Molecular species of phosphatidylcholine in abetalipoproteinemia: effect of lecithin:cholesterol acyltransferase and lysolecithin acyltransferase

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Abstract In order to study the role of very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in determining the molecular species composition of phosphatidylcholine (PC) and the specificity of lecithin:cholesterol acyltransferase (LCAT) in human plasma, we studied the PC species composition in plasma from abetalipoproteinemic (ABL) and control subjects before and after incubation at 37°C. The ABL plasma contained significantly higher percentages of *sn*-2-18:1 species (16:0-18:1, 18:0-18:1, and 18:1-18:1) and lower percentages of *sn*-2-18:2 species (16:0-18:2, 18:0-18:2, and 18:1-18:2) as well as *sn*-2-20:4 species (16:0-20:4, 18:0-20:4, and 18:1-20:4). Similar abnormalities were found in the PC of ABL erythrocytes, while the PE of the erythrocytes was less affected. The relative contribution of various PC species towards LCAT reaction in ABL plasma was significantly different from that found in normal plasma. Thus, while 16:0-18:2 and 16:0-18:1 contributed, respectively, 43.8% and 15.9% of the total acyl groups used for cholesterol esterification in normal plasma, they contributed, respectively, 21.5% and 37.9% in ABL plasma. The relative contribution of 16:0-20:4 was also significantly lower in ABL plasma (4.7% vs. 9.0% in normal), while that of 16:0-16:0 was higher (6.4% vs. 0.5%). However, the selectivity factors of various species (percent contribution/percent concentration) were not significantly different between ABL and normal plasma, indicating that the substrate specificity of LCAT is not altered in the absence of VLDL and LDL. Incubation of ABL plasma in the presence of normal VLDL or LDL resulted in normalization of its molecular species composition and in the stimulation of its LCAT activity. Addition of LDL, but not VLDL, also resulted in the activation of lysolecithin acyltransferase (LAT) activity. The incorporation of [1-^{14}C]palmitoyl lysoPC into various PC species in the presence of LDL was similar to that observed in normal plasma, with the 16:0-16:0 species having the highest specific activity. These results indicate that the absence of apoB-containing lipoproteins significantly affects the molecular species composition of plasma PC as well as its metabolism by LCAT and LAT reactions. —**Banerji, B., P. V. Subbaiah, R. E. Gregg, and J. D. Bagdade.** Molecular species of phosphatidylcholine in abetalipoproteinemia: effect of lecithin:cholesterol acyltransferase and lysolecithin acyltransferase. *J. Lipid Res.* 1989. **30:** 1907-1916.

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Phosphatidylcholine (PC), the major phospholipid of plasma lipoproteins, is not only involved in maintaining the structural integrity of the lipoproteins, but also is the source of fatty acyl groups for the formation of cholesteryl esters present in human plasma. We have recently shown that normal human plasma contains at least 20 molecular species of PC that differ in their fatty acid composition and that the distribution of these species is not uniform among different lipoproteins (1, 2). The metabolic fate and physiological function of these molecular species in plasma lipoproteins is not yet well understood. Our earlier results showed that certain species of PC are preferentially utilized as substrates by the LCAT reaction (1). Moreover, in related studies we have also shown that the acylation of lysoPC to PC by plasma lysolecithin acyltransferase (LAT) results in preferential synthesis of specific PC species (2). Since the LAT reaction requires LDL, it is markedly depressed in abetalipoproteinemia (ABL) (3), a rare genetic disorder characterized by the absence of the apoB-containing plasma lipoproteins VLDL, LDL, and chylomicrons. In this disorder the total amount of PC is decreased and its fatty acid composition is significantly altered as a consequence of patients' inability to absorb dietary long chain unsaturated fatty acids (4, 5).

The absence of apoprotein B-containing lipoproteins in ABL plasma may not only affect the molecular species composition of PC, but also the activity of LCAT (6, 7)

Abbreviations: ABL, abetalipoproteinemia; GLC, gas-liquid chromatography; HDL, high density lipoproteins; HPLC, high performance liquid chromatography; LAT, lysolecithin acyltransferase; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoproteins; PC, phosphatidylcholine; PE, phosphatidylethanolamine; RRT, relative retention time; TLC, thin-layer chromatography; VLDL, very low density lipoproteins.

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because these lipoproteins normally serve as the acceptors of most of the cholesteryl esters produced by the LCAT reaction. It is also possible that the specificity of LCAT towards various molecular species is modified in ABL because of the altered PC and apoprotein compositions. Furthermore, the absence of the LAT reaction may have independent effects of PC composition which in turn may affect LCAT specificity. For these reasons it is important to determine the molecular species composition of PC in ABL plasma and estimate the extent to which the altered composition of PC affects the activity and specificity of LCAT. ABL plasma also provides an important tool to assess the influence of LDL and VLDL on the activity and specificity of LCAT. Since the LAT activity can be generated in ABL plasma in vitro by the addition of LDL, it is also possible to study the role of this activity in influencing the PC molecular species composition.

In the present study, we found that the composition of molecular species of PC is markedly different in ABL and normal plasma. Compositional alterations in the molecular species of PC and PE were also found in ABL erythrocytes (acanthocytes) that have previously been shown to have abnormalities in membrane fluidity and cholesterol content (8, 9). The relative amounts of the various species utilized by the LCAT reaction differed significantly in ABL and normal plasma, but the specificity of the enzyme was unchanged. When the ABL plasma was supplemented with normal LDL or VLDL, there was an exchange of various molecular species between the normal and ABL lipoproteins and a stimulation of the LCAT activity. Although the relative contribution of various PC species to the LCAT reaction changed after supplementation, its specificity was not altered significantly. The addition of LDL, but not VLDL, stimulated the LAT activity and the species of PC it generated were similar to those formed in intact normal plasma.

MATERIALS AND METHODS

Patients

Five patients with phenotypic abetalipoproteinema were studied. Two patients were genetically homozygous familial hypobetalipoproteinemics, while the other three were homozygous familial abetalipoproteinemic. None had detectable levels of apoB-containing lipoproteins. The clinical characteristics of these patients, their lipoprotein abnormalities, and the molecular defects they showed in the synthesis of apoB have been described previously (10, 11). All patients were on self-selected low fat diets that contained 30–35% of total calories as fat. They were all receiving high doses of vitamins A (25,000–50,000 IU/day), E (7,000–13,000 IU/day), and K (5–10 mg/day).

Fasting blood was drawn after an overnight (12–14 h) fast at the Clinical Center of the National Institutes of Health, Bethesda, MD, after informed consent was obtained. It was mixed immediately with EDTA (1 mg/ml) and the plasma was separated by centrifugation at 4°C. In two patients, the erythrocytes were isolated, washed 3 × with phosphate-buffered saline, resuspended in the same buffer, and transported on ice to Chicago for subsequent analyses. The plasma samples in most cases were frozen and transported on dry ice to Chicago, where they were thawed immediately prior to analysis. The plasma samples used in exchange experiments with normal and ABL erythrocytes were not frozen prior to the experiment and the exchange experiments were performed within 48 h of blood drawing. All analyses of plasma were completed within 4 weeks of blood drawing.

Analysis of molecular species of PC and PE

The whole plasma, lipoprotein fractions, and the erythrocytes were extracted by the Bligh and Dyer (12) procedure, and the PC was separated on TLC plates as described before (1). The PC and PE spots (in the case of erythrocytes) were eluted and the molecular species were separated by HPLC on C-18 reverse phase columns as described before (1) excepting that the hydrolysis by phospholipase C was carried out overnight in case of PE. The individual peaks were identified by running standards of known composition under identical conditions, by the analysis of fatty acid composition of the eluted peaks by capillary GLC, and by comparison of the relative retention times (RRT) with the literature values. The RRTs were calculated by dividing the retention times of all peaks with the retention time of 16:0–18:2, the major species of PC in normal human plasma (1). In cases where a positive identification of the peak could not be made, the peak was designated with its RRT only.

Plasma for ABL or normal subject was incubated at 37°C for 24 h in the presence of 0.01% sodium azide and 5 mM mercaptoethanol in screw-cap tubes. Where indicated, LDL, VLDL, or HDL prepared from pooled normal human plasma was added. In some experiments [1-^{14}C]lysopalmitoyl PC (210,000 dpm, 0.2 μmol lipid phosphorus) was added to the reaction mixture as an aqueous suspension in 10 mM Tris-HCl buffer, pH 7.4. After incubation the lipoproteins were separated by heparin-Mn^{2+} precipitation (1) and the precipitate (LDL + VLDL) and the supernatant (HDL) were analyzed for molecular species as described above. A small percentage (less than 10%) of the phospholipids of ABL plasma precipitated by this procedure. When the labeled lysoPC was included in the reaction mixture, aliquots of the eluates from the PC spots were counted for radioactivity in a Beckman LS-7000 liquid scintillation counter. During separation of these labeled PCs by HPLC, 1.0-min fractions were collected using a fraction collector and the fractions were counted in a liquid scintillation counter, following evaporation of the solvent under N_2

and addition of 5 ml of scintillation fluid. The counts were corrected for quenching by using the H number (Beckman Instruments).

Normal VLDL (d < 1.006 g/ml), LDL (d 1.019-1.063 g/ml), and HDL (d 1.063-1.21 g/ml) were isolated from pooled normal human plasma by preparative ultracentrifugation at their respective densities for 18 h (VLDL, LDL) and 44 h (HDL). All lipoproteins were washed once at their floating densities and dialyzed against 0.15 M NaCl, 1 mM EDTA, pH 7.4, and concentrated by using Amicon Centriflo filters.

The percentage contribution of each molecular species towards LCAT reaction was calculated as described before (1) by dividing the net decrease in the species with the decrease in total PC in the reaction mixture. The selectivity factor was obtained by dividing the percentage contribution of each species by its concentration at 0 time (1).

Lipid phosphorus was determined by the modified Bartlett procedure (12). Statistical analysis of significance between normal and ABL plasma was performed by Student's *t*-test.

RESULTS

Molecular species of PC in ABL plasma

Fig. 1 compares the HPLC pattern of PCs in ABL and control plasma analyzed as diacylglycerol benzoates. The identification of the numbered peaks and the percentage composition of the plasma PCs is shown in **Table 1**. Since ABL plasma has essentially no VLDL or LDL and since the HDL PC composition is different from that of VLDL and LDL in normal plasma (1), the composition of normal HDL PC is also provided for purposes of comparison. The values for the normal whole plasma and normal HDL are comparable to those reported by us earlier (1). The most significant difference between the ABL plasma and normal HDL is the predominance of species containing 18:1 in the *sn*-2 position in ABL plasma, as opposed to normal HDL which has predominantly *sn*-2-18:2 species. The relative amounts of 16:0-18:1, 18:1-18:1, and 18:0-18:1 were all higher in ABL plasma compared to normal plasma and HDL. The major PC species in ABL plasma was 16:0-18:1 whereas it was 16:0-18:2 in normal plasma. The concentrations of all 20:4-containing species (16:0-20:4, 18:0-20:4, 18:1-20:4) were lower in ABL plasma compared to normal HDL, although only 16:0-20:4 values were statistically significant. Another significant difference between normal and ABL plasma was the higher percentage of 16:0-16:0. in the latter. The total amount of lecithin was significantly lower in ABL plasma (194 ± 32 nmol/ml) compared to normal plasma (1533 ± 213) reflecting the absence of apoB-containing lipoproteins, and in accordance with previous reports (4, 7, 13, 14).

Molecular species of PC and PE in erythrocytes

In order to determine whether the above changes in the molecular species composition were limited to plasma lipoproteins or also present in cell membranes, we analyzed the composition of PC and PE in erythrocytes isolated from normal and ABL subjects. As shown in **Table 2**, there were also marked alterations in the composition of molecular species of ABL erythrocytes. In PC, the 16:0-18:1 species was markedly increased and replaced 16:0-18:2 as the major species. The 16:0-18:2 and 16:0-20:4 decreased significantly in accordance with the findings in ABL plasma, but the corresponding species containing 18:0 in the *sn*-1 position (18:0-18:2 and 18:0-20:4) actually showed an increase over normal levels. Also in contrast to our findings in plasma, the level of 16:0-16:0 PC was not higher in ABL erythrocytes, although a peak we tentatively identified as 18:0-16:0 was increased. In PE the changes in molecular species were less dramatic. There was little change in 16:0-20:4 and 18:0-20:4 species, but a significant decline in 16:0-18:2, 18:1-18:2, and 18:0-18:2. It is of interest to note that 16:0-18:1 species of PE was not increased in ABL, unlike the corresponding PC species which was increased in both plasma and erythrocytes. The 18:1-20:4 species of both PC and PE were increased in ABL erythrocytes.

In two separate experiments we incubated the ABL erythrocytes derived from 1.0 ml of blood with 1.0 ml of normal human plasma for 24 h at 37°C in the presence of glucose (1 mg/ml) and penicillin and streptomycin (1000 U/ml each) to determine whether the abnormal molecular species composition of the ABL erythrocytes can be normalized. Taking the average of these two experiments, there was an increase in the percentage of 16:0-18:2 PC from 14.1 to 18.9% and a decrease in the percentage of 16:0-18:1 PC from 42.4 to 32.4%. The 16:0-20:4 PC increased from 4.2 to 5.7% and the 18:0-18:2 species decreased from 20.9 to 16.5%. No significant changes occurred in ABL erythrocyte PE composition following incubation with normal plasma (results not presented). These results indicate that at least some of the abnormalities in PC composition of ABL erythrocytes are due to the exchange of the species with abnormal lipoproteins.

Effect of action of LCAT on PC molecular species of ABL plasma

The decrease in the amount of PC that takes place when plasma is incubated results entirely from its being utilized by the LCAT reaction (1). Therefore, the contribution of individual molecular species of PC for cholesterol esterification can be calculated from their decreases following incubation of whole plasma. As shown in **Table 3**, there were significant differences between ABL and normal plasma in the relative contribution of different PC species to the LCAT reaction. In normal plasma the

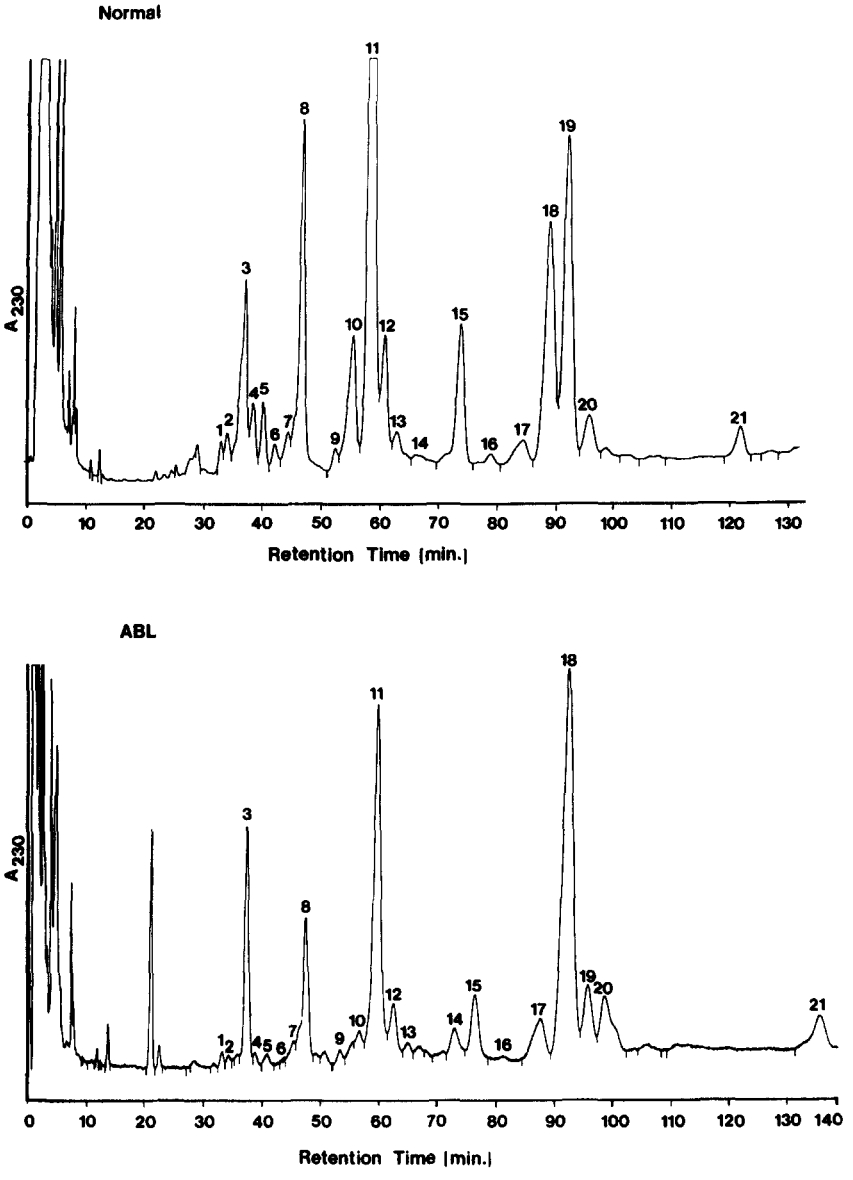

Fig. 1. HPLC pattern of molecular species of PC in normal and abetalipoproteinemic plasma. The PCs from the plasma were first separated from other lipids by TLC, and then converted to diacylglycerol benzoates as described in the text. The benzoates were separated on a C18 ultrasphere ODS column using the solvent system of acetonitrile–isopropanol–water 70:27:5 (by vol) at a flow rate of 1.5 ml/min. The absorbance was monitored at 230 nm and quantitated on a Shimadzu CR3A integrator. The identification of the numbered peaks and their percentage compositions are given in Table 1.

largest contributor for cholesterol esterification was the 16:0–18:2 species (44%) as shown earlier (1), whereas in ABL plasma the largest percentage (38%) of acyl groups were derived from the 16:0–18:1 species, followed by 16:0–18:2 (21%) and 18:0–18:2 (11%). The 16:0–16:0 species contributed about 6% of the acyl group for the LCAT reaction in ABL plasma compared to only 0.5% in normal plasma. The contributions of 16:0–20:4 and 18:1–20:4 were lower in ABL plasma compared to normal plasma. These results indicate that the relative contribution of individual PC species to the LCAT reaction is primarily dependent on their concentrations.

The specificity of LCAT for various molecular species of PC was next determined by calculating the selectivity factors, which were derived by dividing the percentage contribution of each species by its concentration at 0 hour (1). Despite the wide differences in the percentage contribution of various species between ABL and normal plasma, the selectivity factors were similar for most of the species (results not shown). The only statistically signifi-

TABLE 1. Identification of molecular species of PC and their percentage composition in normal and in abetalipoproteinemic plasma

Peak No. in Fig. 1	Species	Percentage Composition (Mean ± SD) ABL Plasma (n = 5)	Normal Plasma (n = 7)	Normal HDL (n = 5)
1	18:1-20:5	0.13 ± 0.26	0.37 ± 0.19	0.67 ± 0.51
2	16:0-20:5	0.18 ± 0.28	0.47 ± 0.24	0.33 ± 0.16
3	16:0-22:6 + 18:2-18:2	3.00 ± 2.42	4.04 ± 0.96	4.30 ± 0.30
4	RRT 0.67[a]	0.08 ± 0.12	0.63 ± 0.56	N.D.
5	RRT 0.70	0.10 ± 0.15	0.55 ± 0.45	0.45 ± 0.40
6	RRT 0.73	0.01 ± 0.03	0.34 ± 0.23	N.D.
7	18:1-20:4 + 18:0-22:6	0.60 ± 0.56	0.93 ± 0.32	1.16 ± 0.35
8	16:0-20:4	6.35 ± 1.35**	9.91 ± 1.48	10.30 ± 1.91
9	18:0-20:5	0.86 ± 0.43	0.65 ± 0.21	0.62 ± 0.36
10	18:1-18:2	1.70 ± 1.23***	4.09 ± 0.83	4.77 ± 0.74
11	16:0-18:2	17.63 ± 3.85***	31.50 ± 2.16	32.12 ± 4.70
12	16:0-20:3	2.97 ± 0.78	4.36 ± 0.90	3.93 ± 2.21
13	16:0-20:3 (n-9)	1.37 ± 1.09	0.81 ± 0.64	0.57 ± 0.60
14	RRT 1.20	1.05 ± 0.69	N.D.	N.D.
15	18:0-20:4	5.40 ± 1.40	5.01 ± 1.06	6.03 ± 1.44
16	RRT 1.35	0.61 ± 0.35	0.29 ± 0.17	0.33 ± 0.38
17	18:1-18:1	4.70 ± 1.16***	1.42 ± 0.13	1.53 ± 0.33
18	16:0-18:1	35.20 ± 4.05***	13.87 ± 3.34	11.25 ± 1.68
19	18:0-18:2	5.23 ± 0.98***	13.56 ± 2.81	14.64 ± 2.17
20	16:0-16:0 + 18:0-20:3	4.34 ± 1.94*	2.07 ± 0.80	2.14 ± 0.63
21	18:0-18:1	3.74 ± 0.80***	1.73 ± 0.35	1.66 ± 0.49

Each peak was identified by comparison of its RRT to that of authentic standards or by GLC analysis of the constituent fatty acids (1); N.D., peaks, not detected.

[a]RRT, relative retention time in relation to that of 16:0-18:2.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ when compared to normal HDL by Student's t test.

cant difference in the selectivity factors between the two types of plasma was for 16:0-16:0 which showed higher selectivity factor in ABL plasma (1.56 ± 1.06 for ABL (n = 5) and 0.59 ± 0.42 normal (n = 7) ($P<0.005$)). The mean values for the selectivity of the 16:0-22:6 species also differed markedly in ABL (0.95 ± 0.93) and control plasmas (0.15 ± 0.76), but the difference did not reach statistical significance because of high standard deviations.

Effect of addition of VLDL and LDL to ABL plasma

The observed differences in the utilization of various PC species for the LCAT reaction may be due either to the presence of compositional differences between normal and ABL plasma, the absence of acceptors for the cholesteryl esters synthesized, or the lack of LAT activity as a consequence of the absence of LDL. In order to distinguish these three possibilities, we determined the effect of addition of normal VLDL or LDL on the relative contribution of various PC species on the specificity of the enzyme. Addition of either of these lipoproteins should provide the acceptor for the formed cholesteryl esters, as well as change the molecular species composition, but only the addition of LDL would activate the LAT. LDL or VLDL from controls, containing 1.0 μmol of total PC were first heated at 56°C for 15 min to inactivate any LCAT, and added to 1.0 ml of ABL plasma and the mixture was incubated for 24 h at 37°C. At the end of 24 h, the added lipoproteins were separated from the ABL plasma by precipitation with heparin-Mn^{2+}, and the molecular species composition of both the supernatant (ABL plasma) and the precipitate (added lipoprotein) were analyzed. The results of a typical experiment are shown in **Table 4**. Similar results were obtained with plasma from two other ABL subjects. In the absence of exogenous lipoprotein, there was a decrease in the percentage of 16:0-18:2 species as seen earlier. However, when the plasma was incubated with either LDL or VLDL, there was an increase in the percentage of 16:0-18:2 as well as 18:0-18:2, and a decrease in 16:0-18:1. These compositional changes are obviously due to the exchange of PCs between the added lipoproteins and ABL plasma, as shown by the reciprocal changes in the composition of the precipitated exogenous lipoproteins. At the end of 24 h the compositions of the supernatant and precipitate were similar indicating that almost complete equilibration of all species had taken place. Because the amount of PC present in the added

TABLE 2. Molecular species of diacyl phosphoglycerides in normal and ABL erythrocytes

	% of Total PC		% of Total PE	
	Normal	ABL	Normal	ABL
18:1-20:5	0.07	0.02	1.84	0.56
16:0-20:5	0.00	0.03	0.67	0.95
16:0-22:6 + 18:2-18:2	2.40	1.09	5.57	4.36
18:1-20:4 + 18:0-22:6	0.15	0.70	7.56	10.90
16:0-20:4	5.43	3.78	12.45	13.57
18:0-20:5	0.12	0.23	0.62	2.11
18:1-18:2	2.91	0.33	6.68	1.45
16:0-18:2	37.11	15.35	11.28	3.97
16:0-20:3	3.45	1.85	3.16	3.65
16:0-20:3 (n-9)	0.22	0.55	3.36	6.17
RRT 1.20	0.13	0.00	0.31	2.11
18:0-20:4	3.23	5.08	9.50	8.72
RRT 1.35	0.00	0.00	0.46	0.70
18:1-18:1	1.22	3.85	4.78	5.92
16:0-18:1	20.22	39.56	17.36	16.88
18:0-18:2	15.60	17.96	3.84	1.25
16:0-16:0 + 18:0-20:3	0.71	1.38	0.99	1.48
RRT 1.7	0.15	0.69	1.53	0.87
18:0-18:1	3.84	4.24	4.08	4.74
18:0-16:0?	1.98	2.32	0.00	0.00

All values are the averages of three ABL subjects and two controls.

lipoprotein was fourfold greater than that present in the ABL plasma, the composition of both fractions resembled that of the added lipoprotein.

It is apparent from the decrease in the total amount of PC present in the precipitate and supernatant that more PC was consumed in the presence of added LDL or VLDL than in their absence, indicating that the LCAT reaction was activated by the presence of exogenous lipoproteins. Since these lipoproteins were heat-treated at 56°C before their addition to the ABL plasma, the observed increase in consumption of PC was not due to additional LCAT associated with the exogenous lipoproteins. The addition of normal HDL resulted in much less activation of the LCAT reaction (results not shown) indicating that the presence of a lipoprotein to accept the newly formed cholesteryl esters is necessary for maximal activation of LCAT.

From the disappearance of individual molecular species after incubation with LDL or VLDL, we calculated the percentage contribution as well as the selectivity factors for each of the major species, as described above. The results in **Fig. 2** show that the percentage of acyl groups derived from the 16:0-18:2 species markedly increased in the presence of both LDL and VLDL, whereas the percentage derived from 16:0-18:1, 18:0-18:1, and 18:0-18:1 decreased. The contribution of 16:0-16:0 decreased in the presence of either VLDL or LDL while the contribution of 16:0-20:4 increased in the presence of LDL but not VLDL.

Although the percentage contribution of various species changed dramatically in presence of added lipoproteins, the selectivity factors were not altered significantly (**Table 5**). These results show that the specificity of the enzyme towards most acyl donors is not altered even in presence of excess exogenous lipoproteins having dissimilar molecular species composition. One exception was the negative selectivity factor obtained for 16:0-16:0 in the presence of LDL, possibly reflecting the resynthesis of this species by the LAT reaction. The selectivity factor for 16:0-16:0 was also lower in the presence of VLDL, although the difference did not reach statistical significance because of small sample size.

Molecular species of PC formed by the LAT reaction

We next studied the effect of addition of LDL and VLDL on the acylation of [1-^{14}C]palmitoyl lysoPC. As shown earlier (3) the LDL stimulated the acylation of labeled lysoPC by ABL plasma, but VLDL had no such effect. The labeled PC synthesized in the presence of LDL was analyzed by HPLC to determine the specificity of LAT reaction. The major species synthesized by the LAT

TABLE 3. Percentage contribution of various lecithin species for LCAT reaction in ABL and normal plasma

	Percentage Contribution (Mean ± SD)	
Species	ABL (n = 5)	Normal (n = 7)
18:1-20:5	0.00 ± 0.00	0.37 ± 0.63
16:0-20:5	−0.02 ± 0.05	0.79 ± 0.39
16:0-22:6 + 18:2-18:2	0.79 ± 2.22	3.53 ± 3.18
RRT 0.7	0.42 ± 1.02	0.47 ± 1.03
18:1-20:4 + 18:0-22:6	−0.10 ± 0.82	0.97 ± 0.57*
16:0-20:4	4.69 ± 1.77	8.97 ± 2.16**
18:0-20:5	0.27 ± 0.34	0.17 ± 0.99
18:1-18:2	1.14 ± 4.00	4.04 ± 1.77
16:0-18:2	21.45 ± 7.07	43.84 ± 10.24**
16:0-20:3	2.05 ± 1.63	2.24 ± 5.67
16:0-20:3 (n-9)	0.71 ± 1.50	−0.92 ± 2.64
18:0-20:4	3.20 ± 2.31	3.08 ± 2.90
RRT 1.35	0.40 ± 1.43	−0.66 ± 1.15
18:1-18:1	4.46 ± 1.08	1.01 ± 0.57**
16:0-18:1	37.90 ± 7.06	15.89 ± 7.70**
18:0-18:2	11.20 ± 7.51	14.67 ± 4.64
16:0-16:0 + 18:0-20:3	6.37 ± 4.82	0.50 ± 3.41*
18:0-18:1	4.37 ± 3.17	0.94 ± 0.69*

The percentage contribution of individual species was calculated by dividing the decreases in the amount of each species by the decrease in the amount of total PC following a 24-h incubation at 37°C.
*$P < 0.05$; **$P < 0.005$.

TABLE 4. Effect of addition of normal LDL and VLDL

Species	% Composition (Supt)				% Composition (Ppt)			
		at 24 h after Addition of			with LDL		with VLDL	
	0 H	None	LDL	VLDL	0 H	24 H	0 H	24 H
16:0-22:6	2.02	3.56	2.79	2.60	3.41	3.16	2.65	2.60
16:0-20:4	6.30	7.39	8.33	9.98	8.05	8.15	8.00	8.64
18:1-18:2	2.50	1.08	3.10	3.09	3.05	2.87	3.64	3.21
16:0-18:2	16.91	12.42	33.24	28.60	33.95	29.12	30.73	27.57
16:0-20:3	3.43	3.98	4.76	4.42	4.21	4.45	5.01	5.24
18:0-20:4	6.27	8.60	6.09	8.27	4.18	4.88	4.78	5.00
18:1-18:1	5.52	5.76	1.79	2.45	1.16	1.76	1.33	1.89
16:0-18:1	38.35	39.42	20.42	17.41	16.65	19.29	15.38	18.67
18:0-18:2	4.08	6.43	12.63	15.45	14.70	16.61	17.40	17.36
16:0-16:0	8.07	3.55	1.25	2.08	2.42	1.08	2.38	2.20
18:0-18:1	3.94	4.71	3.13	2.61	2.39	3.55	3.10	3.72
nmol P/ml	240.00	138.00	279.00	247.00	987.00	709.00	987.00	793.00

One ml of plasma from an ABL patient was incubated for 24 h at 37°C without any added lipoprotein or with heat-treated LDL or VLDL containing 1.0 μmol of PC each. After the incubation the lipoproteins were separated by heparin-Mn^{2+} precipitation. The supernatant containing the HDL and the precipitate containing the added lipoprotein (VLDL, LDL) were then extracted and the molecular species composition was analyzed as described in Methods. Species containing less than 1% total amount of PC at zero time were not included.

reaction in the presence of [1-^{14}C]palmitoyl lysoPC were 16:0-20:4, 16:0-18:2, 16:0-20:3 (n-9), 16:0-18:1, and 16:0-16:0, with the largest percentage of label in 16:0-18:2 (results not shown). This labeling pattern was similar to the one we reported previously in studies with enzyme isolated from normal plasma (2). There was no difference in the labeling pattern of PCs of precipitate (LDL) and supernatant (HDL) separated by heparin-Mn^{2+} precipitation, although the bulk of the radioactivity was associated with the added LDL. When the specific radioactivities of all labeled PCs were calculated, the highest specific activity was found in 16:0-16:0 (**Fig. 3**). The specific activity of 16:0-20:3 (n-9) was also found to be higher than that of 16:0-18:2 and 16:0-18:1. These results show that the LAT reaction preferentially forms specific molecular species of PC and thus its activation by LDL influences the molecular species composition independent of the exchange reaction.

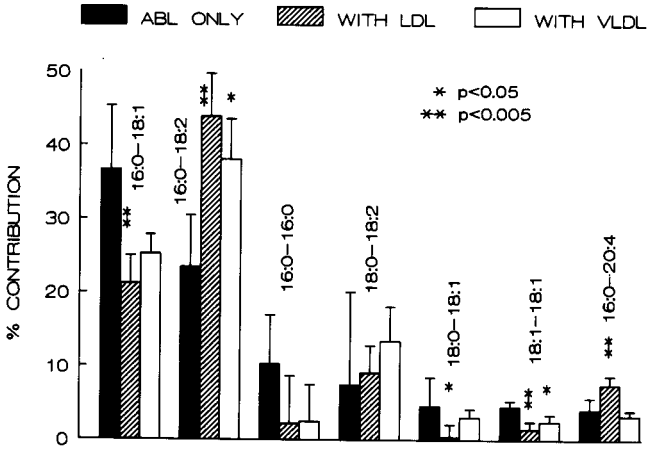

Fig. 2. Effect of addition of LDL and VLDL to ABL plasma on the relative utilization of various PC species by the LCAT reaction. LDL or VLDL isolated from normal plasma and containing 1.0 μmol of total PC was added to 1.0 ml of ABL plasma and the mixture was incubated for 24 h at 37°C. The percentage contribution of individual species toward LCAT reaction was calculated as described in the text. The values given are mean ± SD of five (no addition), four (with LDL), or three (with VLDL) experiments. Where indicated, the values for samples containing VLDL or LDL were significantly different from the control values at $P<0.05$ (*) or $P<0.005$ (**).

DISCUSSION

The abnormal fatty acid composition of plasma lipids in ABL patients has been reported previously (4, 13). Prominent among these abnormalities is the relative increase in 18:1 and a decrease in 18:2 in all the patients studied. A decrease in the content of 20:4 was also reported in some patients (4). While the primary cause of the deficiency of 18:2 and 20:4 in these patients is their inability to absorb dietary long chain unsaturated fatty acids, the presence of other abnormalities such as the decreased concentration of phospholipids in plasma, and increased sphingomyelin/PC and unesterified/esterified cholesterol ratios (4, 7, 13-15), suggest that the absence of VLDL and LDL has profound effects on the metabolism of phospholipids and cholesterol in the plasma.

TABLE 5. Effect of addition of LDL and VLDL on selectivity factors of major PC species

Species	Selectivity Factor (Mean ± SD)		
	No Addition (n = 5)	With LDL (n = 4)	With VLDL (n = 3)
16:0-18:1	1.00 ± 0.20	1.12 ± 0.08	1.11 ± 0.11
16:0-18:2	1.27 ± 0.33	1.43 ± 0.24	1.26 ± 0.05
16:0-16:0	1.56 ± 0.06	−0.50 ± 0.61*	0.22 ± 1.84
18:0-18:2	1.30 ± 0.53	0.86 ± 0.14	0.97 ± 0.27
18:0-18:1	0.96 ± 0.60	0.24 ± 0.45	0.82 ± 0.22
18:1-18:1	0.91 ± 0.19	0.51 ± 1.33	1.07 ± 0.25
16:0-20:4	0.82 ± 0.24	0.90 ± 0.15	0.46 ± 0.09
18:0-20:4	0.58 ± 0.48	0.56 ± 0.36	0.52 ± 0.05
18:1-18:2	0.34 ± 2.26	0.83 ± 0.44	0.99 ± 0.22
16:0-20:3	0.65 ± 0.17	0.51 ± 0.28	0.59 ± 0.19

*$P < 0.05$ compared to control (no addition).

The availability of new techniques to separate individual molecular species of phospholipids makes it possible to study the consequences of a severe deficiency of apoprotein B-containing lipoproteins on the molecular species composition in greater detail than that afforded by the study of fatty acid composition alone. For example, although we found that the species containing 18:2 in the *sn*-2 position are decreased and are replaced by those containing 18:1, thus confirming the published reports (4, 13), the relative decrease in 16:0-18:2 (32.1 to 17.6%) was less than the decrease in 18:0-18:2 (14.6 to 5.2%) or 18:1-18:2 (4.8 to 1.7%). Similarly, the decrease in *sn*-2-20:4 species was not uniform because 16:0-20:4 decreased more than 18:0-20:4. The increase in the percentage of 16:0-20:3 (n-9) in ABL plasma, although not statistically significant, may represent some degree of essential fatty acid deficiency since 20:3 (n-9) has been shown to be increased in experimental animals with essential fatty acid deficiency (16). The presence of a higher percentage of 16:0-16:0 in ABL probably indicates the relatively increased synthesis of this species in liver due to the deficiency of unsaturated fatty acids. Since the LAT reaction is known to preferentially form 16:0-16:0 (when using 1-16:0-lysoPC as substrate) (2), and since this reaction is virtually absent in ABL plasma, it was surprising to find a higher percentage of 16:0-16:0 in ABL plasma. However, if one considers that the total amount of PC is only 10-15% of control plasma, the absolute amount of 16:0-16:0 is still lower than in normal plasma.

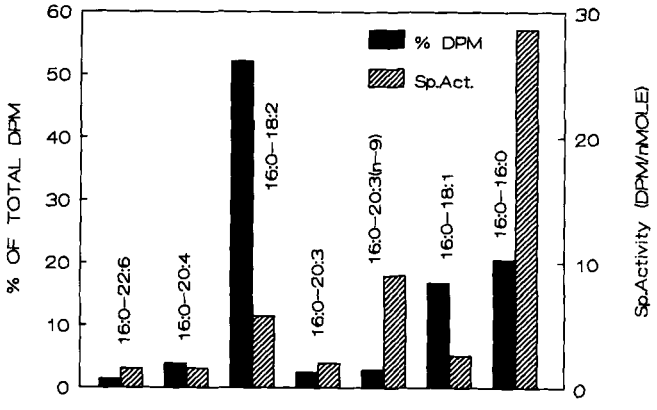

Fig. 3. Molecular species of labeled PC formed after acylation of [1-^{14}C]16:0-lysoPC. The labeled PC obtained after incubation of ABL plasma in the presence of LDL was separated by HPLC and the radioactivity in each PC species was determined, from which the percentage of total PC radioactivity in each species was calculated. The specific activity of each PC species was determined by dividing the total counts in each species by its mass.

The decreased membrane fluidity of erythrocytes from ABL patients has been attributed to the presence of increased amounts of sphingomyelin (8). Our results showing that the PC and PE species of ABL erythrocytes are more saturated than normal cells suggest that these changes in the acyl group composition may also contribute to the observed decrease in membrane fluidity (17). The PC and PE species are apparently affected differently by the absence of apoB-containing lipoproteins because the composition of erythrocyte PC is more abnormal than that of PE. This indicates divergent metabolic fates of the acyl groups of these two diacylglycerophospholipids. It is also likely that since the PC is present mostly in the outer leaflet and the PE in the inner leaflet of the erythrocyte membranes (18), the exchange of PC is more rapid between erythrocytes and lipoproteins compared to the exchange of PE, and therefore the composition of the latter is less affected. However, it should be noted that PC composition of ABL plasma differs from that of the ABL erythrocytes which indicates that not all species of PC are freely exchangeable. Thus, while the ratio of 16:0-18:2/18:0-18:2 was 2.37 in ABL plasma, it was only 0.85 in

ABL erythrocytes (Tables 1 and 2). It has been shown that only about 60% of erythrocyte PC exchanges with plasma PC (19) and it is possible that certain species like 18:0–18:2 are exchanged slower than others or not at all.

VLDL and LDL play several important roles in the LCAT reaction in normal plasma. First, they provide most of the unesterified cholesterol and PC for the reaction (20, 21). Secondly, they serve as the main acceptors of CE synthesized by LCAT (21, 22), thus relieving the product inhibition. Finally, a small but significant amount of cholesterol is esterified on the surface of LDL particles (23). It is therefore not surprising that the LCAT reaction is affected adversely in ABL plasma. Although we have previously shown that the concentration of LCAT protein is decreased in ABL subjects (3), the addition of VLDL or LDL did stimulate the activity several fold, indicating that these lipoproteins are required for optimal LCAT activity. Furthermore, the cholesteryl ester composition in ABL plasma is markedly different from that in normal plasma in having a higher percentage of 16:0 and 18:1 and a lower percentage of 18:2 (4, 24), even when the patients are fed corn oil. The results of Kayden (24) further demonstrated that although the 18:2 content of triglycerides and PC was increased following corn oil therapy, the increase in 18:2 in cholesteryl esters was less significant, suggesting that the specificity of LCAT may be altered in the absence of VLDL and LDL. However, the results presented here show that although the percentage contribution of various species of PC differed in ABL and normal plasma, their selectivity factors were similar. Furthermore, the addition of normal VLDL or LDL to ABL plasma stimulated LCAT activity and altered the percentage contribution of different PC species, but did not have significant effect on the selectivity factors. One notable exception was the higher selectivity factor observed for 16:0–16:0 in the unsupplemented ABL plasma. This appears to be due to its resynthesis in normal plasma by the LDL-stimulated LAT reaction (2), which results in replenishment of this species used by LCAT reaction and thus in an apparently low selectivity factor. This is supported by the observation that when LDL was added to ABL plasma the selectivity factor for 16:0–16:0 decreased and in fact became negative. However, the selectivity factor also decreased in presence of VLDL which does not stimulate LAT activity, suggesting that other factors might also be responsible for this discrepancy.

Since ABL plasma does not have LAT activity, it provides a valuable experimental tool to study the physiological importance of this enzyme activity. In confirmation of our earlier results (3) we found that the addition of LDL, but not VLDL, to ABL plasma stimulated the LAT activity. Incorporation of [1-^{14}C]16:0 lysoPC into various species of PC followed the same pattern as found in normal plasma (2). The highest specific activity was found for 16:0–16:0 species providing additional evidence that LAT reaction preferentially synthesizes disaturated PC when using saturated lysoPC as substrate. Our recent results also indicate that the types and amounts of cholesteryl esters formed by ABL plasma in presence of LDL and VLDL are not the same, indicating the possible influence of LAT activity on cholesteryl ester composition (Subbaiah, P. V., B. Banerji, R. E. Gregg, and J. D. Bagdade, unpublished observations).

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