Molecular Species of Glycerophospholipids and Sphingomyelins of Human Plasma: Comparison to Red Blood Cells

J.J. Myher, A. Kuksis* and S. Pind
Banting and Best Department of Medical Research and Department of Biochemistry, University of Toronto, Toronto, Canada M5G 1L6

In addition to diacyl glycerophosphocholine and sphingomyelin, human plasma also contains small amounts of other glycerophospholipids, which may have special metabolic function. The structure and origin of these minor plasma lipids has not been determined. Knowledge of the detailed composition of the phospholipids of red blood cells (Myher *et al., Lipids 24,* 1989) permits evaluation of one of the possible sources. This study reports the detailed analyses of plasma glycerophospholipids made in parallel to those of the erythrocyte lipids obtained from the same blood using HPLC and GLC methods. The proportions of the major phospholipid classes in the plasma and erythrocytes were similar to published values, including the essential absence of diradyl glycerophosphoserine from plasma. Plasma diradyl glycerophosphocholine contained 93.0% diacyl, 3.4% alkylkacyl and 3.6% alkenylacyl, whereas the diradyl glycerophosphoethanolamine consisted of 71.8% alkenylacyl, 19.9% diacyl and 8.3% alkylacyl subclasses. The diradyl glycerophosphoinositol was 100% diacyl. The content of the minor subclasses of plasma diradyl glycerophosphocholine is similar to that of the red cells, but the ether content of the diradyl glycerophosphoethanolamine is higher in plasma than in cells. The lipid ether subclasses of plasma glycerophospholipids also contained a higher proportion of the C_{20}, C_{22} and C_{24} alkyl and alkenyl chains than those of the cells. Furthermore, the C_{16} and C_{18}-containing species in diradyl glycerophosphoethanolamine subclasses varied with the nature of the polyunsaturated acid, whereas in diradyl glycerophosphocholine subclasses the polyunsaturated acids were combined with the C_{16} and C_{18} acids in equal proportions. The significant differences in the molecular species of glycerophospholipids and sphingomyelin between plasma and red cells would appear to limit any direct transfer or equilibration of their lipid components.
***Lipids 24,* 408-418 (1989).**

The phospholipid class composition of plasma lipoproteins is unusual. It differs from that of the red blood cell membranes and from the plasma membranes of the cells lining the vascular bed. Thus, although diradyl glycerophosphocholine (GPC) and sphingomyelin (SPH) comprise the bulk and the diradyl glycerophosphoethanolamine (GPE) and glycerophosphoinositol (GPI) constitute minor components, the red cell membrane contains the diradyl GPC, GPE, glycerophosphoserine (GPS) and SPH as major and GPI as minor components (1). The glycerophospholipids (GPL) from both sources contain a wide variety of fatty chains, attached by ester or ether linkages, with chain lengths of 16-22 carbons and up to 6 double bonds, whereas SPH is made up of a separate pool of fatty acids and nitrogenous bases joined by amide linkages. This results in several hundreds of chemically distinct species of phospholipids. We have recently determined the detailed molecular species composition of the red blood cells of man (2). The present study extends this work to the plasma GPL and SPH and, along with the accompanying study (2), constitutes the first comprehensive assessment of the composition of GPL species of plasma and erythrocytes from the same blood. A preliminary account on the comparative composition of plasma and red cell GPL has appeared (3). A summary of the SPH data has been published previously (4).

MATERIALS AND METHODS

Blood plasma. The plasma for the diradyl GPL analyses was obtained from the same sample of blood as the erythrocytes analyzed in the accompanying paper (2). This subject also provided plasma SPH, which, however, was analyzed only for fatty acid composition and the carbon number distribution of the ceramide moieties. The molecular species analyses of the sphingomyelins had been performed on 4 other samples of plasma from 3 normolipemic subjects in the fasting state (VLDL, LDL and HDL_3) and one normolipemic subject in the postprandial state (chylomicrons and VLDL), who had shown fatty acid and ceramide compositions of VLDL and LDL similar to those obtained for plasma total SPH from the subject supplying the diradyl GPL. Plasma and cells were separated by centrifugation and cells were washed to remove the buffy coat. Some of the plasma samples were resolved into the major lipoprotein classes by ultracentrifugation as described elsewhere (5).

Lipid analyses. The methods of lipid extraction and chromatographic analyses of molecular species were as previously described in detail (2). Plasma and lipoprotein total lipid profiles were determined by capillary GLC as reported (6).

RESULTS

Total lipid composition. The phospholipid class composition of whole plasma was of the order reported previously on basis of TLC separation and phosphorus analyses (7) or GLC analyses of the component fatty acids (8), with 67.0% diradyl GPC, 17.7% SPH, 2.5% diradyl GPE and 2.1% diradyl GPI. Figure 1 shows the separation of alkenylacyl, alkylacyl and diacyl subclasses of the diradylglycerol moieties of the plasma ethanolamine GPL. A similar separation of the diradylglycerol moieties of plasma choline GPL gave much smaller but readily detectable fractions for the ether-linked species. These separations were performed with the TMS ethers of the diradylglycerols. Based on GLC quantitation the diradyl GPC contained 93.0% diacyl, 3.4% alkylacyl and 3.6% alkenylacyl, whereas the diradyl GPE contained 71.8%

*To whom correspondence should be addressed at: Banting and Best Dept. Medical Research, University of Toronto, 112 College St., Toronto, Canada M5G 1L6.

Abbreviations: GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; GPI, glycerophosphoinositol; GPL, glycerophospholipids; GPS, glycerophosphoserine; SPH, sphingomyelin; PC° and PE°, diacyl GPC and GPE; PC′ and PE′, alkylacyl GPC and GPE; PC″ and PE″, alkenylacyl GPC and GPE.

alkenylacyl, 19.9% diacyl and 8.3% alkylacylglycerol subclasses. The diradyl GPI was 100% diacyl. Although the ether-linked GPL content was not measured in the individual lipoprotein classes, it has been observed that each lipoprotein contained at least some of this lipid class (9). The overall fatty acid composition of the various plasma phospholipid classes was similar to those reported in the literature (7,8,10). The detailed fatty acid composition of the various diradylglycerol fractions derived from them is discussed below under molecular species.

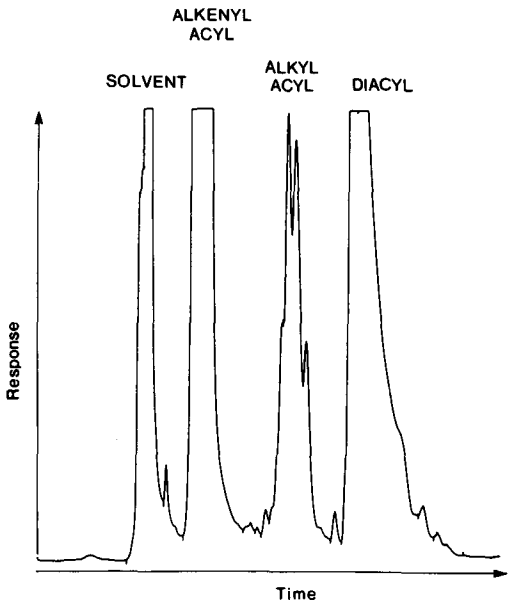

FIG. 1. Normal-phase HPLC separation of alkenylacyl, alkylacyl and diacylglycerol moieties of human plasma diradyl GPE. Peak identification as given in figure. HPLC conditions: column, Supelcosil LC-Si (5 μ), 250 cm × 4.6 mm i.d.; solvent, hexane-isopropanol 99.7:0.3 (v/v), 1 ml/min; temperature, 30°C, isothermal. Sample: diradylglycerol TMS ethers.

Analyses of molecular species. Figure 2 shows the carbon number resolution of the diacyl, alkylacyl and alkenylacyl subclasses of the diradyl GPC. The corresponding quantitative values are given in Table 1. It is seen that the subclasses differ significantly in the relative proportions of the chain lengths, with the alkylacylglycerol species possessing a much greater proportion of the longer chain lengths. Of particular interest here is the presence of appreciable amounts of C_{20}, C_{22} and C_{24} alkyl chains. A comparable increase in chain length was not seen for the alkenylacyl GPC. There were also chain length differences among the diradyl GPE, as well as among the diacyl GPC, GPE and GPI, as shown in Table 1. These data were used for reconstitution of the quantitative composition of the molecular species of the diradylglycerols derived from polar capillary GLC (see following discussion).

Figure 3 shows the polar capillary GLC profile of the diacylglycerol moieties of ethanolamine GPL, whereas Figure 4 shows the polar capillary GLC profile of the diacylglycerol moieties of diacyl GPI. The quantitative composition of the molecular species of the diacyl GPC, GPE and GPI is given in Table 2. The corresponding fatty acid compositions are given in Table 3. It is seen that the diacylglycerol moieties of the three GPL classes differ greatly from each other and from the alkylacyl and alkenylacylglycerol moieties of diradyl GPC and GPE. Thus, combinations of 16:0 with 18:1 and 18:2 form the most abundant species of diacyl GPC, whereas

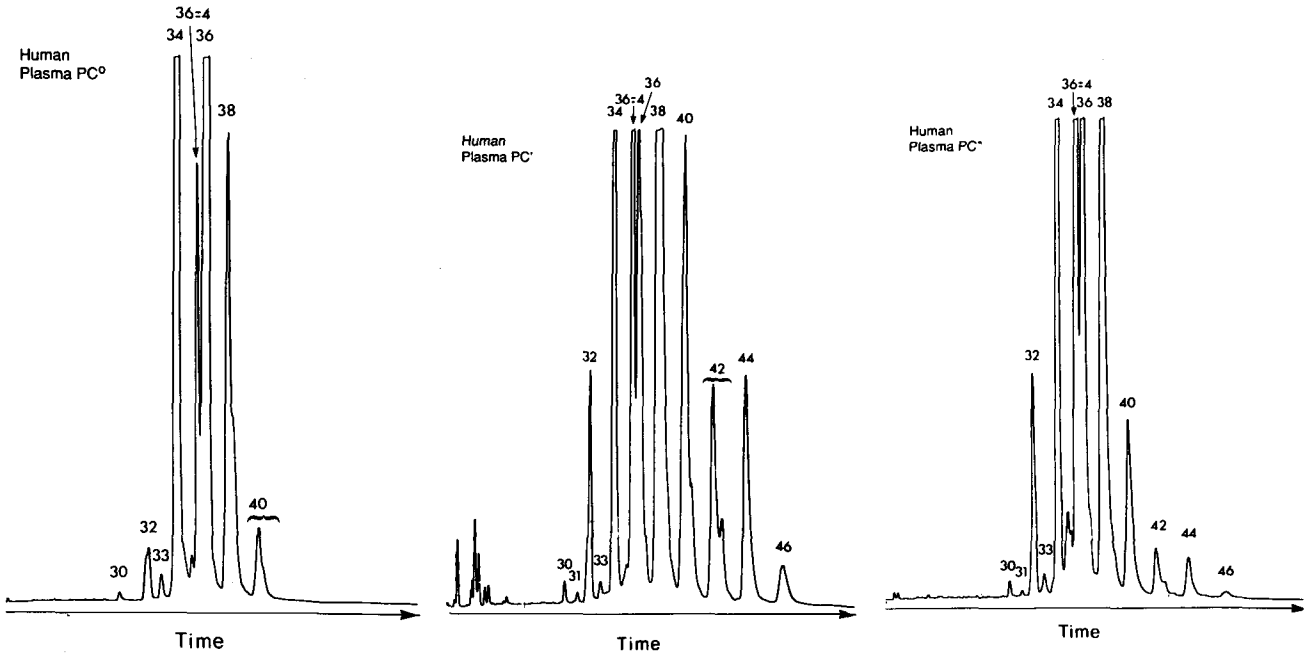

FIG. 2. Carbon number resolution of diacyl (PC°), alkylacyl (PC') and alkenylacylglycerol (PC") moieties of human plasma diradyl GPC. Peak identification as given in figure. GLC conditions: column 8 m × 0.32 mm fused silica capillary coated with cross-linked 5% phenylmethyl silicone (HP-5), 0.17 μ film thickness; carrier gas H_2, 6 psi; instrument and other operating conditions as previously described (2). Sample: 1 μl of 0.1% diradylglycerol TMS ethers in hexane.

TABLE 1

Carbon Number Distribution of Diacyl, Alkylacyl and Alkenylacyl Subclasses in Choline and Ethanolamine Phosphatides of Human Plasma

Carbon number	PC			PE			PI
	Diacyl	Alkylacyl	Alkenylacyl	Diacyl	Alkylacyl	Alkenylacyl	Diacyl
				Mole %			
30	0.2	0.3	0.2	—	—	—	
31		0.2	0.1	—	—	—	
32	1.6	3.8	3.6	0.6	0.3	0.2	0.5
33	0.5	0.4	0.5	0.2	0.3	0.1	—
34	42.4	13.5	24.3	11.1	6.2	4.9	8.2
35	0.7	—	2.5	0.4	1.3	0.8	0.3
36:4	7.7	13.9	21.3	7.5	12.5	11.1	4.0
36	30.4	11.3	17.1	27.4	10.4	14.6	21.5
37	—	—	—	—	—	1.2	1.6
38	14.4	31.6	22.0	43.2	42.1	46.3	59.6
40	2.3	12.1	5.5	9.7	23.3	18.1	3.9
42	—	6.3	1.5	—	2.9	2.0	—
44	—	5.2	1.1	—	0.7	0.7	—
46	—	1.4	0.2	—	—	0.1	—

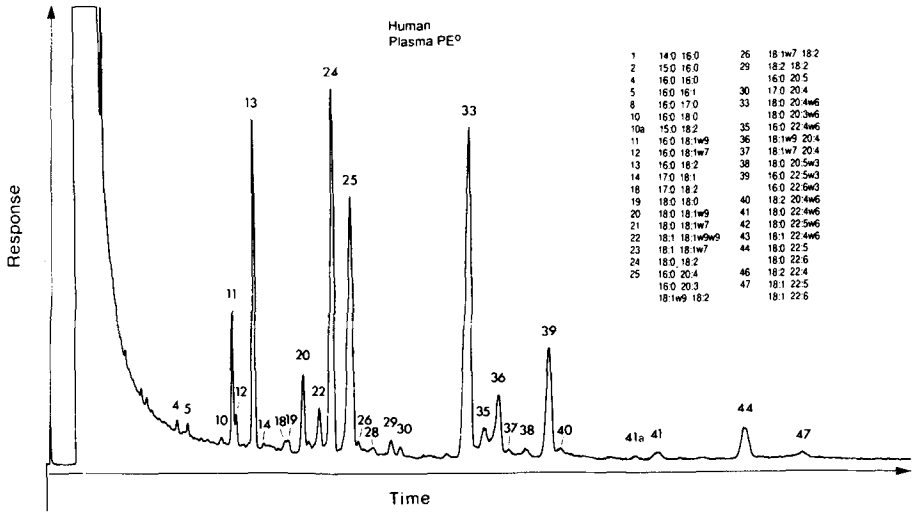

FIG. 3. Polar capillary GLC profile of the diacylglycerol moieties of human plasma diacyl GPE. Peak identification as given in figure. GLC conditions: column, 15 m × 0.32 mm fused silica capillary coated with cross-bonded RTx 2330; carrier gas, H_2, 3 psi; temperature, 250°C, isothermal; instrument and other operating conditions as given (2). Split ratio 7:1. Sample: 1 μl of 0.1% diacylglycerol TMS ethers in hexane.

combinations of 18:0 with 18:2 and/or 20:4 are favored in diacyl GPE and GPI.

Figures 5 and 6 show the elution patterns obtained on polar capillary GLC for the TMS ethers of the alkylacylglycerols derived from plasma choline and ethanolamine GPL, respectively. The corresponding quantitative values are given in Table 4. There are marked differences in both qualitative and quantitative composition, which indicates that the alkylacylglycerols represent different subcellular pools of biosynthetic precursors. Although both choline and ethanolamine GPL contain nearly identical proportions of 16:0′ 20:4 and 18:0′ 20:4 species, the alkylacyl GPC contain much more of the saturated, monoenoic and 18:1′ 20:4 and 16:0′ 22:4, and less of other polyunsaturated species than alkylacyl GPE. Figures 7 and 8 show the separation obtained for the alkylglycerol moieties of the alkylacyl GPC on the nonpolar and polar capillary columns, respectively. From Figure 7, it is seen that the major carbon numbers range from 16 to 24 and that all, except 16, show partial splitting due to a slightly earlier elution of the corresponding mono- and diunsaturated species. From Figure 8, it is seen that the saturated, monounsaturated and diunsaturated alkylglycerol chains are resolved. It can now be appreciated that the 18:1′ peak contains a significant proportion of the *trans* isomer, which is eluted earlier, and the ω7 *cis* isomer, which is eluted later, than the 18:1 ω9 *cis* isomer. The identity of the plasma alkylglycerol diacetates on the polar column was confirmed by GLC analysis of the alkylglycerol fractions resolved by argentation TLC. The

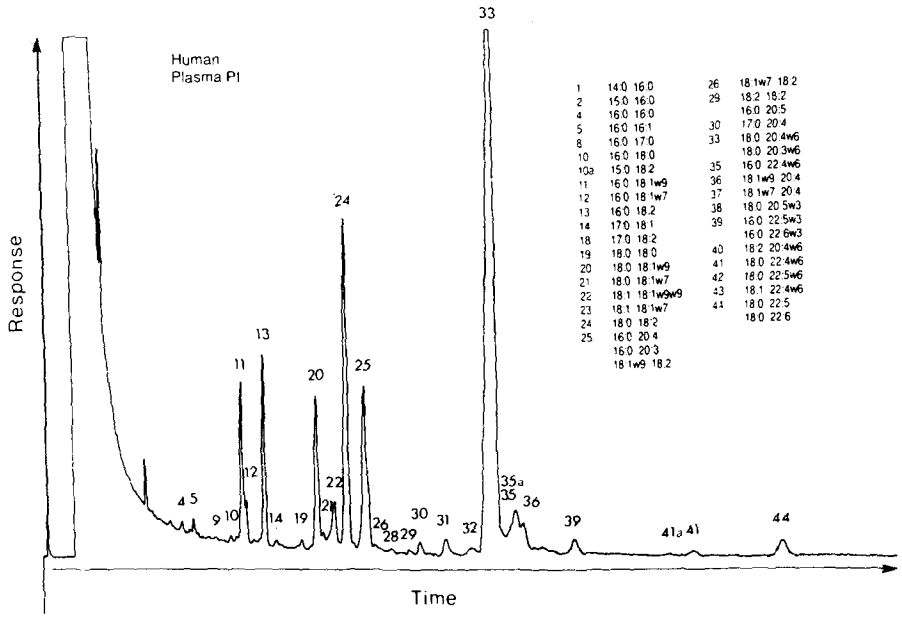

FIG. 4. Polar capillary GLC profile of the diacylglycerol moieties of human plasma diacyl GPI. Peak identification as given in figure. GLC conditions as given in Figure 3. Sample: 1 μl of 0.1% diacylglycerol TMS ethers in hexane.

TABLE 2

Molecular Species of Diacyl Glycerophospholipids of Human Plasma

GLC peak	Molecular species	Phospholipid classes PC	PE	PI	GLC peak	Molecular species	Phospholipid classes PC	PE	PI
		Mole %					Mole %		
1	14:0-16:0	0.2			30	17:0-20:4	0.2	0.3	0.5
4	16:0-16:0	0.8	0.3	0.2	33	18:0-20:4ω6	6.7	22.7	50.0
5	16:0-16:1	0.7	0.3	0.4		18:0-20:3ω6			
8	16:0-17:0	0.2	—	—		16:0-22:4ω6	0.9	2.7	
10	16:0-18:0	0.2	0.2	0.1	35A	18:1-20:3 + 18:1t-20:4			3.9
11	16:0-18:1ω9	9.3	2.8	3.4	36	18:1ω9-20:4	1.3	5.5	1.6
12	16:0-18:1ω7	2.1	0.7	0.8	37	18:1ω7-20:4	0.2	0.7	
13	16:0-18:2	29.4	7.7	4.0	38	18:0-20:5ω3	0.3	1.2	
14	17:0-18:1	0.4	0.1	0.2	39	16:0-22:5ω3	3.5	7.8	1.1
18	17:0-18:2	0.3	0.3			16:0-22:6ω3			
19	18:0-18:0	0.5	0.3	0.2	40	18:2-20:4	0.5	1.1	
20	18:0-18:1ω9	2.1	2.6	5.0	41	18:0-22:4ω6	0.4	1.1	0.8
21	18:0-18:1ω7	0.6	0.3	0.5	42	18:0-22:5ω6	—	—	—
22	18:1ω9-18:1ω9	1.3	1.9	2.5	43	18:1-22:4ω6	0.4	—	—
23	18:1-18:1ω7	—	—	—	44	18:0-22:5	1.5	4.5	2.8
24	18:0-18:2	15.4	13.8	11.6		18:0-22:6			
25	16:0-20:4	7.7	7.5	4.0	46	18:2-22:4		—	—
	16:0-20:3	7.5	5.9	3.5	47	18:1-22:5	1.3		
	18:1ω9-18:2					18:1-22:6			
26	18:1ω7-18:2	1.0	0.5	—	Other		2.9	4.8	3.5
29	18:2-18:2	1.9	0.7	—					
	16:0-20:5			0.2	% PL class		93.0	19.9	100

peaks identified as *trans* isomers migrated well above the long and short chain *cis*-monoenes on the silver nitrate plates. Thus, the GLC and argentation TLC behavior of these compounds is consistent with the known chromatographic properties of the corresponding *trans* acids (11). The GLC retention of alkylglycerol esters was consistent with their known chromatographic properties (12) and the elution of reference standards. The corresponding quantitative composition of the alkylglycerol and the fatty acid methyl esters is given in Table 5.

Figures 9 and 10 give the polar capillary GLC elution patterns recorded for the alkenylacylglycerol moieties of

TABLE 3

Fatty Acid Composition of Diacyl GPC, GPE and GPI From Human Plasma

Fatty acids	Diacylglycerophospholipids			Fatty acids	Diacylglycerophospholipids		
	Choline	Ethanolamine	Inositol		Choline	Ethanolamine	Inositol
	Mole %				Mole %		
14:0	0.3	0.4	0.9	18:3ω3	0.1	—	—
15:0	0.3	0.2	0.3	20:1ω9	0.4	0.2	—
16:0	28.8	14.4	7.7	20:2ω6	0.3	0.3	0.6
16:1ω9	0.4	0.4	0.3	20:3ω6	2.6	1.4	3.3
16:1ω7	0.5		0.5	20:4ω6	7.6	18.6	28.0
17:0	0.2	0.3	0.6	20:5ω3	0.4	0.3	0.1
18:0	12.9	21.1	37.3	22:4ω6	0.2		0.3
18:1t	0.7	1.2	2.3	22:5ω6	0.3		0.2
18:1ω9	11.2	8.7	7.9	22:5ω3	0.7	2.0	0.8
18:1ω7	2.3	1.5	—	22:6ω3	2.5	6.9	1.1
18:2ω6	27.3	20.0	7.8				

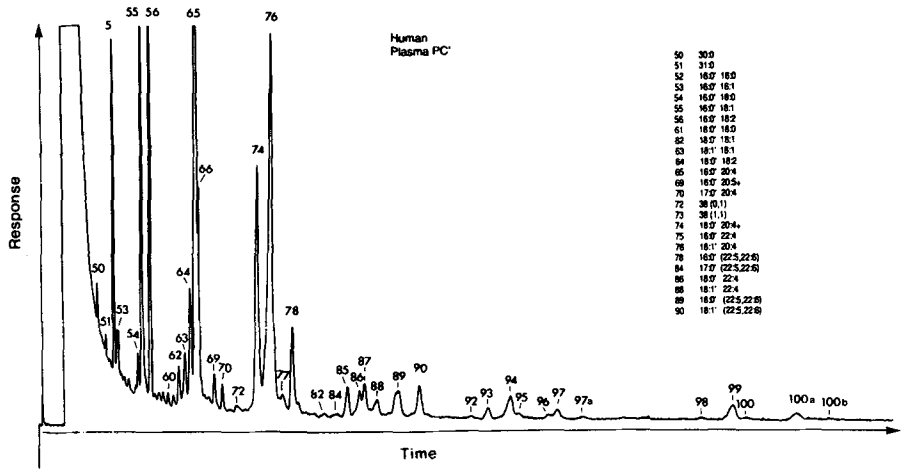

FIG. 5. Polar capillary GLC profile of the alkylacylglycerol moieties of human plasma diradyl GPC. Peak identification as given in figure. GLC conditions as given in Figure 3. Sample: 1 μl of 0.1% diradylglycerol TMS ethers in hexane.

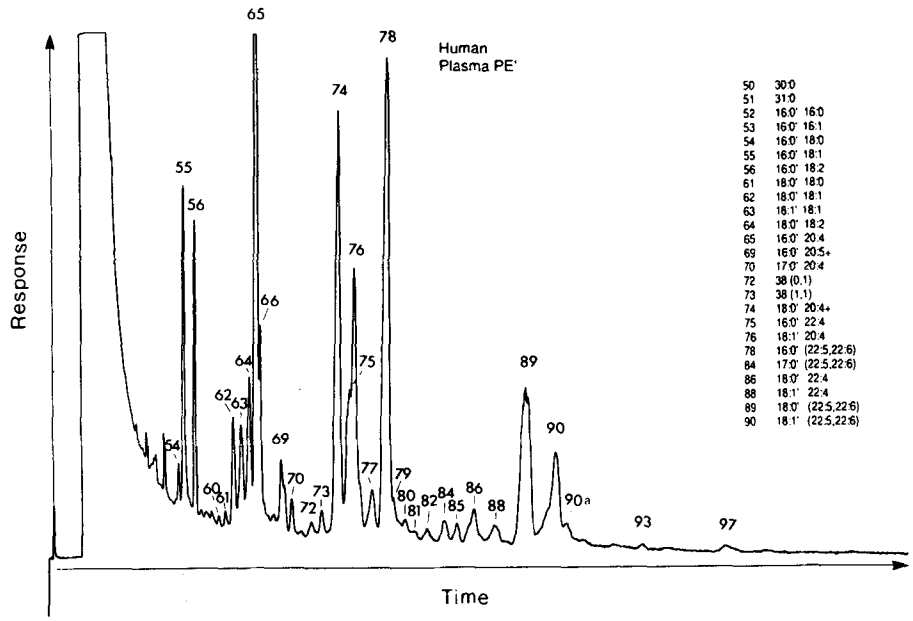

FIG. 6. Polar capillary GLC profile of the alkylacylglycerol moieties of human plasma diradyl GPE. Peak identification as given in figure. GLC conditions as given in Figure 3. Sample: 1 μl of 0.1% diradylglycerol TMS ethers in hexane.

TABLE 4

Molecular Species of Alkylacyl Glycerophospholipids of Human Plasma

GLC peak	Molecular species	Phospholipid classes PC′	PE′	GLC peak	Molecular species	Phospholipid classes PC′	PE′
		Mole %				Mole %	
50	30:0	0.3		74	18:0-20:4+	9.1	11.1
51	31:0	0.2		75	16:0-22:4	17.9	3.2
52	16:0-16:0	3.0		76	18:1-20:4		8.2
53	16:0-16:1	1.0		77		0.9	2.0
54	16:0-18:0	0.6	0.5	78	16:0-22:5 + 16:0-22:6	3.3	16.3
55	16:0-18:1	6.5	3.8	79-83		0.4	3.7
56	16:0-18:2	5.7	2.9	84	17:0-22:5 + 17:0-22:6+	0.2	1.0
57-60		0.7	0.4	85		1.6	0.7
61	18:0-18:0	0.2	0.2	86	18:0-22:4	1.9	1.9
62	18:0-18:1	0.9	1.5	87		1.9	
63	18:1-18:1	1.5	1.9	88	18:1ω9-22:4 + 18:1ω7-22:4	1.2	1.2
64	18:0-18:2	3.1	2.5	89	18:0-22:5 + 18:0-22:6	2.0	8.7
65	16:0-20:4	13.9	11.7	90	18:1-22:5 + 18:1-22:6	2.3	7.0
66		5.9	2.5	91-98	42	6.3	2.9
69	16:0-20:5+	0.9	1.6	99-102	44	4.9	0.7
70	17:0-20:4	0.6	0.5	Other		0.8	0.9
72	38 (0,1)	0.3	0.1				
73	38 (1,1)		0.4	% PL class		3.4	8.3

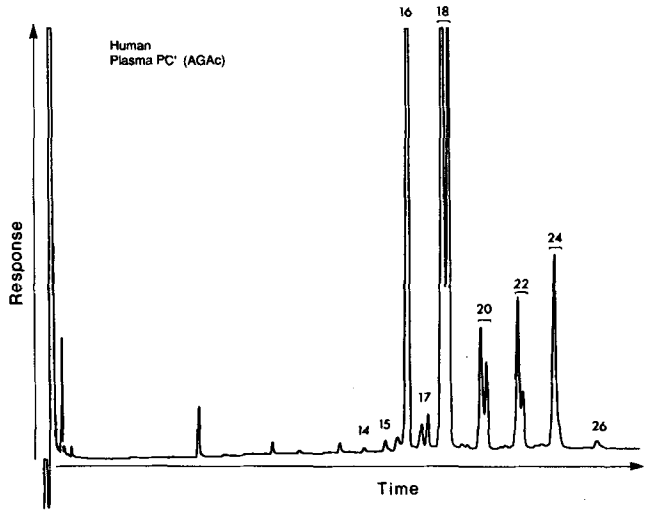

FIG. 7. Nonpolar capillary GLC resolution of alkylglycerol moieties of alkylacyl GPC of human plasma. Peak identification as given in figure. Column and carrier gas as given in Figure 2. Sample: 1 μl of 0.1% alkylglycerol diacetates in hexane.

plasma diradyl GPC and GPE. The corresponding quantitative values are given in Table 6. There are significant differences between the two lipids in the composition of the alkenylacyl species indicating the existence of separate pools of precursors or independent transformation mechanisms. The alkenylacyl GPC is much richer in species in which a 16:0″ alkenylglycerol is combined with saturated, monoeneoic, dienoic and tetraenoic fatty acids, whereas alkenylacyl GPE is much richer in 18:0″ alkenylglycerol combined with dienoic, tetraenoic and hexaenoic fatty acids. The above identities of the molecular species of the alkenylacylglycerol moieties of the diradyl GPC and

TABLE 5

Quantitative Composition of Alkylglycerols and Fatty Acids of Alkylacyl Glycerophosphocholine From Human Plasma

Fatty chains	PC′ Alkylglycerols	PC′ Fatty acids
	Mole %	
14:0	0.16	0.22
15:0	0.43	0.1
16:0	33.14	8.39
16:1ω9	0.89	0.32
16:1ω7		0.39
17:0	1.0	
18:0	16.54	1.8
18:1t	3.59	0.2
18:1ω9	21.37	6.6
18:1ω7	2.10	0.6
18:2	0.41	14.5
20:0	2.48	
20:1	3.0	
20:2ω6	1.8	0.29
20:3ω6		4.14
20:4ω6		42.14
20:5ω3		1.01
22:0	1.53	
22:1	3.0	
22:2	1.5	
22:4ω6		3.2
22:5ω6		1.2
22:5ω3		2.8
22:6ω3		6.4
24:0	0.6	
24:1	3.3	
24:2	2.3	

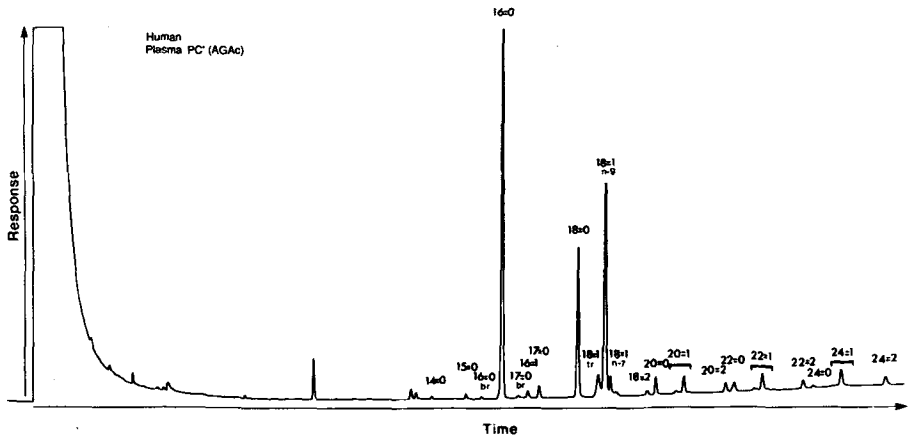

FIG. 8. Polar capillary GLC resolution of alkylglycerol moieties of alkylacyl GPC. Peak identification as given in figure. Column and carrier gas as in Figure 3. Temperature programmed from 100 (0.5 min) to 180°C at 20°C/min, then to 240°C at 5°C/min. Instrument and other GLC conditions as given (2). 24:2 eluted in 17.5 min. Sample: 1 μl of 0.1% alkylglycerol diacetates in hexane.

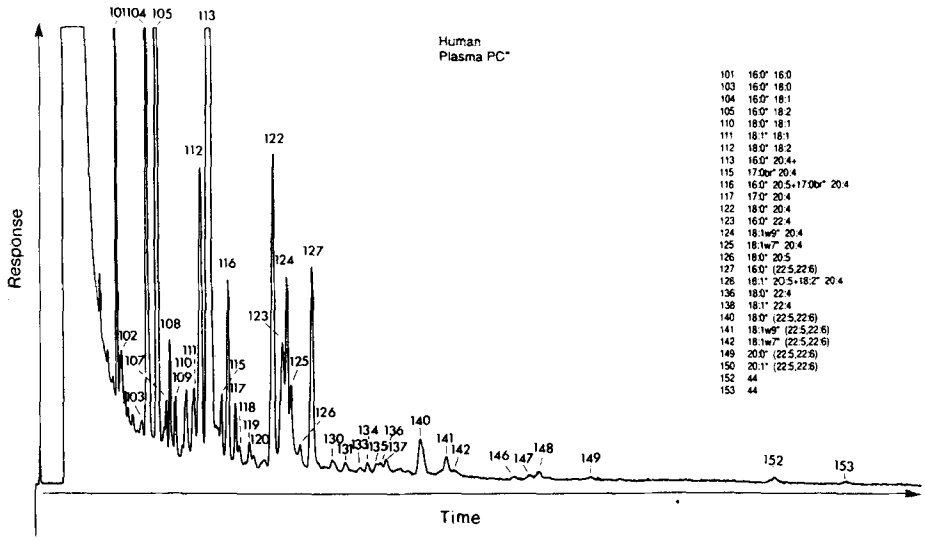

FIG. 9. Polar capillary GLC profile of the alkenylacylglycerol moieties of human plasma diradyl GPC. Peak identification as given in figure. GLC conditions as given in Figure 3. Sample: 1 μl of 0.1% diradylglycerol TMS ethers in hexane.

GPE are supported by the composition of the alkenyl chains, determined independently by polar capillary GLC of the dimethylacetals, and the fatty acid methyl esters derived from the corresponding alkenylacylglycerol fractions, shown in Table 7.

Table 8 gives the molecular species composition of the ceramide moieties of the SPH of VLDL, LDL, HDL_3 and chylomicrons. The SPH from the fasting plasma samples had been obtained from 3 normolipemic subjects other than the one who supplied the plasma diradyl GPL. The SPH from the postprandial plasma was obtained from another normolipemic subject 2 hr after ingesting a fatty meal. Because the plasma total SPH of the present subject had fatty acid and ceramide composition closely similar to that of the VLDL and LDL of the previously analyzed subjects (data not shown), it was assumed that the molecular species would be similar to those already determined, but not previously reported. As a result the data in Table 8 show minor interlipoprotein and intersubject variation, except for HDL_3. They possess closely similar ranges of carbon numbers, but differ significantly in the relative proportions of the short and long chain lengths. The major components are the even carbon number species, especially C_{34} and C_{42}, but odd carbon number components are also present in readily detectable amounts. We have previously pointed out the differences between HDL and LDL in the chain length distribution of the plasma SPH (4,9), but the present report gives the first full documentation of the composition of the major and minor species including VLDL and chylomicrons.

DISCUSSION

The present study provides the first detailed description of the molecular species of the different classes of GPL

TABLE 6

Molecular Species of Alkenylacyl Glycerophospholipids of Human Plasma

GLC peak	Molecular species	Phospholipid classes PC*	PE*	GLC peak	Molecular species	Phospholipid classes PC*	PE*
		Mole %				Mole %	
101	16:0-16:0	3.3		126	18:0-20:5	0.7	2.0
102		0.8		127	16:0-22:5 + 16:0-22:6	4.8	7.9
103	16:0-18:0	0.2	0.1	128	18:1 20:5 + 18:2-20:4	0.2	0.6
104	16:0-18:1	5.7	1.6	129-135		1.5	4.1
105	16:0-18:2	19.4	3.3	136	18:0-22:4	0.4	0.8
107-109		2.1	0.7	138	18:1-22:4	0.3	0.7
110	18:0-18:1	1.1	1.9	139		0.2	0.3
111	18:1-18:1	1.2	1.3	140	18:0-22:5 + 18:0-22:6	1.5	6.6
112	18:0-18:2	4.6	6.7	141	18:1ω9-22:5 + 18:1ω9-22:6	1.0	3.3
113	16:0-20:4+	21.3	11.1	142	18:1ω7-22:5 + 18:1ω7-22:6	0.3	0.8
	18:1-18:2 + 16:0-20:3	6.0	3.2	143-148		0.9	1.2
115	17:0br-20:4	0.8	0.2	149	20:0-22:5 + 20:0-22:6	0.2	0.6
116	16:0-20:5 + 17:0br-20:4	2.1	1.1	150	20:1-22:5 + 20:1-22:6		0.3
117	17:0-20:4	1.0	0.7	152-153	44	1.1	0.7
118-121		1.2	0.6		46		0.1
122	18:0-20:4	6.7	21.8	Other		0.7	2.2
123	16:0-22:4	3.0					
124	18:1ω9-20:4	3.8	13.5				
125	18:1ω7-20:4	1.9		% PL class		3.6	71.8

br, branched

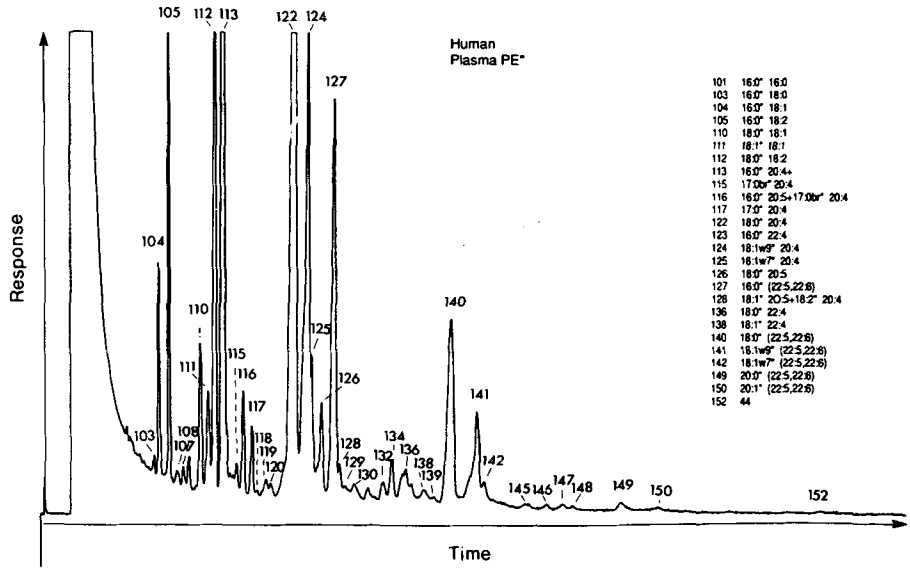

FIG. 10. Polar capillary GLC profile of the alkenylacylglycerol moieties of human plasma diradyl GPE. Peak identification as given figure. GLC conditions as given in Figure 3. Sample: 1 μl of 0.1% diradylglycerol TMS ethers in hexane.

from human plasma isolated from the same subject and of the molecular species of SPH isolated from different lipoprotein classes. Previously only the major diacyl GPC species had been identified (13,14) in pooled or mismatched samples, whereas the analyses of plasma diradyl GPE (15) and GPI (10) had been confined to the estimation of the total lipid amount and fatty acid composition. The presence of ether-linked phospholipids, especially the high proportion of the alkenylacyl GPE, has not been appreciated. The characteristic composition of the molecular species of the diacyl GPI, however, could have been approximately predicted from the fatty acid composition (10). The presence of phospholipids having *trans*-monoenoic alkyl and alkenyl chain in both plasma and erythrocytes presumably arises from dietary *trans*-fatty acid precursors. The metabolic consequences of such substitution, if any, are unknown. We had previously reported (9) the ceramide carbon numbers of the VLDL, LDL and HDL fractions, but molecular species had been given for only 4 of the total of 12 carbon numbers. In another study (4) the ceramide carbon numbers of VLDL, LDL and HDL were determined for subjects on saturated

TABLE 7

Quantitative Composition of Fatty Acids and Dimethylacetals of the Alkenylacyl Glycerophospholipids of Human Plasma

Fatty chains	PC″ Fatty acids	PC″ Dimethylacetals	PE″ Fatty acids	PE″ Dimethylacetals	Fatty chains	PC″ Fatty acids	PC″ Dimethylacetals	PE″ Fatty acids	PE″ Dimethylacetals
	Mole %		Mole %			Mole %		Mole %	
16:0	9.7	66.9	2.3	27.4	20:3ω6	3.7		2.1	
16:1ω9		1.9	0.1	1.4	20:4ω6	33.2		42.7	
16:1ω7					20:5ω6	1.0		2.6	
17:0		2.0	1.3	2.1	22:0				0.6
18:0	3.0	15.3	0.7	40.4	22:1ω9				0.6
18:1t				3.0	22:4ω6	2.7		2.3	
18:1ω9	8.1	10.6	5.9	16.3	22:5ω6	1.0		1.1	
18:1ω7	0.7	3.3	0.1	3.7	22:5ω3	2.1		4.7	
18:2	29.3		17.8		22:6ω3	5.0		16.5	
20:0		tr		2.7	24:0				0.2
20:1ω9				1.2	24:1				0.3
20:2ω6	0.5		0.6						

TABLE 8

Molecular Species of Sphingomyelins of Different Lipoprotein Classes of Human Plasma

Molecular species	Lipoprotein classes Chylos[a]	VLDL[a]	VLDL[b]	LDL[b]	HDL_3[b]	Molecular species	Lipoprotein classes Chylos[a]	VLDL[a]	VLDL[b]	LDL[b]	HDL_3[b]
			Mole %						Mole %		
32:0	0.052	0.043	0.037	0.040	0.070	38:0	0.066	0.072	0.127	0.119	0.071
d16:1-16:0	1.382	1.279	0.976	1.073	0.170	d16:1-22:0	0.910	1.035	1.186	1.714	2.016
d16:1-16:1	0.061	0.054	0.018	0.006	—	d16:1-22:1	0.085	0.074	0.126	0.167	0.083
d17:1-15:0	0.010	0.005	—	0.007	0.029	d17:1-21:0	0.048	0.040	0.100	0.038	0.057
d18:1-14:0	0.750	0.688	0.501	0.607	1.002	d18:1-20:0	1.862	2.054	1.429	2.209	2.280
d18:2-14:0	0.155	0.111	0.057	0.062	0.310	d18:1-20:1	0.120	0.084	0.125	0.154	0.133
33:0	0.089	0.004	0.011	—	0.010	d18:2-20:0	0.879	0.953	0.755	0.792	1.716
d16:1-17:0	0.082	0.070	0.024	0.009	0.030	d18:2-20:1	0.31	0.128	0.051	0.107	0.144
d17:1-16:0	1.461	1.387	0.951	0.491	0.404	39:0	0.017	0.014	0.018	0.010	0.070
d17:1-16:1	0.031	0.027	0.012	0.003	0.015	d16:1-23:0	0.350	0.344	0.512	0.707	0.605
d18:1-15:0	0.086	0.080	—	0.034	0.052	d16:1-23:1	0.067	0.013	0.041	0.049	0.125
d18:2-15:0	0.050	0.030	0.001	—	0.020	d17:1-22:0	0.485	0.384	0.510	0.612	0.622
34:0	0.678	0.676	1.169	1.052	0.383	d17:1-22:1	0.054	0.050	0.078	0.085	0.095
d16:1-18:0	1.909	2.018	1.813	1.324	1.540	d18:1-21:0	0.269	0.229	0.150	0.249	0.239
d16:1-18:1	0.285	0.267	0.190	0.190	0.220	d18:2-21:0	0.190	0.139	0.090	0.089	0.343
d17:1-17:0	0.010	0.053	0.058	0.001	0.028	40:0	0.060	0.042	0.16	0.17	0.04
d18:1-16:0	30.830	29.899	30.453	28.519	17.301	d16:1-24:0	0.169	0.262	0.315	0.723	1.132
d18:1-16:1	0.394	0.392	0.266	0.305	0.297	d16:1-24:1	1.274	1.314	1.499	1.929	2.628
d18:2-16:0	4.104	3.784	3.251	2.217	3.330	d17:1-23:0	0.403	0.425	0.239	0.340	0.438
d18:2-16:1	—	—				d17:1-23:1	0.068	0.098	0.018	0.073	0.047
35:0	0.173	0.025	—	0.019	0.160	d18:1-22:0	5.804	5.062	5.479	8.052	8.835
d16:1-19:0	0.246	0.148	—	—	0.100	d18:1-22:1	0.115	0.161	0.636	0.655	0.417
d17:1-18:0	0.671	0.541	0.729	0.540	0.433	d18:2-22:0	1.934	1.821	2.363	2.241	4.716
d17:1-18:1	0.118	0.046	0.067	0.031	0.008	d18:2-22:1	0.472	0.460	0.388	0.620	0.849
d18:1-17:0	0.940	0.610	0.751	0.828	0.242	41:0	0.014	0.006	—	0.009	—
d18:2-17:0	0.190	0.160	0.064	0.081	0.201	d17:1-24:0	0.365	0.338	0.440	0.487	0.620
36:0	0.225	0.267	0.274	0.200	0.171	d17:1-24:1	0.554	0.549	0.694	0.688	0.996
d16:1-20:0	0.953	1.105	1.168	1.001	0.905	d17:1-24:2	0.051	0.064	0.053	0.076	0.082
d16:1-20:1	0.121	0.098	0.077	0.109	0.026	d18:1-23:0	2.130	2.033	2.633	3.020	2.810
d18:1-18:0	5.766	6.229	4.668	4.165	2.975	d18:1-23:1	0.075	0.219	0.307	0.264	0.384
d18:1-18:1	0.646	0.737	0.690	0.539	0.674	d18:2-23:0	0.777	0.751	0.893	0.814	1.463
d18:2-18:0	2.502	2.487	1.808	1.326	1.941	d18:2-23:1	0.227	0.161	0.080	0.142	0.244
d18:2-18:1	0.338	0.337	0.213	0.159	0.308	42:0			0.042	—	0.015
37:0	0.032	0.041	0.060	0.026	0.029	d18:1-24:0	5.186	4.817	5.046	6.306	5.233
37:1	0.776	0.885	0.740	0.670	0.471	d18:1-24:1	12.885	12.907	12.945	12.076	13.420
37:2	0.202	0.184				d18:1-24:2	0.376	0.551	0.948	0.987	2.043
						d18:2-24:0	2.277	2.130	3.200	1.595	4.588
						d18:2-24:1	3.406	4.076	3.394	3.234	4.799
						d18:2-24:2	0.890	0.648	0.325	0.401	—
						43	nd	nd	1.5	1.5	1.8

[a]Postprandial plasma of a normolipemic subject other than the one supplying the erythrocyte diradylglycerophospholipids.
[b]Fasting plasma from three different normolipemic subjects other than the one supplying the erythrocyte diradylglycerophospholipids.
nd, Not detectable.

and unsaturated fat diets, but molecular species were reported only for LDL. Neither of the previous publications gave the composition of the chylomicron SPH. The present study shows that the chylomicrons and VLDL possess SPH species, which are closely similar to those of LDL and differ from those of HDL by a lower proportion of the longer chain lengths.

The present study also permits the first systematic comparison of the molecular species of GPL and SPH of erythrocytes and plasma isolated from the same blood. The identification and quantitation of the species is based on polar capillary GLC resolution performed in parallel on purified subclasses and total diradylglycerol moieties of the GPL. The method allowed a resolution of most species on the basis of carbon number and degree of unsaturation, as well as of certain positional and geometric double bond isomers. The determined composition of the molecular species was found to be consistent with the independently measured fatty acid methyl ester, dimethylacetal and alkylglycerol composition.

Although the qualitative compositions are similar, marked differences are seen among the quantitative proportions of both major and minor molecular species between the corresponding plasma and red cell GPL, excluding extensive equilibration. The quantitative differences are as great between the choline and ethanolamine GPL within plasma or cells, as between the corresponding GPL of plasma and cells. However, the similarity in the composition of long chain saturated and unsaturated alkyl groups in the alkylacyl GPC from plasma and red cells suggests a common origin. In contrast to the other GPL, the diacyl GPI of plasma and cells possess closely similar qualitative and quantitative composition of molecular species. The serine phosphatide, which was detected in measurable amounts only in the red cells, possessed molecular species that differed greatly from those of all other GPL, and were characterized by a high proportion of the polyunsaturated long chain length species. The use of whole plasma GPL for the comparison is justified on the basis of the known similarity of the major molecular species of choline GPL in the different lipoprotein classes (16). The use of GPL pooled from the inner and outer halves of the red cell membrane is justified on the basis of the known equilibration of the major diacyl GPC and GPE species between the inner and outer halves of the erythrocyte membranes (17). Such an equilibration, however, does not appear to exist for the SPH either between the inner and outer half of the red cell membrane (18) or among the different lipoprotein classes (4,19).

Red cells could be a potential source of longer chain SPH species in HDL, but a comparison of species indicates that it is unlikely. For C_{42} the ratio of d18:1 to d18:2 containing species is slightly lower in HDL (2.3) than in LDL (3.7). If red cells were the source of elevated C_{42} (d18:1/d18:2 = 8.4) an increase of this ratio rather than the observed decrease would be anticipated. The same argument applies to the ratio of d18:1 24:0 to d18:1 24:1. The chain length differences in SPH between HDL and LDL, therefore, must be attributed to the origin of these lipoproteins in liver and intestine, respectively (20).

The major differences in the quantitative and to a lesser extent in the qualitative composition of the GPL species between plasma and red cells can be explained on the basis of differences in the metabolic origin of the species and the absence of significant mass equilibration among the GPL of plasma and red cells. The present results do not exclude, however, selective exchanges of major (21) or nonselective exchange of minor species. The study would appear to eliminate red cell PE as the only source of plasma PE, including the alkylacyl and alkenylacyl subclasses. Dietary fatty acids affect plasma lipids much more rapidly and extensively than the red cell lipids (22), which are subject to much slower turnover (23). Some of the molecular species of GPL and SPH are clearly derived from the clearance of intestinal VLDL and chylomicrons (24), whereas other GPL species are derived from the nascent HDL secreted by liver (25). The GPL transformations resulting from the activity of plasma lipoprotein (26) and hepatic (27) lipases, and lecithin-cholesterol acyltransferase (28) must also be considered. Although the intestinal GPL is relatively rich in the alkylacyl and the alkenylacyl species (29), the liver GPL is essentially free of them (30).

Although the detailed comparisons were performed on blood from one subject only, these results were representative of the GPL analyses from other subjects. The present analyses are of interest in view of the increasing importance of the minor species of GPL in lipoprotein and cell membrane structure and function. A more complete assessment of the origin of the GPL and SPH of plasma must await studies of the kinetics of appropriately labeled molecular species of GPL and SPH.

ACKNOWLEDGMENTS

These studies were supported by grants from the Heart and Stroke Foundation of Ontario, Toronto, Ontario; and the Medical Research Council of Canada, Ottawa, Canada.

REFERENCES

1. Nelson, G.J. (1972) in *Blood lipids and lipoproteins* (G.J. Nelson, ed.), pp. 317-386, J. Wiley and Sons, New York, NY.
2. Myher, J.J., Kuksis, A. and Pind, S. (1989) *Lipids 24,* 396-407.
3. Kuksis, A., Pind, S., and Myher, J.J. (1988) *J. Am. Oil Chem. Soc. 65,* 538-539.
4. Myher, J.J., Kuksis, A., Breckenridge, W.C., and Little, J.A. (1981) *Can. J. Biochem. 59,* 626-636.
5. Breckenridge, W.C., Kakis, G., and Kuksis, A. (1979) *Can. J. Biochem. 57,* 72-82.
6. Myher, J.J., and Kuksis, A. (1984) *J. Biochem. Biophys. Methods 10,* 13-23.
7. Phillips, G.B., and Dodge, J.T. (1967) *J. Lipid Res. 8,* 676-681.
8. Bowyer, D.E., and King, J.P. (1977) *J. Chromatogr. 143,* 473-490.
9. Myher, J.J., Kuksis, A., Shepherd, J., Packard, C.J., Morrisett, J.D., and Gotto, A.M. (1981) *Biochim. Biophys. Acta 666,* 110-119.
10. Breckenridge, W.C., and Palmer, F.B.St.C. (1982) *Biochim. Biophys. Acta 712,* 707-711.
11. Gunstone, F.D., Ismail, I.A., and Lie Ken Jie, M. (1967) *Chem. Phys. Lipids 1,* 376-385.
12. Myher, J.J., Marai, L., and Kuksis, A. (1974) *J. Lipid Res. 15,* 586-592.
13. Van Golde, L.M.G., Tomasi, V., and Van Deenen, L.L.M. (1967) *Chem. Phys. Lipids 1,* 282-293.
14. Marai, L., and Kuksis, A. (1969) *J. Lipid Res. 10,* 141-152.
15. Diagne, A., Fauvel, J., Record, M., Chap, H., and Douste-Blazy, L. (1984) *Biochim. Biophys. Acta 793,* 221-231.
16. Kuksis, A., Myher, J.J., Geher, K., Breckenridge, W.C., Jones, G.J.L., and Little, J.A. (1981) *J. Chromatogr. Biomed. Applic. 224,* 1-123.
17. Op den Kamp, J.A.F., Roelofsen, B., and Van Deenen, L.M.M. (1985) *Trends Biochem. Sci. 10,* 320-323.

18. Boegheim, J.P.J. Jr., Van Linde, M., Op den Kamp, J.A.F., and Roelofsen, B. (1983) *Biochim. Biophys. Acta 735,* 438-442.
19. Kuksis, A., Myher, J.J., Breckenridge, W.C., and Little, J.A. (1979) in *Report of the High Density Lipoprotein Methodology Workshop* (Lippel, K., ed.), NIH Publication No. 79-1661, pp. 142-161, Bethesda, Maryland.
20. Dolphin, P.J. (1985) *Can. J. Biochem. Cell Biol. 63,* 850-869.
21. Child, P., Myher, J.J., Kuypers, F.A., Op den Kamp, J.A.F., Kuksis, A., and Van Deenen, L.L.M. (1985) *Biochim. Biophys. Acta 812,* 321-332.
22. Clandinin, M.T., Field, C.J., Hargraves, K., Morson, L., and Zsigmond, E. (1985) *Can. J. Physiol. Pharmacol. 63,* 546-556.
23. Shohet, S.B. (1970) *J. Clin. Invest. 49,* 1668-1678.
24. Gotto, A.M. Jr., Pownall, H.J., and Havel, R.J. (1986) *Methods Enzymol. 128,* 3-41.
25. Eisenberg, S. (1984) *J. Lipid Res. 25,* 1017-1058.
26. McLean, L.R., Demel, R.A., Socorro, L., Shinomiya, M., and Jackson, R.L. (1986) *Methods Enzymol. 129,* 738-783.
27. Ehnholm, C., and Kuusi, T. (1986) *Methods Enzymol. 129,* 716-738.
28. Albers, J.J., Chen, C.H., and Lacko, A.G. (1986) *Methods Enzymol. 129,* 263-283.
29. Pind, S., and Kuksis, A. (1986) in *Fat Absorption* (Kuksis, A., ed.), Vol. 1, pp. 43-82, CRC Press, Boca Raton, FL.
30. Holub, B.J., and Kuksis, A. (1978) *Adv. Lipid Res. 16,* 1-125.

[Received September 6, 1988; Revision accepted January 13, 1989]