (lane 6). The molar ratios of each Lp2A-I were determined after chromatographic reisolation and are indicated in Table 1. Stokes' diameters were determined by comparison to high molecular weight standards. Lp2A-I particles exhibited a single discrete band, the size of which ranges between 7.6 and 7.9 nm.

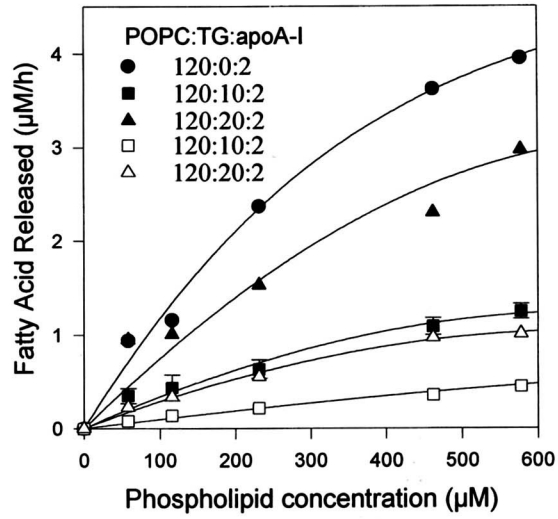

Fig. 3. Effect of the triacylglycerol content of spherical Lp2A-I on HL activation. HL-mediated hydrolysis of triacylglycerol, TG (open symbols) and POPC (solid symbols) in Lp2A-I particles containing various amounts of TG is shown. Lp2A-I were prepared by co-sonication (stoichiometries after reisolation are shown in Table 1) and were incubated with purified HL as described in the text. Hydrolytic values are the mean ± SD of triplicate determinations and are representative of three different preparations of Lp2A-I (SD error bars were generally smaller than the size of symbols and are therefore not shown for all curves).

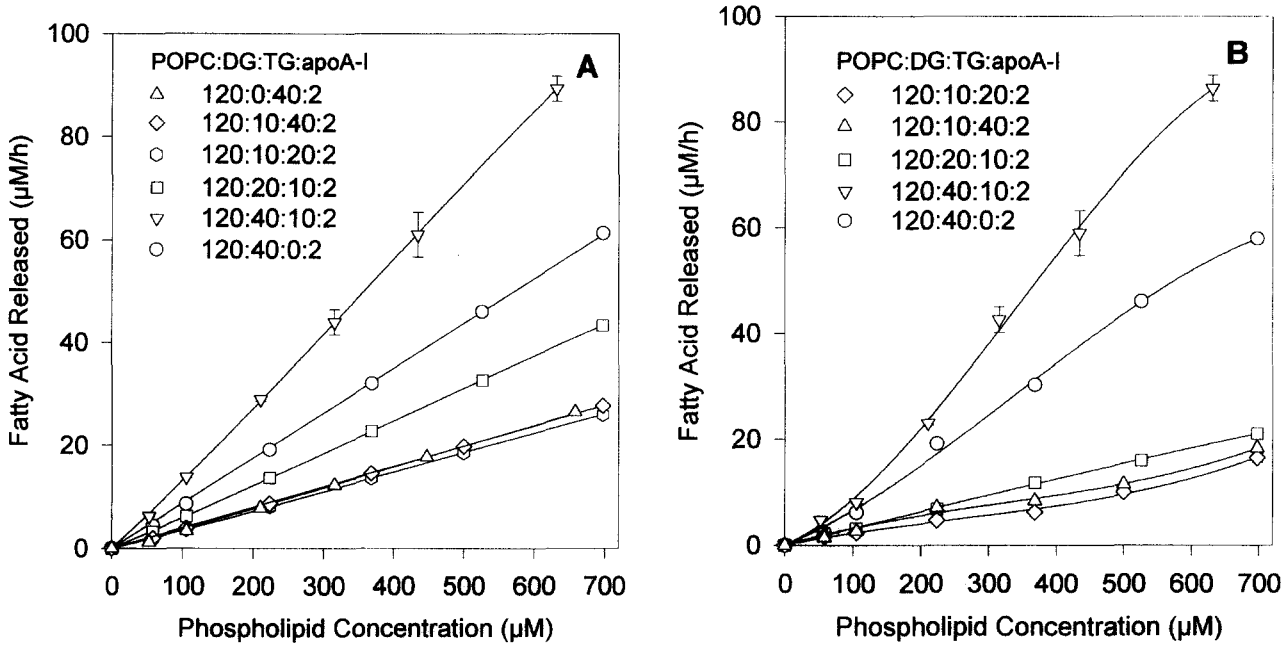

Fig. 4. Effect of the diacylglycerol content of spherical Lp2A-I on HL activation. HL-mediated hydrolysis of total lipids (panel A) and diacylglycerol, DG (panel B) in Lp2A-I particles containing various amounts of DG and TG is shown. Lp2A-I stoichiometries after reisolation are shown in Table 1. Hydrolytic values are the mean ± SD of triplicate determinations and are representative of three different preparations of Lp2A-I.

strate concentration studied (**Fig. 4**). The hydrolytic rates described in Table 1 are therefore expressed as pseudo first-order rate constants. While incorporation of 10 or 20 molecules of TG into the Lp2A-I particles has different effects on the absolute rates of PC and TG hydrolysis, it is striking that with the different lipoprotein particles, exactly the same relative rates of lipid hydrolysis are evident, 74% PC and 26% TG hydrolysis. In contrast, further TG enrichment of the Lp2A-I particle significantly stimulates overall lipid hydrolytic rates and also modifies relative lipid hydrolytic rates (Fig. 4A). The Lp2A-I particle with the composition of 120:40:2, POPC:TG:apoA-I (mol:mol) has the highest TG hydrolytic rate of all particles characterized. The TG hydrolytic rate for this particle is 16.7 nM FFA/h per μM POPC and represents 43% of the total hydrolysis. Doubling the TG content of the Lp2A-I from 20 to 40 molecules is therefore associated with a 6-fold increase in the total lipid hydrolytic rate and a shift in the contribution of each lipid substrate, constituting a 17% reduction in PC hydrolysis and a parallel increase in TG hydrolysis.

Effect of Lp2A-I diacylglycerol content on hepatic lipase activity

Inclusion of a small amount (<10 molecules) of DG in Lp2A-I has no effect on overall rates of hydrolysis, relative to a particle with a similar amount of PC and TG (Fig. 4A and Table 1). However, incorporation of this lipid significantly affects the substrate specificity of HL. Inclusion of 10 molecules of DG into a particle containing 40 molecules of TG significantly reduces the rate of hydrolysis for both PC and TG, in favor of a major increase in DG hydrolysis; 61% of the fatty acids liberated are from DG (Table 1 and Fig. 4B). When the amount of TG is reduced from 40 to 20 molecules, but the DG maintained at 10 moles, the total hydrolytic activity remains unaffected but the relative hydrolytic rates for different lipids are modified. DG hydrolytic rates are slightly reduced, while TG hydrolysis is reduced by approximately 50% and PC hydrolysis is increased 1.6-fold. When the DG content is increased and TG reduced to 120:20:10:2, POPC:DG:TG:apoA-I (mol:mol) the percentage of total hydrolysis is increased almost 2-fold and constitutes an increase in the hydrolysis of PC and a parallel reduction in both DG and TG hydrolysis. The inclusion of DG in Lp2A-I shifts the hydrolysis from the phospholipids and triacylglycerols to almost exclusively DG. The most dramatic results can be seen with the 120:40:10:2 POPC:DG:TG:apoA-I (mol:mol) particle, where most of the fatty acids liberated were from DG hydrolysis. Increasing the DG content to 40 molecules results in the highest total hydrolytic rate of 143 nM FFA/h per μM POPC, 96% of

which is due to DG hydrolysis. The same percentage could be seen with the particle that had no TG content. The particle with the highest DG content but devoid in TG, 120:40:2 POPC:DG:apoA-I mol:mol ratio, had a total hydrolytic rate of 89 nM fatty acids released/h per μM POPC that was almost entirely due to DG hydrolysis.

DISCUSSION

Observations from a number of laboratories have shown an association between plasma HL activities and HDL particle size distribution and thus have suggested that HL plays a major role in the remodeling of HDL particles (7, 22–26). Several reports show that the substrate specificity and activity of this enzyme can be modulated by various constituents of HDL (27–30). The results from substrate specificity studies have varied depending on the model system used and the physical environment in which the substrate is presented. Laboda, Glick, and Phillips (31) have shown that in lipid monolayers, triolein and dioleinphoshatidylethanolamine (PE) hydrolysis were similar and much greater than dioleinphosphatidlycholine (PC). They proposed that the polar group of the phospholipids may have an effect on the rate of hydrolysis and possibly on the formation of the enzyme–substrate complex. Azéma et al. (27) have studied radiolabeled native HDL and have shown that the rates of hydrolysis of [^{14}C]dilinoleoyl PE and [^{3}H]triolein are approximately 30- to 50-fold higher than that of [^{14}C]dioleoyl PC. They interpreted these results to mean that PE and TG are the preferred substrates for this enzyme in HDL, while PC is a less preferred substrate. This explanation appears correct only if the percentage of substrate hydrolyzed is examined rather than the absolute amount. When one considers the very small amounts of both TG and PE in an HDL particle, this hydrolysis appears to be minimal. More recent data suggest that the amount of fatty acids liberated from HDL-PC is greater than that for PE and TG combined (15, 28). Data reported by Thuren et al. (15) suggest that PC is the preferred substrate of HL in HDL. In their study, the hydrolysis of phospholipids in HDL was 4- to 9-fold greater than that of TG. These data are consistent with that observed in our laboratory. Experiments with both native and reconstituted HDL show that HL predominantly acts as a phospholipase with normolipidemic HDL.

HDL from normolipidemic patients exhibit similar initial hydrolytic rates (10–36 nM/h per μM PL) to the Lp2A-I particles shown. Experiments have further shown that with native HDL, HL primarily hydrolyzes DG and PL; only 1–5% of the fatty acids liberated came from TG. This may explain why introduction of human HL into an HL-deficient mouse had very little effect on plasma TG levels (32). The rates of TG hydrolysis for normotriglyceridemic Lp2A-I particles (that contain DG and <20 mol TG/particle) were generally less than 10% of total hydrolytic rates and less than 5% in the presence of substantial amounts of DG. Only when HDL is enriched in TG does HL appear to be a TG lipase. TG-enrichment of Lp2A-I stimulates an increased rate of both TG and DG hydrolysis by HL and results in a concomitant reduction in PL hydrolysis. Azéma et al. (27) also showed that enrichment of HDL with TG stimulates TG hydrolysis. As HDL from both hypertriglyceridemic and combined hyperlipidemic patients are enriched in TG (7, 33), this compositional modification may partially account for the increased HL hydrolytic rates observed in incubations with HDL from these patients (7). However, while HDL-TG can affect the rates of TG hydrolysis, the relative rates of TG and PL hydrolysis in HDL are much more significantly affected by the particle DG content (Fig. 3).

A recent report from Vieu et al. (11) has suggested that DG may be an important constituent of HDL and may play an important role in the metabolism of these lipoproteins. These authors have shown that DG is the most abundant glyceride in HDL and that the molar ratios of DG/TG in HDL particles were from 2 to 7 for HDL_2 and HDL_3, respectively. Our analyses of HDL glycerides confirm that DG is a major consituent of HDL, however, we observed much lower ratios of DG/TG than that reported by Vieu et al. (11) These differences may be due to inter-patient variation, as in preliminary studies we have observed considerable variation in this measurement among subjects. Vieu et al. (11) proposed that most of the DG molecules were associated with the lipoprotein surface lipids, as 60% of the DG was accessible to cationic pancreatic lipase. HDL-DG also appears to be the preferred substrate for human hepatic lipase in vitro. We have consistently observed an increase in total hydrolytic activity of HL with an increase in DG content of the Lp2A-I particles and this increase in lipid hydrolysis is primarily due to an increased hydrolysis of DG by HL. As DG significantly affects the initial hydrolytic rates of HL, this suggests that the stimulation of HL by DG may be due to an increased affinity of this enzyme for this lipid. This is similar to the data reported by Waite et al. (34) which suggests that rat HL also prefers substrates without polar head groups and may have a high affinity for DG. We show that DG hydrolytic rates represent about 49% of the total lipid hydrolysis in normolipidemic HDL. This is also close to what we have observed for a normo-

lipidemic, reconstituted HDL particle containing 20 and 10 molecules of DG and TG, respectively.

Increases in the rates of DG hydrolysis by HL appear to be inversely proportional to the hydrolysis of PL. When a small amount of TG was included in a particle containing DG and PL, there was a dramatic increase in the DG hydrolysis rate (Table 1). This increase in hydrolysis could be due to an increase in DG at the surface of the Lp2A-I particle. If TG can displace a portion of the 40% of DG in the core of the lipoprotein, DG may move to the particle surface and become more accessible to HL. This effect is probably due more to an increase in substrate accessibility rather than an increase in the surface area. A corresponding increase in phospholipid, in the absence of DG, only resulted in a 5% increase of the hydrolytic rate (data not shown). If DG is predominantly located at the particle surface as previously proposed (11, 35), this would confirm the hypothesis that HL predominantly acts as a surface lipid lipase with normolipidemic HDL particles (15).

The origin of DG in plasma lipoprotein particles is unclear. Vieu et al. (11) showed evidence to suggest that it is unlikely that a plasma phospholipase C could be responsible for DG synthesis in the plasma. Instead they suggested that DG may accumulate in HDL due to a reduced capability of HL to hydrolyze DG relative to TG. This hypothesis, however, is not supported by the studies of Waite and colleagues (34) or by our observation that human HL can hydrolyze DG much more rapidly than TG. Alternatively, HL may be involved in DG accumulation in HDL through its ability to promote the synthesis of DG. Early reports showed that HL also has a monoacylglycerol acyltransferase (MGAT) activity that can produce DG from two monoacylglycerol molecules or from one monoacylglycerol and a phospholipid (36). HL MGAT activity may act cooperatively with lipoprotein lipase and convert its lipolytic degradative products in HDL into DG.

As with other interfacial enzymes, HL is extremely sensitive to the composition and structure of its lipoprotein substrates. This study has shown that the DG content of HDL particles can regulate the hydrolytic rates and lipid substrate specificity of HL. Through its propensity to promote the disassociation and clearance of apoA-I from HDL, HL can reduce plasma HDL levels and, thus, add to the risk of atherogenesis (37–39). Numerous lines of evidence suggest that changes in HDL structure have significant and important consequences with regard to the function of this lipoprotein and the fate of its hydrolytic products. Recent investigations in this laboratory show that DG has major effects on both charge and stability of HDL particles (C. R. Coffill and D. L. Sparks, unpublished observation). However, further studies are required to determine how DG may be involved in HDL lipid metabolism and cholesterol homeostasis.

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