Comparative analysis of lipid composition of normal and acute-phase high density lipoproteins

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Abstract **In the acute-phase response and in diseases with prolonged acute phases, normal HDL (NHDL) is converted into acute-phase HDL (APHDL) and becomes proinflammatory and unable to protect LDL against oxidative modification. Earlier work had demonstrated that these changes are associated with alterations in apolipoprotein composition and enzymatic activity of APHDL, but the effect of the acute-phase condition on the lipid composition of APHDL had remained obscure. The present study shows marked quantitative differences in lipid composition between NHDL and APHDL. Specifically, APHDL contained 25% less total lipid per milligram of protein. Up to 50% of cholesteryl ester in the lipid core of APHDL was replaced by triacylglycerol; however, the total phospholipid/total neutral lipid ratios were the same as in NHDL, both lipoproteins giving similar calculated lipid core radii. Furthermore, the phosphatidylcholine/sphingomyelin ratio in APHDL was nearly double that in NHDL, indicating a relative loss of sphingomyelin. A decrease was also seen in diacyl and alkenylacyl glycerophosphatidylethanolamine as well as in phosphatidylinositol of APHDL when compared with NHDL. APHDL contained proportionally more saturated and less polyunsaturated and isoprostane-containing species of phosphatidylcholine, as well as more saturated than unsaturated cholesteryl esters. APHDL also contained significantly more free fatty acids, lysophosphatidylcholine, and free cholesterol. These changes in the lipid composition of HDL are consistent with the alterations in the apoprotein composition and enzymatic activity of APHDL and indicate proinflammatory and proatherogenic roles for APHDL.**—Pruzanski, W., E. Stefanski, F. C. de Beer, M. C. de Beer, A. Ravandi, and A. Kuksis. **Comparative analysis of lipid composition of normal and acute-phase high density lipoproteins.** ***J. Lipid Res.*** **2000.** 41: **1035–1047.**

Supplementary key words normal and acute phase • high density lipoprotein • lipid composition • functional impact

Atherosclerosis results from a complex interaction of humoral and cellular elements in the arterial wall (1–5). Lipoproteins play a crucial role in atherosclerosis, with low density lipoprotein (LDL), especially in oxidized form, being proatherogenic (6) and high density lipoprotein (HDL) being protective, inhibiting LDL oxidation (7). To exert its protective action, HDL must maintain its structural integrity (8). It was shown that in the acute phase (9, 10) and in chronic inflammatory conditions (11) normal HDL (NHDL) undergoes marked alteration by the acquisition of acute-phase reactant serum amyloid A (SAA) and by losing a major part of apolipoproteins A-I and A-II (12–14) and thus converting into acute-phase HDL (APHDL). SAA synthesis and extracellular release are transiently increased in the acute-phase reaction (11–14); however, overexpression of SAA may last a long time in chronic inflammatory diseases (11).

Only a few reports have described the lipid composition of human or animal APHDL (12, 13, 15–17). These studies have not analyzed in detail the relative content of various lipids in APHDL. In humans, APHDL was reported to be similar to NHDL in terms of relative lipid and protein content. No significant difference in phospholipid and triacylglycerol content was observed (12, 15). In rabbits and baboons, but not in mice, APHDL was found to be enriched in triacylglycerols (16). Yet, in another study by the same group, rabbit APHDL was found to be depleted of triacylglycerols, cholesterol, and phospholipid (17).

The need for more extensive comparative analysis of NHDL and APHDL assumed more significance after a recent report that APHDL is proinflammatory and possibly proatherogenic, losing the protective role of physiologic NHDL (8). The proinflammatory property of APHDL has been linked to the loss of paraoxonase and PAF-acetylhydrolase and to the relative enrichment in ceruloplasmin (8). Paraoxonase was found to protect LDL from oxidation (18).

Abbreviations: APHDL, acute-phase HDL; C, cholesterol; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; FAME, fatty acid methyl ester; FC, free cholesterol; FFA, free fatty acid; GLC, gas–liquid chromatography; GroPCho, glycerophosphocholine; MG, monoacylglycerol; NHDL, normal HDL; PL, phospholipid; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdIns, phosphatidylinositol; SAA, serum amyloid A; SM, sphingomyelin; SR-B1, scavenger receptor B1; TC, total cholesterol; TG, triacylglycerol; TLC, thin-layer chromatography.

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We reported that acute-phase SAA increases the hydrolytic activity of proinflammatory secretory group IIA phospholipase A_2 ($sPLA_2$) (19), an enzyme that is overexpressed with SAA in the acute-phase reaction (20) and in some chronic inflammatory diseases (21). Group IIA $sPLA_2$ per se, and especially after enhancement by SAA, was found to hydrolyze lipoproteins, converting phospholipids into lyso forms and liberating oxygenated and nonoxygenated fatty acids (22). These compounds are known proinflammatory agents and are most probably proatherogenic. APHDL was hydrolyzed by $sPLA_2$ faster and more extensively than NHDL (22).

Herein, we report a detailed analysis of the lipid composition of human NHDL and APHDL. It was found that these lipoproteins differ in the content of triacylglycerol, cholesteryl ester, sphingomyelin, oxophospholipids, choline, ethanolamine, and inositol phosphatides. These differences may be attributed, at least in part, to the loss of apolipoprotein A-I (apoA-I) and replacement by SAA in APHDL.

MATERIALS AND METHODS

Preparation of normal and acute-phase HDL

Blood samples for the study of normal HDL (NHDL) were obtained with informed consent from healthy volunteers. APHDL was obtained 34–38 h after surgery from patients who underwent bypass surgical procedures and had an uncomplicated postoperative course. NHDL and APHDL were isolated and purified as described (23–25). NHDL and APHDL pools were centrifuged under identical conditions to minimize the possibility of ultracentrifugation-related artifacts, and total NHDL and APHDL (1.06 –1.25 g/mL) were tested. Four pools of total NHDL and four pools of APHDL, each from 8 to 10 individuals, were tested. Protein concentration was determined by the method of Lowry et al. (26) and individual apolipoprotein content was established by pyridine extraction of Coomassie blue-stained bands from sodium dodecyl sulfate (SDS)-polyacrylamide gels as described (27). NHDL contained about 68% apolipoprotein A-I and 32% apolipoprotein A-II, including other minor apolipoproteins. APHDL contained on average about 56% apolipoprotein A-I, 17% apolipoprotein A-II, and 27% acute-phase SAA. Various batches did not vary significantly in terms of these components.

Preparation of total lipid extracts

Total lipids of the NHDL and APHDL were obtained by chloroform–methanol 2:1 (v/v) extraction (28) without acidification to avoid plasmalogen decomposition. The chloroform phase was blown down to dryness under nitrogen and redissolved in 2 mL of chloroform–methanol 2:1 (v/v).

Separation of lipid classes

The total lipid extracts were resolved into the component lipid classes by thin-layer chromatography, using neutral and polar solvent systems. The neutral lipids and free fatty acids (FFAs) were separated with heptane–isopropyl ether–acetic acid 60:40:4 (v/v/v), which retained the polar phospholipids at the origin. The neutral lipid and FFA bands were detected by brief exposure to iodine vapor, and the neutral lipid fractions were recovered by extracting with chloroform the gel scrapings from appropriate areas of the TLC plates. The phospholipids were recovered from the origin of the plate with chloroform–methanol 2:1 (v/v) and were rechromatographed on another TLC plate, using chloroform–methanol–ammonia–water 65:35:1:3 (v/v/v/v) as previously described (29). The individual phospholipid classes were recovered by extracting with chloroform–methanol 2:1 (v/v). Aliquots of the various lipid extracts were reduced to dryness and redissolved in chloroform for liquid chromatography/mass spectrometry (LC/MS).

Analysis of fatty acids

The lipid classes were methylated with 6% sulfuric acid in methanol by heating in sealed vials at 80°C for 2 h. The methyl esters were recovered by repeat extraction with hexane after diluting the reaction mixture with distilled water. The solvents were blown down under nitrogen and the samples redissolved in hexane. The fatty acid methyl esters were analyzed on a polar capillary column (SP 2380, 15 m × 0.32 mm i.d.; Supelco, Mississauga, ON, Canada) installed in a Hewlett-Packard (Palo Alto, CA) model 5880 gas chromatograph equipped with a hydrogen flame ionization detector. Hydrogen was the carrier gas at 3 psi. Injections were made at 100°C and after 0.5 min the oven temperature was programmed at 20°C/min to either 130 or 180°C and then to 240°C at 5°C/min (29). The fatty acid peaks were identified by comparing the relative retention times of the unknowns with those of standards run before and after analyzing the unknowns.

Analysis of total lipids

Total lipid content of NHDL and APHDL was estimated by high-temperature gas–liquid chromatography (GLC) after dephosphorylation of the total lipid extracts of the lipoproteins by phospholipase C (*Clostridium welchii*, type I; Sigma, St. Louis, MO) as previously described (30). To the lipid was added 1 mL of diethyl ether and 2 mL of a buffer solution (17.5 mM Tris buffer, pH 7.3) containing 8 mg of $CaCl_2$ and 2 units of phospholipase C. The suspension was incubated with stirring for 2 h in a tightly closed screw-cap vial under nitrogen at 37°C. Reaction mixtures were extracted with chloroform–methanol 2:1 (v/v) containing 100 μg of tridecanoylglycerol as internal standard. The lower phase was passed through a Pasteur pipette containing anhydrous Na_2SO_4 and the solvent was evaporated to dryness under a stream of nitrogen. The lipids were then reacted for 30 min at 20°C with [*N,O*-bis(trimethylsilyl)]trifluoroacetamide containing 1% trimethylchlorosilane (Sylon BFT) with 1 part of dry pyridine. This reaction mixture was evaporated to dryness, diluted with hexane, and used for GLC analysis by carbon number with a nonpolar capillary column (quartz capillary, 8 m × 0.32 mm i.d., coated with permanently bonded SE-54 liquid phase; Hewlett-Packard) as previously described (31). Hydrogen was the carrier gas and the column was temperature programmed. The peaks were identified by comparing the relative retention times of the unknowns with those of standards run before and after analyzing the unknowns. The peak areas generated by the hydrogen flame ionization detector were quantified in relation to the peak area of the internal standard.

Analysis of glycerophospholipids and sphingomyelins

Normal-phase HPLC separations of the total lipid extracts were performed as previously described (22, 23), using Spherisorb 3 micron columns (100 mm × 4.6 mm i.d.; Alltech Associates, Deerfield, IL) installed into a Hewlett-Packard model 1060 Liquid Chromatograph (LC) connected to a Hewlett-Packard model 5988B quadruple mass spectrometer (MS) equipped with a nebulizer-assisted electrospray ionization (ESI) interface (HP 59987A). The capillary exit voltage was set at 150 V, with the electron multiplier at 1,795 V. By injecting each sample two times, both positive and negative ion spectra were obtained. Positive ESI spectra were taken in the mass range 350–1,100 and negative

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spectra in the mass range 300–1,100. Selected ion chromatograms were retrieved from the total ion spectra by computer. The masses given in the tables and figures are actual masses of the $[M + 1]^+$ (positive ion mode) or $[M-1]^-$ (negative ion mode) ions, rounded to the nearest integer. The nominal masses are 1 mass unit lower or higher, respectively.

The molecular species of the various phospholipids were identified on the basis of the molecular mass provided by ESI/MS, the knowledge of the fatty acid composition of the phospholipid classes, and the relative HPLC retention time (longer chain species migrated ahead of the shorter chain species) of the glycerophospholipids and sphingomyelins.

The isoprostanoic acid-containing glycerophosphocholines were identified on the basis of the masses determined by ESI/MS and the retention times of standards prepared by peroxidation of palmitoyl-arachidonoylglycerophosphocholine (32, 33). The molecular species of the isoprostane esters were quantified in relation to either palmitoylsphingosinephosphocholine derived from endogenous SM (m/z 703), or in relation to dipentadecanoylglycerophosphocholine added as internal standard at the level of 50 nmol/mg of protein.

Analysis of oxygenated fatty acids

The oxygenated acids were specifically identified and quantified by LC/ESI/MS in the negative ion mode during total lipid profiling (see above). The oxygenated fatty acids were eluted as separate peaks after the elution of the nonoxygenated saturated and unsaturated fatty acids. The oxofatty acids were identified on the basis of the molecular weight and the HPLC retention time of appropriate standards as previously described (22).

Calculation of particle size

The radius of the particle core was calculated by dividing the volume of the core by the surface area. The surface area = $4\pi r^2 = \kappa[\text{Apl(PL)} + \text{Achol(C)}]$ and the volume of the core = $4/3\pi^3 r = \kappa[\text{Vtg(TG)} + \text{Vce(CE)}]$, where κ is a proportionality constant dependent on the number of particles per unit lipid mass; PL, C, TG, and CE are the molar percentages of phospholipid, free cholesterol, triacylglycerol, and cholesteryl ester, respectively; Apl = 0.685 nm^2 and Achol = 0.31 nm^2 are the molecular surface areas of phospholipid and free cholesterol, respectively; and Vtg = 1.556 nm^3 and Vce = 1.068 nm^3 are molecular volumes of triacylglycerol and cholesteryl ester, respectively. Total radii of the particles are obtained by adding the thickness of the surface monolayer (2.0 nm) to the core radii (34).

RESULTS

Total lipid profile and particle size

The lipid profile of representative total NHDL and total APHDL is shown in **Fig. 1** (top and bottom, respectively). Qualitatively, the lipid profiles of NHDL and APHDL are similar to those recorded earlier for normolipemic subjects (34), but quantitatively there are discernible differences. **Table 1** gives the quantitative values (mg%) and ratios (mol/mol). There is much more free fatty acid ($P < 0.01$), lysoPtdCho (lysophosphatidylcholine, estimated as monoacylglycerol [MG]), and free cholesterol ($P < 0.05$) in APHDL in comparison with NHDL. APHDL contained about 25% less lipid per milligram of protein than NHDL. The PtdCho/sphingomyelin (SM) ratio in APHDL was nearly double that recorded for NHDL ($P < 0.01$) in the present analysis and in earlier studies (34, 35). The higher ratio was due to the decreased content of SM in APHDL.

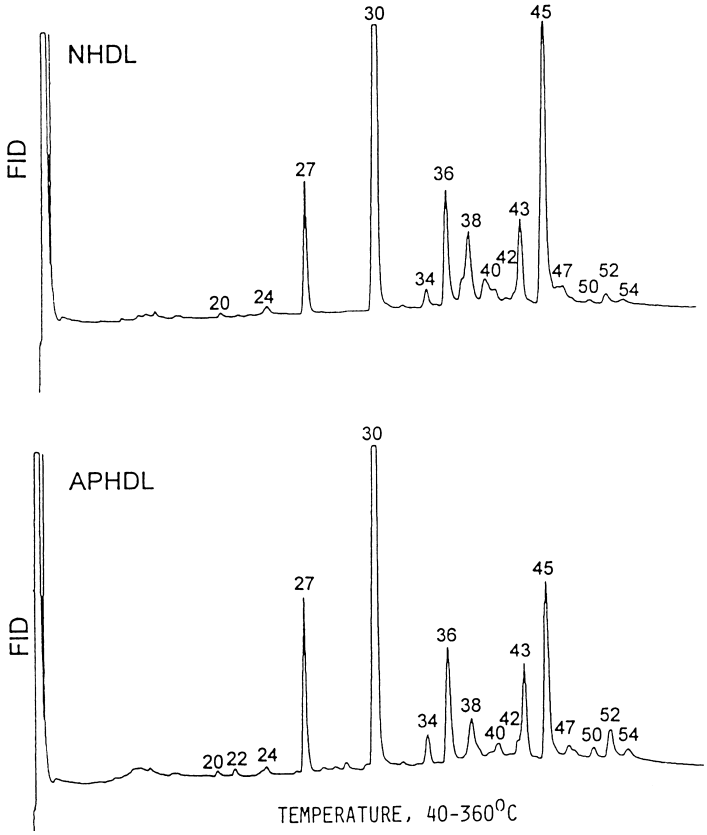

Fig. 1. Total lipid profiles of NHDL (top) and APHDL (bottom) as obtained by gas–liquid chromatography after dephosphorylation of the lipid extracts with phospholipase C and trimethylsilylation. Peak identification: 20–24, di-TMS ethers of C_{16}–C_{20} monoacylglycerols; 27, TMS ether of cholesterol; 30, tridecanoylglycerol (internal standard); 34, TMS ether of C_{34} ceramide (palmitoylsphingosine); 36–40, TMS ethers of diacylglycerols with C_{34}–C_{38} acyl carbons; 43–47, cholesteryl esters with C_{16}–C_{20} fatty acids; 50–54, triacylglycerols with C_{50}–C_{54} acyl carbons. Instrument: Hewlett-Packard (Palo Alto, CA) model 5880 gas chromatograph equipped with a flexible quartz capillary column (8 m × 0.32 mm i.d.) coated with a nonpolar phenylmethylsilicone (DB-50; Hewlett-Packard) liquid phase; carrier gas, H_2; detector, flame ionization; temperature, programmed from 40 to 220°C ballistically; from 220 to 320°C at 10°C/min; and from 320 to 360°C at 2°C/min.

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TABLE 1. Lipid composition and particle size of NHDL, APHDL, and control HDL[a]

Variable	NHDL (n = 4)	APHDL (n = 4)	HDL[b] (n = 8)
FFA (mg%)	2.7 ± 0.5	8.0 ± 0.5[c]	2.5 ± 0.5
MG (mg%)	6.2 ± 1.0	10.6 ± 1[d]	2.5 ± 0.5
FC (mg%)	21.8 ± 1.0	25.6 ± 2[d]	21.9 ± 4
CE (mg%)	138.3 ± 6	95.2 ± 5[e]	141.1 ± 10
TC (mg%)	104.8 ± 5	85.0 ± 8[e]	106.6 ± 10
TPL (mg%)	165.0 ± 10	189.4 ± 10	157.9 ± 11
TG (mg%)	41.1 ± 5	76.8 ± 5[e]	41.6 ± 4
PtdCho (mg%)	140.7 ± 10	166.4 ± 12	141.6 ± 11
SM (mg%)	14.7 ± 1	9.6 ± 3[d]	16.3 ± 3
Total lipid (mg%)	375.1 ± 20	377.0 ± 20	368 ± 30
Lipid/protein (mg/mg protein)[c]	0.92	0.67	0.90
PtdCho/FC (mol/mol)	3.14 ± 0.5	3.18 ± 0.5	3.16 ± 0.5
PtdCho/SM (mol/mol)	9.6 ± 0.5	17.3 ± 1[e]	8.7 ± 0.5
CE/TG (mol/mol)	3.4 ± 1	1.3 ± 0.5[e]	3.4 ± 1
FC/TC	0.20 ± 0.01	0.30 ± 0.05[d]	0.21 ± 0.01
Lipid core radius (nm)	4.59 ± 0.1	5.05 ± 0.2	4.5 ± 0.3

Abbreviations: CE, cholesteryl ester; FC, free cholesterol; FFA, free fatty acid; MG, monoacylglycerol; PtdCho, phosphatidylcholine; SM, sphingomyelin; TC, total cholesterol; TG, triacylglycerol; TL, total lipid.

[a] Means ± SD.

[b] Adapted from ref. 34.

c Protein content of NHDL (52%) and APHDL (60%).

[d] $P < 0.05$.

[e] $P < 0.01$.

There was a significant decrease in the cholesteryl ester/triacylglycerol (CE/TG) ratio in APHDL (1.2 ± 0.5) compared with NHDL (3.4 ± 1), which was due to a decrease in CE and an increase in TG content in APHDL, as seen from the increased FC/TC ratio in APHDL (0.30 ± 0.05) versus NHDL (0.20 ± 0.01). Therefore, it can be concluded that up to 50% of the CE in the lipid core of APHDL has been replaced by TG.

Despite the discrepancies in the total lipid classes, the phospholipid/total neutral lipid (CE plus TG) ratios remained the same in all NHDL and APHDL samples (1.0 ± 0.1 vs. 1.2 ± 0.1), indicating that APHDL and NHDL had similar calculated lipid core radii (4.59 vs. 5.05 nm). These values are of the order observed for $APHDL_3$ (4.2 nm) by electron microscopy (16), whereas on nondenaturing gel electrophoresis a larger particle radius (5.2 nm) had been observed (12). Cabana et al. (16) concluded that this was probably due to the presence of SAA in the surface of the particle, resulting in an increase in the thickness of the shell of the particle, which would be consistent with the increase in the density of the particle as well as with the finding of unchanged radii of the lipid cores.

Neutral lipid profiles

In addition to the GLC profiles of total lipids shown in Fig. 1, GLC profiles of neutral lipids of NHDL and APHDL were determined in order to determine if free diacylglycerols and free ceramides were present in the total lipid extracts (chromatograms not shown). Vieu et al. (36) had reported that HDL may contain as much free diacylglycerol as free cholesterol, or twice as much as triacylglycerol. In view of the elevated triacylglycerols in APHDL the diacylglycerol content became a matter of concern, because it could have led to an overestimation of the PtdCho content by the GLC method and an underestimation of the particle size. The GLC profiles of the neutral lipids obtained after removal of the phospholipids by Zeolite or Florisil cartridges, however, gave no evidence of any increase in the free ceramide and free diacylglycerol content of NHDL or APHDL. Although both contained small peaks in the combined diacylglycerol–ceramide region, the estimated quantities were well below those reported by Vieu et al. (36) and approached those reported by Skipski et al. (35) and by us (37). Skipski et al. (35) had estimated that HDL_2 and HDL_3 from fasting normolipemic subjects could contain up to 20% of total neutral acylglycerol mixture as free diacylglycerol. In view of the low triacylglycerol content of HDL, this would result in an increase of a maximum of 5% of total HDL phospholipid, which would not affect the various phospholipid and neutral lipid ratios.

Composition of neutral lipids and free fatty acids

Table 2 gives the average fatty acid composition of FFA and neutral lipid fractions in NHDL and APHDL. The composition of the FFA of APHDL was similar to that of NHDL. In comparison with the fatty acids present in the TG and CE of APHDL, the FFA were characterized by greater proportions of the more saturated species, especially the shorter chain-length saturated and stearic acid. The FFA of both NHDL and APHDL had notably less linoleic acid, which was the major fatty acid in TG and CE. The FFA fraction was rich in 24:1 acid, the origin of which was not established. Obviously the FFA may not derive exclusively from TG or even the *sn*-2-position of PtdCho, which contains an excess of the polyunsaturated fatty acids (see below). The composition of the FFA of NHDL in our study differed from that reported for human total serum FFA (38), which reflected more or less the composition of the depot fat TG. The fatty acid compositions of TG and CE of NHDL and APHDL were similar to those reported by Sattler et al. (39), who measured, however, only a few fatty acids.

Carbon number distribution of neutral lipids and phospholipids

The total lipid profiles in Fig. 1 show the carbon number distribution of the major neutral and phospholipid classes of NHDL and APHDL. The diacylglycerol moieties of PtdCho in both NHDL and APHDL were made up qualitatively of the same fatty acid pairings, e.g., C_{16}/C_{18} (C_{36}), C_{18}/C_{18} (C_{38}) and C_{16}/C_{20} (C_{38}), C_{18}/C_{20} (C_{40}) and C_{18}/C_{22} (C_{42}). However, differences were noted in the relative proportions of the resulting carbon numbers of the total phospholipids. In comparison with NHDL, APHDL contained a higher proportion of the C_{34} species, which represented mainly palmitoylsphingosine, and fewer of the C_{38} and C_{42} species, which represented largely polyunsaturated species of PtdCho. More detailed documentation of the molecular species was obtained by LC/ESI/MS (see below).

The relative proportion of esterified to free cholesterol was decreased in APHDL in comparison with NHDL, sug-

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TABLE 2. Fatty acid composition of the free fatty acid, triacylglycerol, and cholesteryl ester fractions of NHDL and APHDL[a]

	NHLD (n = 4)			APHDL (n = 4)		
FAME	FFA	TG	CE	FFA	TG	CE
12:0	4.2 ± 0.5	Trace	ND	5.7 ± 0.5	Trace	ND
14:0	8.1 ± 1.0	2.7 ± 0.5	0.5 ± 0.1	9.4 ± 0.5	2.3 ± 0.3	0.5 ± 0.1
16:0	33.7 ± 3.5	40.5 ± 3.5	13.7 ± 2.1	33.3 ± 5.1	36.3 ± 3.1	11.0 ± 2.1
16:1n7	1.7 ± 0.5	1.0 ± 0.3	3.4 ± 0.3	1.8 ± 0.5	0.9 ± 0.3	3.4 ± 0.3
18:0	15.4 ± 2.1	4.5 ± 0.3	1.1 ± 0.1	12.6 ± 2.5	5.2 ± 0.3	1.1 ± 0.1
18:1n9	11.9 ± 2.1	17.0 ± 2.1	29.6 ± 2.2	16.9 ± 2.1[c]	21.6 ± 2.1	21.1 ± 0.3
18:1n7						
18:2n6	1.9 ± 0.3	25.3 ± 3	33.7 ± 5.4	3.1 ± 0.2[c]	28.9 ± 3	53.7 ± 5.4[c]
18:3n3	0.4 ± 0.3	2.1 ± 0.3	1.0 ± 0.3	0.6 ± 0.1	Trace	1.0 ± 0.3
20:4n6	Trace	3.0 ± 0.3	7.3 ± 1.5	0.2 ± 0.3	4.0 ± 0.3	5.3 ± 1.5
20:5n3	0.1 ± 0.1	Trace	0.1 ± 0.3	Trace	Trace	0.3 ± 0.3[b]
24:1n13	9.3 ± 1.5	Trace	Trace	5.9 ± 1.5[b]	Trace	Trace
22:5n3	0.6 ± 0.1	0.8 ± 0.1	Trace	Trace	0.4 ± 0.1[c]	Trace
22:6n3	Trace	2.8 ± 0.3	0.2 ± 0.1	0.3 ± 0.1	Trace	0.4 ± 0.1[b]
Other	8.8	0.3	9.4	10.2	0.8	2.2

Abbreviations: FAME, fatty acid methyl esters; FFA, free fatty acids; TG, triacylglycerols; CE, cholesteryl esters; ND, not detected.

[a] By weight %. Values represent means ± SD.

[b] $P < 0.05$.

[c] $P < 0.01$.

gesting that LCAT activity may have been impaired. This possibility is supported by the finding (see Fig. 1) that the cholesteryl esters of APHDL were more saturated than those of NHDL, as they contained relatively more palmitate (C_{43}) than the oleate and linoleate (C_{45}). Furthermore, APHDL contained less cholesteryl arachidonate (C_{47}) compared with the oleate and linoleate. In contrast, the major carbon numbers of the triacylglycerols were similar in NHDL and APHDL, although APHDL contained more total triacylglycerol when compared with total cholesteryl ester.

Quantification of phospholipid classes

Figure 2 compares the total positive ion current profiles of total lipid extracts of NHDL (Fig. 2, top) and APHDL (Fig. 2, bottom). Only choline-containing phospoholipids, i.e., PtdCho (PC), lysoPtdCho (LPC), and sphingomy-

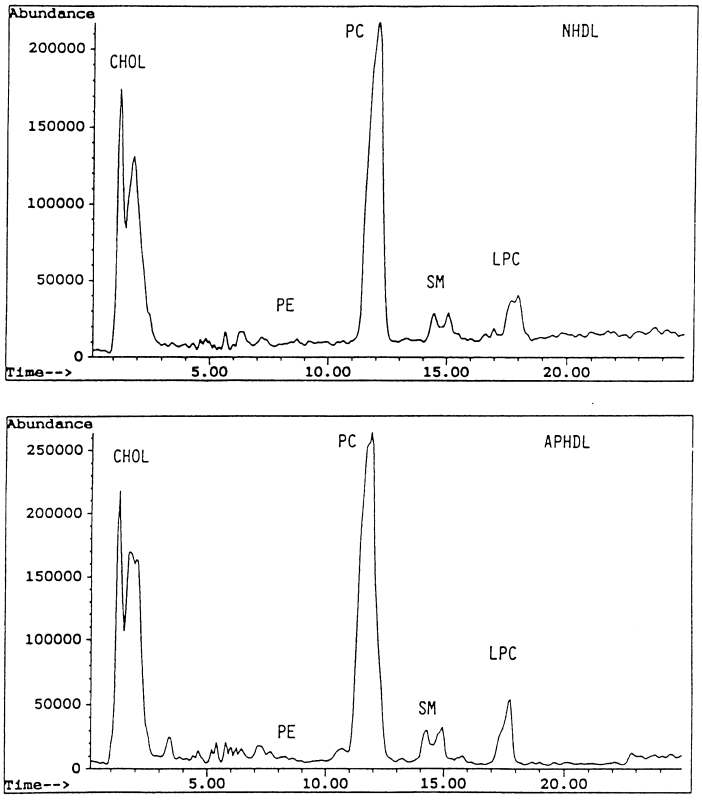

Fig. 2. Total positive ion current profiles of NHDL (top) and APHDL (bottom) as obtained by normal-phase liquid chromatography/mass spectrometry with electrospray ionization. Chol, Free cholesterol along with other neutral lipids (cholesteryl esters and triacylglycerols); PE, phosphatidylethanolamine including its alkyl and alkenyl ether homologs and peroxidation products; PC, phosphatidylcholine including alkyl ether homologs and peroxidation products; SM, sphingomyelin; LPC, lysophosphatidylcholine including alkyl ether homologs. Column, Spherisorb 3 μ (100 × 4.6 mm i.d.; Alltech Associates, Deerfield, IL); solvent, linear gradient of 100% solvent A to 100% solvent B in 14 min and then solvent B for 10 min (solvent A, chloroform–methanol–30% ammonium hydroxide [80:19.5:0.5, by volume]; solvent B, chloroform–methanol–water–30% ammonium hydroxide [60:34:5:0.5, by volume]); instrumentation, Hewlett-Packard model 1060 liquid chromatograph interfaced with Hewlett-Packard model 5988B quadruple mass spectrometer equipped with a nebulizer-assisted electrospray interface. Positive ion spectra were taken in the mass range 350–1,100. The capillary exit voltage was set at 150 V, with the electron multiplier at 1,795 V. The phospholipids were quantified on the basis of standard curves established for each phospholipid class.

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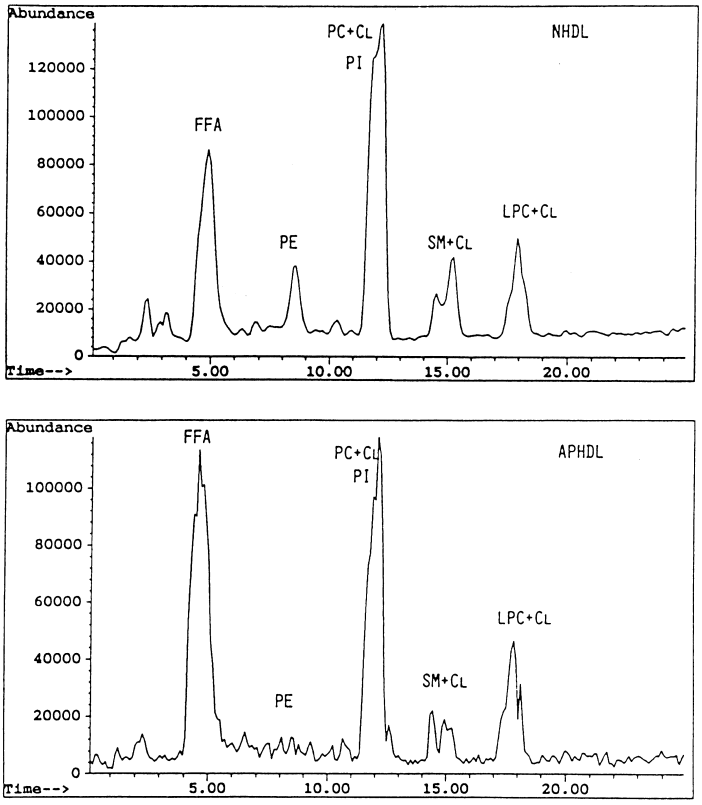

Fig. 3. Total negative ion current profiles of NHDL (top) and APHDL (bottom) as obtained by normal-phase liquid chromatography/mass spectrometry with electrospray ionization. FFA, Free fatty acids; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PC + Cl, chloride adduct of phosphatidylcholine; SM + Cl, chloride adduct of sphinogomyelin; LPC + Cl, chloride adduct of lysophosphatidylcholine. HPLC column, solvent gradient, and mass spectrometer as in Fig. 2; negative ion spectra were taken in the mass range 300–1,100 with the capillary exit voltage set at 160 V. The phospholipids were quantified on the basis of standard curves established for each phospholipid class.

elin (SM), were effectively detected by ESI in this mode. There was no obvious difference in the total positive ion current profiles between NHDL and APHDL. There was some peak tailing and splitting, which was due to the more or less complete resolution of the molecular species within each choline-containing phospholipid class. Also, there were no marked differences in the full mass spectra averaged over the corresponding phospholipid peaks between NHDL and APHDL (spectra not shown). The large peaks preceding phosphatidylethanolamine (PtdEtn; PE in Fig. 2) were due to cholesterol, other neutral lipids, and unknown contaminants. The molecular species making up the various choline-containing phospholipid classes are presented below.

Figure 3 compares the total negative ion current profiles of total lipid extracts of NHDL (Fig. 3, top) and APHDL (Fig. 3, bottom). In addition to the peaks for PtdEtn (PE) and PtdIns (PI) there are peaks due to the chloride adducts of the PtdCho (PC + Cl), which overlapped extensively with PtdIns (PI), SM (SM + Cl) and lysoPtdCho (LPC + Cl), which were detected at much lower sensitivity than the acidic phospholipids.

There was some peak splitting due to the presence of different molecular species (see below). Except for the decreased peak for PtdEtn (PE) in the APHDL profile, there were no differences in the peak patterns between the NHDL and APHDL. The molecular species making up the PtdEtn and PtdIns peaks are presented below. **Table 3** gives the phospholipid class composition, in micromoles per 2 mg of protein, for NHDL and APHDL. In general, APHDL contained significantly less PtdEtn and PtdIns than NHDL ($P < 0.01$) (see below).

TABLE 3. Quantitative analysis of phospholipid classes in NHDL and APHDL

Lipoprotein Class	Phospholipid Class (μmol/2 mg protein)[a]						
	PtdCho	LysoPtdCho	SM[b]	PtdEtn	LysoPtdEtn	PtdIns	LysoPtdIns
NHDL (n = 4)	1.15 ± 0.1	0.035 ± 0.005	0.165 ± 0.01	0.050 ± 0.005	ND	0.030 ± 0.005	ND
APHDL (n = 4)	1.03 ± 0.03	0.050 ±0.002[c]	0.130 ± 0.01	0.020 ± 0.005[d]	Trace	0.015 ± 0.005[d]	ND

Abbreviations: PtdCho, phosphatidylcholine; LysoPtdCho, lysophosphatidylcholine; SM, sphingomyelin; PtdEtn, phosphatidylethanolamine; LysoPtdEtn, lysophosphatidylethanolamine; PtdIns, phosphatidylinositol; LysoPtdIns, lysophosphatidylinositol; ND, not detected.

[a] Means ± SD.

[b] SM estimated by GLC as ceramide released by phospholipase C. The molecular weight of SM was calculated as stearoylsphingosinephosphocholine (MW 731); the molecular weight of PtdCho was calculated as distearoylglycerophosphocholine (MW 790); both PtdCho and lysoPtdCho were estimated by LC/ESI/MS as molar ratios to SM; all values were recalculated as μmol/2 mg of protein.

[c] $P < 0.05$.

[d] $P < 0.01$.

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TABLE 4. Molecular species of sphingomyelin from NHDL, APHDL, and control HDL_3 and HDL_2[a]

CN:DBN[b]	m/z	Molecular Species[b]	NHDL[c] (n = 4)	APHDL[c] (n = 4)	HDL_3[d] (n = 2)	HDL_2[d] (n = 2)
32:1	675	d18:1–14:0 d16:1–16:0	1.2 ± 0.1	1.3 ± 0.3	1.5 ± 0.1	1.0 ± 0.1
33:1	689	d17:1–16:0 d18:1–15:0	0.7 ± 0.5	0.4 ± 0.1[e]	0.6 ± 0.1	1.0 ± 0.1
34:2	701	d18:1–16:1 d16:1–18:1	2.7 ± 2.3	3.6 ± 1.4	3.8 ± 0.5	3.7 ± 0.5
34:1	703	d16:1–18:0 d18:1–16:0	29.7 ± 4.0	31.9 ± 2.2	18.6 ± 3.0	27.4 ± 3.0
36:2	729	d18:2–18:0 d18:1–18:1	4.7 ± 1.3	5.5 ± 1.8	2.8 ± 0.5	2.5 ± 0.5
36:1	731	d18:1–18:0 d16:1–20:0	6.2 ± 2.0	8.7 ± 1.6	3.9 ± 1.0	4.2 ± 0.5
38:2	757	d18:1–20:1 d16:1–22:1	1.7 ± 1.0	1.2 ± 0.5	3.1 ± 0.1	6.5 ± 1.0
38:1	759	d18:1–20:0 d16:1–22:0	5.0 ± 2.0	2.9 ± 0.5[e]	4.2 ± 0.5	5.2 ± 1.0
39:1	773	d16:1–23:0 d18:1–21:0	0.6 ± 0.05	0.7 ± 0.7	1.7 ± 0.1	2.0 ± 0.1
40:2	785	d18:1–22:1 d16:1–24:1	5.8 ± 0.4	6.2 ± 0.3	7.7 ± 0.5	6.4 ± 0.5
40:1	787	d16:1–24:0 d18:1–22:0	8.6 ± 0.9	6.7 ± 2.6	10.3 ± 1.0	9.9 ± 1.0
41:2	799	d18:2–23:0	1.6 ± 0.1	1.2 ± 0.2	4.3 ± 0.1	6.5 ± 0.5
41:1	801	d18:1–23:0 d17:1–24:0	1.7 ± 1.2	1.7 ± 0.4	3.3 ± 0.5	3.0 ± 0.5
42:3	811	d18:1–24:2 d18:2–24:1	9.5 ± 0.8	7.9 ± 1.0	6.8 ± 1.0	7.4 ± 1.0
42:2	813	d18:2–24:0 d18:1–24:1	13.9 ± 2.0	14.6 ± 3.0	18.2 ± 2.0	14.7 ± 2.0
42:1	815	d18:1–24:0	5.6 ± 1.9	4.5 ± 0.7	7.7 ± 1.0	6.5 ± 1.0

[a] In mol%.
[b] CN:DBN, caron number:double bond number; molecular species are identified by carbon number of sphingosine base (d18:1, etc.) and carbon and double bond number of fatty acid (14:0, etc.).
[c] Means ± SD (n = 4).
[d] Average ± range of two samples from the same subject (30).
[e] $P < 0.05$.

Molecular species of sphingomyelins

Mass chromatograms for the major molecular species of NHDL and APHDL were retrieved from the total positive ion current spectra. There was considerable resolution among the molecular species, with the longer chain species being completely separated from the shorter chain species. **Table 4** gives the composition of the major SM species as obtained by LC/ESI/MS in comparison with the molecular species of SM of normal HDL_2 and HDL_3 analyzed previously by GLC as the *tert*-BDMS ethers of the derived ceramides (39). The major molecular species are as follows: *m/z* 703 (d18:1/16:0); *m/z* 813 (d18:1/24:1); and others (d18:1/18:0; d18:1/24:0; d18:1/22:0; and d18:1/23:0). There were only minor differences in the relative proportions of some of the species between NHDL and APHDL. These differences were probably due to discrepancies in the d16:1, d18:1, and d18:2 base composition (40), and were not investigated further.

Molecular species of glycerophospholipids

For quantitative analysis, mass chromatograms of the major molecular species of PtdCho in NHDL and APHDL were obtained from the total positive ion current profile. All the species were eluted from the normal-phase column as symmetrical peaks with closely similar retention times and exhibited characteristic masses. When present, the alkylacyl and alkenylacyl glycerophosphocholine (GroPCho) emerged with the ascending limb of the PtdCho peak. The relative proportions of the major molecular species of

TABLE 5. Molecular species of phosphatidylcholine of NHDL and APHDL[a]

CN:DBN[b]	$[M + 1]^+$	Molecular Species	NHDL (n = 4)	APHDL (n = 4)
32:1	732	16:0/16:1	1.2 ± 0.2	1.8 ± 0.4[c]
32:0	734	16:0/16:0	2.5 ± 0.3	2.4 ± 0.7
34:2	758	16:0/18:2	28.3 ± 3.5	25.9 ± 2.6
34:1	760	16:0/18:1	13.4 ± 1.5	19.7 ± 0.9[c]
36:5	780	16:0/20:5	0.5 ± 1.5	0.3 ± 0.1
36:4	782	16:0/20:4 & 18:2/18:2	10.9 ± 2.8	11.6 ± 1.8
36:3	784	18:1/18:2	10.1 ± 1.9	8.5 ± 1.5
36:2	786	18:1/18:1 & 18:0/18:2	13.0 ± 1.3	12.0 ± 2.6
38:6	806	16:0/22:6	4.4 ± 0.8	3.3 ± 0.8
38:5	808	16:0/22:5	5.9 ±1.7	3.9 ± 1.3[c]
38:4	810	18:0/20:4	9.7 ± 2.0	9.5 ± 0.3
40:6	834	18:0/22:6	2.4 ± 0.8	1.2 ± 0.7[d]

[a] By mole %. Values represent mean ± SD (n = 4).
[b] CN:DBN, carbon number:double bond number.
[c] $P < 0.05$.
[d] $P < 0.01$.

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the PtdCho in NHDL and APHDL are given in **Table 5**. APHDL contained a significantly smaller proportion of the polyunsaturated species (16:0/22:5 and 18:0/22:6) in comparison with NHDL. A relative in the saturated species (16:0/18:1 and 16:0/16:1) was accompanied by a relative increase in the molecular species containing palmitic (16:0) compared with stearic (18:0) fatty acid. These differences in the molecular species correspond to those noted in the total lipid profiles based on carbon number distribution.

A similar LC/ESI/MS examination of the composition of the lysoPtdCho peak showed ions at *m/z* 496, 524, and 522, corresponding to the 16:0, 18:0, and 18:1 lysoPtdCho, respectively, as the major or sole components of both NHDL and APHDL. There were no significant differences in the composition of lysoPtdCho between them, although APHDL contained more of the lysoPtdCho than did NHDL.

Figure 4 shows the single ion mass chromatograms for the major isoprostane esters of GroPCho as retrieved from the total positive ion current spectrum of NHDL. The major ions correspond to 36:4 iso-PGE_2epoxyGroPCho (*m/z* 828), 36:4 iso-PGE_2/D_2GroPCho (*m/z* 830), and 36:4 iso-PGF_2GroPCho (*m/z* 832). The 38:4 isoiso-PGE_2/D_2GroPCho (*m/z* 858) and 38:4 iso-PGF_2GroPCho (*m/z* 860) species were minor and appeared erratically in the APHDL samples. Likewise, the dehydration product of 36:4 iso-PGE_2GroPCho (*m/z* 810, not shown) was found to be present in small and variable amounts only. No evidence was obtained for the presence of the neuroprostanes derived from the 16:0/22:6 GroPCho and 18:0/22:6 GroPCho. Figure 4 also shows an ion at *m/z* 703, which corresponds to palmitoylsphingosinePCho (d18:1/16:0) used as internal standard for quantification. **Table 6** gives the estimates for the isoprostane derivatives from NHDL and APHDL. The isoprostane content of NHDL was much higher, more consistent

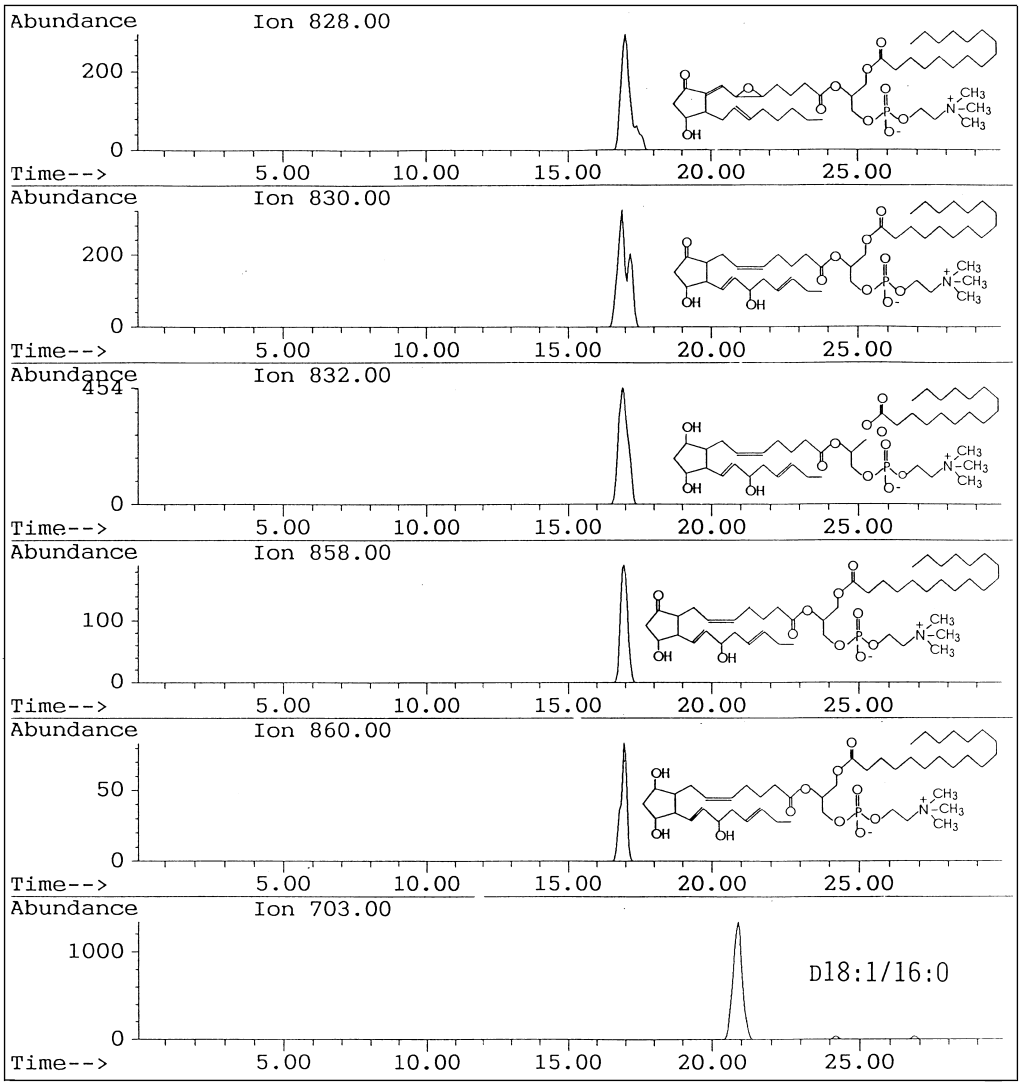

Fig. 4. Single ion mass chromatograms of the isoprostane GroPCho in NHDL. Peaks are identified and structural formulas given. Carbon number and isoprostane symbols are as given in Table 6. 36:3, Palmitoyleicosanoyl; 36:4, palmitoylarachidonoyl; 38:4, stearoylarachidonoyl; D18:1/16:0, palmitoylsphingosine Pcho used as internal standard. LC/ESI/MS conditions were as described in Materials and Methods.

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TABLE 6. Isoprostane-containing phosphatidylcholine of NHDL and APHDL[a]

CN:DB	Ions $[M + 1]^+$	Isoprostane-Containing PtdCho	NHDL (n = 4)	APHDL (n = 4)
36:3	810	5,6-Epoxy Iso-PGA_2GroPCho	Trace	Trace
36:4	828	5,6-Epoxy Iso-PGE_2GroPCho	9.3 ± 1.0	1.1 ± 0.5[c]
36:4	830	Iso-PGE_2/D_2GroPCho	13.8 ± 1.5	0.9 ± 0.4[c]
36:4	832	Iso-PGF_2GroPCho	11.7 ± 1.5	1.9 ± 0.5[c]
38:4	858	Iso-PGE_2/D_2GroPCho	3.6 ± 0.5	1.9 ± 0.5[b]
38:4	860	Iso-PGF_2GroPCho	3.0 ± 0.5	0.3 ± 0.3[c]

[a] Means ± SD (n = 4); estimated on the basis of single ion mass chromatograms extracted by computer from total positive ion current profiles recorded by LC/ES/MS, as shown in Fig. 4.
[b] $P < 0.05$.
[c] $P < 0.001$.

and represented a wider range of molecular species than that of APHDL.

Mass chromatograms of the major molecular species of ethanolamine phospholipids of NHDL and APHDL showed that the major species were the plasmalogens, which were eluted with the front portion of the ascending limb of the PtdEtn peak. Examination of the proportions of the major molecular species of the PtdEtn on NHDL and APHDL detected 16:0/20:4pl (3.4 vs. 3.0%); 16:0/18:2pl (2.8 vs. 6.0%), 18:0/20:4 (3.2 vs. 20.0%), 18:0/20:4pl (59.4 vs. 40.0%), and 16:0/22:5pl (28.7 vs. 6.0%). Other species appeared in trace amounts with the corresponding alkyl derivatives at 14 amu lower and the corresponding alkenyl derivatives at 16 amu lower than the diacyl GroPEtn.

Comparison also was made between the mass chromatograms of the major molecular species of PtdIns from NHDL and APHDL as retrieved from the total negative ion current profiles. Only a few ions were detected in the elution range of PtdIns ($(M - 1)^-$, 833–911 amu) from any of the available samples. The major species of PtdIns were 18:0/20:4 (78.7 vs 57.5%), 18:0/20:3 (7.9 vs 21.7%), and 18:0/18:2 (6.2 vs 17.2%). In general, the levels of the PtdIns species in APHDL were too low to make a reliable quantitative comparison to those in NHDL. There was no evidence for the presence of PtdSer species in APHDL with the available sample sizes. Small amounts of PtdSer made up of palmitoyl linoleates and oleates as the major components, however, could be recognized in NHDL by direct single ion monitoring in the negative ion mode (data not shown).

DISCUSSION

Studies have shown functional differences between APHDL and NHDL (8). Accelerated clearance of APHDL from circulation was noted during inflammation (13). Increased association of APHDL with neutrophils and macrophages (15) reduced affinity for hepatocytes (41), and a shift in HDL-cholesterol carrying capacity toward the macrophages were reported. Our previous studies had shown that APHDL but not NHDL markedly enhanced hydrolytic activity of proinflammatory group IIA secretory phospholipase A_2 (19, 42), an enzyme that hydrolyzes lipoproteins (22). The enhancement seems to depend at least in part on the presence of SAA because purified acute-phase SAA was a strong enhancer per se (42). In the acute-phase reaction, SAA content may vary from 15% to more than 80% of the apoproteins of HDL (13). Furthermore, anchoring of SAA to HDL is associated with significant, albeit variable, loss of apoA-I and apoA-II. Finally, APHDL is unable to protect LDL against oxidative modification (8). This was attributed to a loss of paraoxonase and PAF-acetylhydrolase, and it was concluded that APHDL may actually become proinflammatory (8).

Previous studies of human $APHDL_3$ and $NHDL_3$ had reported equal proportions of proteins, phospholipids, triacylglycerols, free cholesterol, and cholesteryl esters (12, 15). However, a comparison of $NHDL_2$ with $APHDL_2$ had shown more protein and less cholesterol ester in the latter (12). The fact that the acute-phase reaction markedly alters structural and functional properties of HDL prompted us to compare in greater detail the lipid composition of NHDL and APHDL.

The present study showed that the lipid composition of APHDL was normal in terms of the polar/nonpolar lipid ratio, although the CE/TG ratio was low. Assuming that the phospholipids along with free cholesterol make up the surface of the particle and that the cholesteryl esters along with the triacylglycerols make up the core of the particle, it was possible to calculate the lipid core diameters and to show that APHDL and NHDL possessed lipid cores of similar radii (4.59–5.05 nm). These values are on the order observed for APHDL (4.2 nm) by electron microscopy (16), whereas on nondenaturing gels a larger particle radius (5.95 nm) had been observed. Thus, the increased size and density of APHDL reported previously (12) must have been related to the presence on the particle surface of extra protein (SAA), the mass of which was not fully compensated for by the expulsion of apoA-I and apoA-II. Cabana et al. (16) accounted for the smaller size of APHDL seen by electron microscopy by postulating that the SAA particles on the APHDL surface were held in a spokelike arrangement radiating away from the surface. Clearly, the content of the surface lipid is not reduced in relation to the core lipid as a result of the attachment of SAA.

The decreased cholesteryl ester content of APHDL was compensated for by an increased proportion of triacylglycerol, which reached 50% of total neutral lipid. The decrease in cholesteryl ester content is consistent with the

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observation of reduced LCAT activity in APHDL due to a displacement of apoA-I from the particle surface by SAA (43). Because neither lipoprotein lipase nor hepatic lipase were measured, it was not determined whether the increased TG levels were due to decreased activity of these enzymes. Cabana et al. (16) had suggested that it might be due to inhibition of hepatic lipase or to up-regulation of cholesteryl ester transfer protein (CETP) mediating more rapid exchange of TG. However, mice transgenic for the human CETP gene show that hepatic CETP mRNA and plasma CETP activity decrease in inflammation after lipopolysaccharide (LPS) administration (44). Furthermore, the major mediators of the acute-phase response, interleukin 1 (IL-1), tumor necrosis factor (TNF), and IL-6, have been shown to inhibit lipoprotein lipase (45). The cytokines may also induce lipid synthesis and secretion, and de novo fatty acid synthesis (46). Despite induction of the acute phase, no change was observed in plasma lipids in mice. It remains to be established whether murine SAA has the same propensity to displace murine apoA-I in HDL as in other species (16).

Quantitative analysis of the phospholipid class composition of APHDL revealed reduction in the minor components, PtdEtn and PtdIns, when compared with NHDL. The PtdEtn and PtdIns levels of NHDL were similar to those reported by others (35) and averaged about 25% of the levels of SM. It was possible that the minor phospholipids had been removed from APHDL by the apoA-I and apoA-II displacement by SAA (12). Forte et al. (47) have demonstrated that apoA-II preferentially recruits PtdEtn, whereas apoA-I preferentially recruits SM, although both apoproteins contain PtdCho as the most abundant phospholipid. It is possible that the endogenous sphingomyelinase (48–50) also contributes to the partial loss of SM of APHDL (see below).

The present study reports the first analysis of molecular species of phospholipids from APHDL. Detailed comparison of the molecular species of intact SM, PtdCho, and lysoPtdCho by normal-phase LC/ESI/MS revealed good correspondence between NHDL and APHDL in the composition of both major and minor components, although the content of the total phospholipid per milligram of protein was somewhat lower in the APHDL. The molecular species of SM were closely similar in NHDL and APHDL and not unlike those of control HDL_3 analyzed earlier (30). This is consistent with the belief that APHDL is more closely related to HDL_3 than to HDL_2 (12, 15). If there had been any loss of SM due to displacement of apoA-I by SAA or to the action of acute-phase sphingomyelinase, all the molecular species must have been affected in proportion to their mass. There was no significant decrease seen in the polyunsaturated PtdCho in APHDL, although a GLC determination of the carbon number distribution of the derived diacylglycerols had suggested some decrease in the long-chain, and an increase in the short-chain, diacylglycerol moieties. The absence of alterations in the composition of the molecular species of PtdCho in APHDL was consistent with the earlier observation that the secretory group IIA phospholipase A_2 attacks all major species indiscriminately (22).

The molecular species of PtdEtn and PtdIns, however, differed markedly between NHDL and APHDL. Because of the greatly decreased amounts of the acidic phospholipids in APHDL only the major molecular species could be compared with NHDL. Interestingly, both NHDL and APHDL contained largely plasmenyl PtdEtn. The PtdIns was represented mainly by the palmitoyl and stearoyl arachidonates in both NHDL and APHDL. Analyses of the molecular species of the lysoPtdCho showed mainly palmitoyl, stearoyl, and oleoyl species in both NHDL and APHDL. There was somewhat more lysoPtdCho in APHDL than in NHDL.

In a previous study (22) we had demonstrated that secretory group IIA phospholipase A_2 released oxygenated fatty acids from both choline and ethanolamine glycerophospholipids, which were identified as the hydroperoxides and hydroxides mainly of linoleic acid. The present study shows that the peroxidation products of the arachidonic acid esters are present in both NHDL and APHDL as the isoprostane derivatives. The isoprostane-containing PtdCho was identified in relation to the standards prepared by oxidation of pure 16:0/20:4 GroPCho, e.g., iso-PGE_2/D_2 (m/z 830) and iso-PGF_2 (m/z 832). These standards possessed HPLC retention times and molecular masses identical to those detected in the lipid extracts of NHDL and APHDL. Both NHDL and APHDL also contained the 18:0/20:4 isoprostane homologs. The isoprostane content of APHDL ranged from 0 to 15 nmol/mg of protein, whereas that of NHDL was 10 to 20 times higher, consistent with a wider range of molecular species than that of APHDL. Because the analyses were performed on fresh samples of NHDL it is unlikely that these compounds would have accumulated during storage, which is known to result in an up to 50-fold increase in the isoprostane content in plasma (33). The quantities of the isoprostanes detected varied with the arachidonate content, which was significantly lower in APHDL. It is not clear whether low isoprostane levels in APHDL are related to the hydrolytic activity of $sPLA_2$ or whether other factors related to the acute-phase reaction contribute to their release. Berliner and colleagues (51, 52) have recognized the proinflammatory activity of isoprostanes in mildly oxidized LDL. Our finding of markedly lower active isoprostane levels in APHDL as compared with NHDL may suggest that during the conversion of NHDL to APHDL active isoprostanes are released to the surrounding medium inducing inflammatory reaction.

The present demonstration of the isoprostanes in NHDL and APHDL complements the results of our earlier study (22), which showed that $sPLA_2$ releases hydroxy and hydroperoxy linoleates and other oxygenated acids from these lipoproteins along with normal free fatty acids and lysophospholipids. The lyso lipids are highly inflammatory, increase protooncogene expression and AP-1-binding activity, and activate MAP kinase in the vascular smooth muscle and other cells (53, 54). LysoPtdCho was found to induce adhesion molecules VCAM-1 and ICAM-1 in the arterial endothelial cells (55) and to enhance chemotaxis of monocytes (56). Likewise, the toxic role of free fatty acids has been de-

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scribed (57, 58) and phospholipid hydroperoxides isolated from minimally oxidized LDL were found to inhibit LCAT activity (59).

According to Van Lenten et al. (8), NHDL contains paraoxonase and the PAF acetylhydrolase, which function as antioxidants preventing the cell-mediated oxidation of LDL. APHDL had low levels of paraoxonase and PAF acetylhydrolase and had essentially no antioxidant effect on the monocyte transmigration (8). In fact, APHDL showed an increased level of the prooxidant ceruloplasmin (8, 60). Inactivation of human serum paraoxonase by lysoPtdCho has been reported (61). In transgenic mice overexpressing $sPLA_2$ there was an increase in the formation of the bioactive oxo fatty acids, probably because of action of the enzyme on HDL and LDL (62). These mice, which have low circulating plasma paraoxonase and high levels of nonesterified cholesterol, develop advanced atherosclerosis (63). Thus, in animals prolonged overexpression of $sPLA_2$ is associated with atherosclerosis. The preceding observation clearly links the hydrolysis of APHDL to the generation of metabolically active proatherogenic products, which in chronic human diseases (in which prolonged overexpression of SAA and $sPLA_2$ takes place) may induce accelerated atherosclerosis.

The scavenger receptor SR-B1 has been identified as an HDL receptor (64). This receptor has been reported to interact with the anionic phospholipid moiety rather than the apolipoproteins (65). Because the present study shows that APHDL is depleted in PtdEtn, PtdIns, and possibly PtdSer, its interaction with the SR-B1 receptor may be impaired. Further impairment may arise from the partial replacement of apoA-I and apoA-II, which along with other HDL apoproteins are known to mediate the binding of HDL to SR-B1 (66). It is conceivable that in the acute phase two types of APHDL are present, pre-receptor-linked and post-receptor-detached molecules.

In conclusion, the present study shows that the conversion of NHDL into APHDL induced by acute-phase reactants leads to changes in lipid content and composition in addition to the previously established partial replacement of the normal apolipoproteins by SAA, loss of enzymes, and enrichment in ceruloplasmin. The preceding observations emphasize the complexity of the interaction among apolipoproteins, lipids, enzymes, and receptors of HDL. Clearly, APHDL is not merely a passive molecule unable to protect LDL against oxidative transformation. The increase in lyso compounds, liberation of free fatty acids and oxo fatty acids, along with loss of acidic phospholipids in the acute phase converts APHDL into an active proinflammatory and proatherogenic molecule. Translation of the above-described structural changes into functional language is necessary to understand the role of APHDL in pathology.

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