ASBMB
JOURNAL OF LIPID RESEARCH

minireview

Separation of phospholipid molecular species by high performance liquid chromatography: potentials for use in metabolic studies

Sander J. Robins and George M. Patton

Department of Medicine, Boston Veterans Administration Medical Center, Boston, MA 02130

Abstract Different molecular species of phospholipids exhibit distinctly different patterns of biologic behavior. In this minireview, the utility of HPLC for analysis of molecular species of phospholipids is illustrated in studies in which it has been demonstrated that molecular species are selectively synthesized, selectively transported, and selectively participate in enzymatic reactions. HPLC appears to be more adaptable for routine use than older procedures used to separate phospholipid molecular species. Since the metabolism of intact molecules can be characterized with HPLC, this procedure promises to provide particularly novel information with respect to changes in composition brought about by remodelling reactions during the biologic life of specific phospholipids. —**Robins, S. J., and G. M. Patton.** Separation of phospholipid molecular species by high performance liquid chromatography: potentials for use in metabolic studies. *J. Lipid Res.* 1986. **27:** 131-139.

Supplementary key words lecithins • turnover • transport

For any naturally occurring phospholipid, a large number of molecular species may exist. A variety of different fatty acids or combinations of fatty acids are commonly present in phospholipids with the same general structure that are isolated from any animal tissue. Thus, although a phospholipid such as lecithin may have a certain prototypic structure, the lecithin that is isolated from a natural source, in reality, is a family of related molecules that should be more properly referred to as "lecithins." In this review of an emerging area of research, we would hope to demonstrate that this distinction is more than semantic. Phospholipids that have the same base component but different fatty acids are truly different molecules, with different metabolic and physical properties. Thus, for example, it is not possible to compare lecithin turnover in different tissues or under different metabolic conditions without determining which specific molecular species of lecithin are present in these particular tissues and to what extent each lecithin participates in turnover.

Molecular species analysis at the present time can be undertaken using HPLC with much greater facility and certainty than was previously possible using a more indirect and multi-step chromatographic approach. Furthermore, in contrast to the older techniques, only with the use of HPLC can intact phospholipid molecules be isolated. Thus, multiple portions of the same molecule can be labeled and traced at the same time and, consequently, any change that is brought about by remodelling of single molecules during their biologic life can be readily detected. In this review we will suggest the potentials for use of phospholipid molecular species analysis in a wide range of metabolic studies. In three specific areas we will illustrate the utility of this approach in characterizing lipid behavior in complex biologic systems. This review will be selective with a relatively narrow focus and is intended more to provide a prospective than a complete survey of the published literature.

Evolution of methodology and advantages of HPLC

Several generally similar schemes were employed prior to the introduction of HPLC methods to separate individual molecular species of diacylglycerophospholipids (reviewed by Holub and Kuksis (1) and Christie (2). None, however, appear to be particularly adaptable for routine use in metabolic studies requiring repetitive analysis. The

Abbreviations: HPLC, high performance liquid chromatography; GLC, gas-liquid chromatography; HDL, high density lipoprotein(s); VLDL, very low density lipoprotein(s); LDL, low density lipoprotein(s).

Downloaded from www.jlr.org by guest, on June 19, 2012

most discriminating of these non-HPLC procedures require degradative analysis in which diacylglycerophospholipids are first hydrolyzed to their diglycerides which are then derivatized and quantitated by GLC-mass spectrometry (3-5) or, with less elaborate instrumentation, are separated into groups of molecules with the same total number of double bonds by argentation TLC and the fatty acids of these groups of molecules are then quantitated by conventional GLC (6, 7). This last procedure requires independent quantitation of fatty acid methyl esters of the total phospholipid population (usually at several stages during the procedure) and, finally, certain assumptions to be made regarding the probability of associations of acyl groups in individual molecules.

Although a technical possibility, analysis of radioactivity in addition to mass in individual molecular species using any of the non-HPLC methods is not readily accomplished. (Molecular species can of course be labeled with deuterium, and this label can be independently quantitated by GLC-mass spectrometry. However, this method is costly and, because of the need for high specific activities, it is not generally as adaptable for biologic studies as the use of radioisotopes in tracer amounts.)

In contrast to these earlier approaches, molecular species analysis by HPLC appears to possess all of the desirable features necessary to conduct a metabolic study. HPLC is essentially a single-step procedure in which amounts of mass and radioactivity can be determined in individual molecular species at the same time. Recoveries are complete and analysis can be performed without incurring any degradative losses (8). With conventional (bonded octadecylsilyl) reverse phase columns, individual molecular species elute in a predictable sequence (8, 9), according to their relative hydrophilic strengths (as illustrated in **Fig. 1** by HPLC of a homologous series of lecithins containing 18:2).[1] Thus, any analysis can routinely be undertaken without using standards for identification. When confirmation of the identity of molecular species is desirable, this can readily be accomplished by collecting any peak eluted from an HPLC column for GLC analysis of its component fatty acids. HPLC of phospholipids can ordinarily be completed in 2 to 3 hr, at which time a fully saturated molecule with acyl groups of 36 carbons (or its equivalent) can be detected. As illustrated (**Fig. 2**) by the

[1]By convention, the fatty acid listed first is in the *sn*-1 position of a diacylglycerophospholipid and the fatty acid listed second is in the *sn*-2 position. The distribution of fatty acids in single molecules must be determined by selective enzymatic hydrolysis, but will neither affect the overall hydrophilic strength of a molecule nor its retention time on a reverse phase column. Where not specifically determined, we have generally listed the more saturated fatty acid in the *sn*-1 position and the more unsaturated fatty acid in the *sn*-2 position of single molecular species.

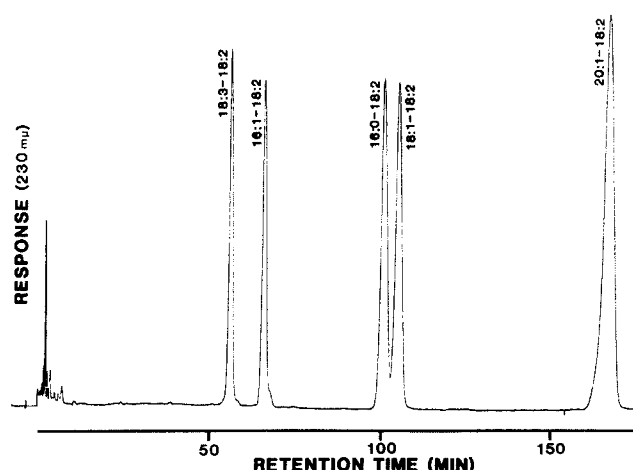

Fig. 1. Relative rates of elution from an HPLC reverse phase column of lecithin molecular species containing an 18:2 acyl group. HPLC was performed after converting lecithins to their diacylbenzoates (13), using a 4.6 × 250 mm Ultrasphere ODS column (5-μ packing) and a solvent of methanol-water-acetonitrile 95.5:3.5:1 with a flow rate of 1.5 ml/min. The positional distribution of fatty acids was not determined for the molecular species shown, although for each of these species 18:2 is similarly listed in the *sn*-2 position.

chromatograms of phospholipids isolated from a normal rat liver, a large number and diversity of molecular species may be present in native phospholipids obtained from any single biologic source. Complete descriptions of the conditions of HPLC for phospholipid molecular species analysis can be found in several recent publications (8-11).

Quantitation of molecular species can be accomplished by phosphorus analysis or, perhaps more expediently, by integration of peak areas. Although quantitation by integration is "on-line" and inherently more desirable, detection of intact phospholipids in (optimally) the 200-nm range (12) is a function of double bond number and is, therefore, not uniform for different molecular species. Quantitation by integration, however, can be readily accomplished by converting diacylphospholipid species to diglyceride derivatives (such as benzyl esters of diglycerides) (13) which will then detect at 230 nm, where the usual variable response of different acyl groups is not apparent. We have used HPLC with only minor changes in the conditions of chromatography to separate intact lecithins as well as their derivatized diglycerides. Both procedures may be used to advantage. Whereas in metabolic studies intact lecithins may need to be chromatographed to retain the phosphorylcholine group for radioactivity measurement, for other studies derivatized diglycerides may be chromatographed, since individual species of diglyceride benzoates can be separated by HPLC with better definition than intact lecithin species and can be detected with greater sensitivity (when quantitated by integration) than is possible for intact lecithins (when quantitated by phosphorus).

Downloaded from www.jlr.org by guest, on June 19, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR

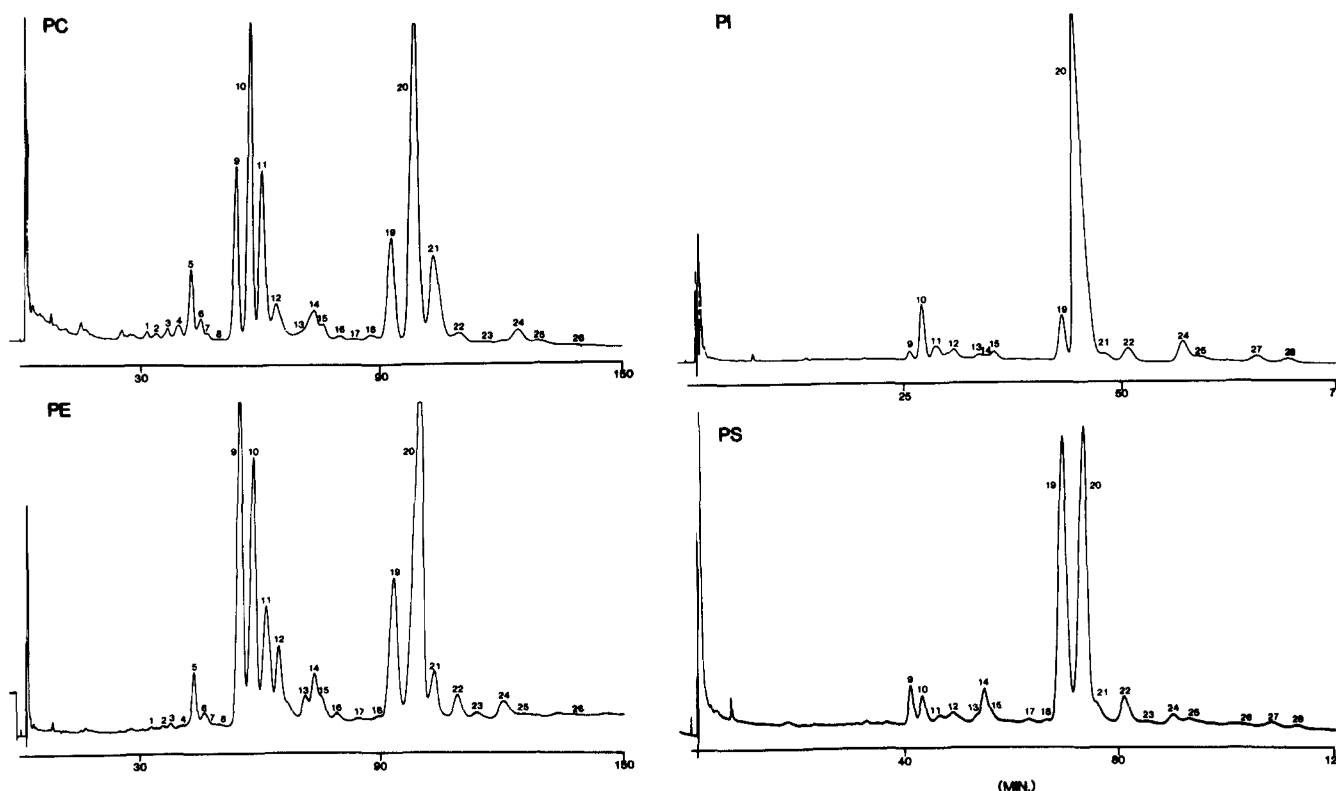

Fig. 2. HPLC of molecular species of intact hepatic lecithin (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS) from a normal (chow-fed) rat. Peaks are numbered in order of elution, using PC as the reference chromatogram. Molecular species of PC, PE, and PI were eluted from an Ultrasphere ODS column with a solvent of 20 mM choline chloride in methanol-water-acetonitrile 90.5:7:2.5 with a flow of 2.0 ml/min, or for PS, with a solvent of 30 mM choline chloride in methanol-KH_2PO_4-acetonitrile-acetic acid 90.5:7:2.5:0.8 with a flow also of 2.0 ml/min. Peaks in different phospholipid chromatograms that are similarly numbered contain species with the same acyl groups, identified in a previous publication (8). (Reprinted from ref. (8) with permission of the Journal of Lipid Research.)

Recognition of selective synthesis of phospholipid molecular species

The initial information regarding the distinct biologic behavior of individual molecular species of phospholipids pertained to the turnover in isolated tissues of lecithins that were ordinarily present in the largest amounts, with a conventional structure—that is, lecithins with a saturated fatty acid in the *sn*-1 acyl group position and an unsaturated fatty acid in the *sn*-2 position. In studies reported almost 20 years ago (14, 15), there is clear evidence for selectivity in the de novo synthesis of individual lecithins. In tissues such as the liver, where lecithins may be synthesized by either the incorporation of choline into diglycerides or the methylation of phosphatidylethanolamines, individual lecithins are preferentially synthesized by one or the other pathway. As demonstrated for a normal chow-fed rat (**Table 1**), which was injected at the same time with [methyl-^{3}H]methionine and [1,2-^{14}C]choline chloride, methyl groups are preferentially incorporated into 18:0–20:4 lecithin, whereas choline is preferentially incorporated into lecithins with 16:0 in the *sn*-1 position, especially 16:0–18:2 lecithin. Relative rates of turnover have been determined for several of the lecithins that are ordinarily the most prevalent in animal tissues (reviewed by Holub and Kuksis (1)), demonstrating that the substitution of a single fatty acid may result in a remarkable change in the turnover rate of the whole lecithin molecule. For example, in the liver where 16:0–18:2 and 16:0–20:4 lecithins turn over with half-times of 0.9 and 3.9 hours, respectively, 18:0–18:2 and 18:0–20:4 lecithins turn over with half-times of 6.5 and 11.3 hours, respectively. (In view of these differences, it should be appreciated that a comparison of lecithin turnover cannot be undertaken using only argentation chromatography to isolate lecithins in groups according to their overall degree of unsaturation.)

During their biologic life, phospholipids that have been selectively synthesized may also be selectively remodelled. As a result of deacylation–reacylation (or transacylation) reactions, entirely new molecular forms of a phospholipid may be evolved. For example, there is growing evidence that 16:0–16:0 lecithin, the predominant lecithin of pulmonary surfactant, is not synthesized de novo but is the

Downloaded from www.jlr.org by guest, on June 19, 2012
ASBMB JOURNAL OF LIPID RESEARCH

TABLE 1. Relative rates of utilization of choline and methionine for the synthesis of hepatic lecithins

Lecithin Species	% Distribution	% of Radiolabel Incorporated from Choline	from Methionine
16:0-22:6	3.9	4.1	14.1
16:0-20:4	9.7	9.6	14.3
16:0-18:2	14.4	40.8	11.9
16:0-18:1	6.4	20.6	2.3
18:0-22:6	3.6	2.5	9.8
18:0-20:4	16:1	4.3	27.6
18:0-18:2	14.8	4.3	7.0
All others	31.1	13.8	13.0

Intact lecithins were isolated by HPLC from the liver of a chow-fed rat that was injected via the portal vein with a bolus of [methyl-^{3}H]methionine and [1,2-^{14}C]choline chloride. Amounts of phosphorus and radiolabels were determined for each of the major lecithin species shown.

product of remodelling by specialized pulmonary cells that preferentially utilize 1-palmitoyl-2-lysolecithin and 16:0-CoA as the substrates for reacylation (16, 17). Although the functional significance of 16:0-16:0 lecithin or any other single phospholipid may be debated, it is now clear that particular molecular species of phospholipids may be preferentially evolved and/or preferentially utilized in a diversity of biologic reactions. Thus, particular molecular species of lecithin are preferentially solubilized in bile salt micelles (18); particular molecular species of lecithin participate in the complex series of events in which platelet activating factor (1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is formed and degraded (19); particular lecithins and lysolecithins participate in lecithin: cholesterol acyltransferase-mediated reactions in the plasma (20); and particular lecithins are preferentially exchanged between lipoproteins and other lipid particles in an aqueous environment (21).

Utility of HPLC in molecular species analysis

In three general areas of phospholipid research, molecular species analysis should prove to be of major benefit. In each case, the overriding advantage of this analysis relates to the facility with which naturally occurring phospholipids that are present in usual amounts and in their usual surroundings can be manipulated and traced. Studies can therefore be performed without having to add to a biologic system exogenous phospholipids that, in kind or by their particular associations with other tissue components, may not appropriately reflect the natural state.

In studies of phospholipid turnover. First, the turnover of specific molecular species of potentially all naturally occurring phospholipids can be determined in the whole animal or in isolated tissues. Turnover, which may reflect both de novo synthesis and remodelling, can be assessed by radiolabeling one or several portions of phospholipid molecules and determining the change in the specific activity of these labels during a finite period of study. For example, we have measured relative rates of turnover of individual lecithins utilized in the formation of newly synthesized intestinal lipoproteins (22). This study was undertaken with the dual purpose of, first, contrasting turnover of individual lecithins in native lipoproteins before these lipoproteins entered the systemic circulation and were appreciably equilibrated; and, second, determining whether there were differences in the turnover of individual lecithins in different intestinal lipoproteins—a finding that would suggest that these lipoproteins are formed by different mechanisms, in which different metabolic pools of lecithin are utilized. In this study we followed the changes in the specific activity of choline-labeled lecithins that were isolated from rat bile, solubilized in bile salt, and infused into the duodenum of non-radiolabeled rats with a lymph fistula. As shown in **Fig. 3**, the specific activities of all infused lecithins decreased after their absorption and reutilization for lipoprotein synthesis. The decline in specific activity was highly variable for individual molecular species and reflects variable utilization of endogenous, non-radioactive lecithins or lecithin precursors (derived from

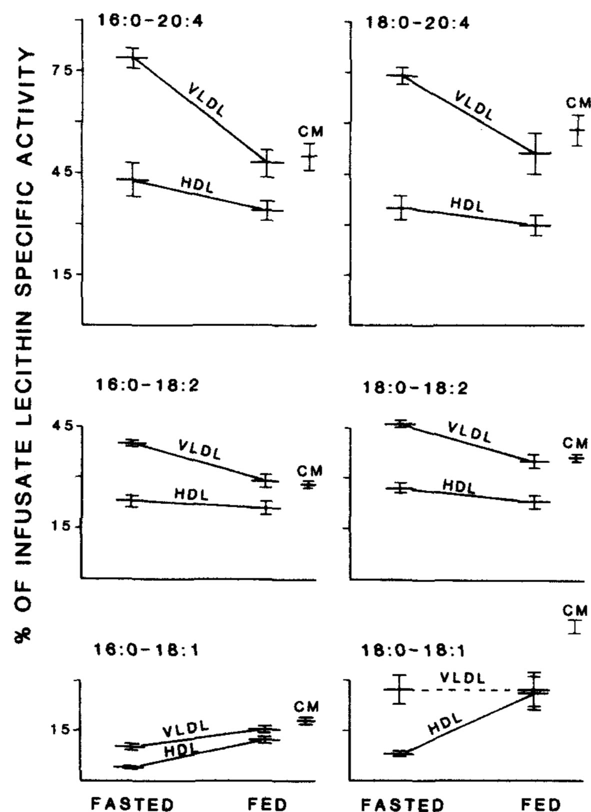

Fig. 3 Changes in specific activities of six major molecular species of lecithin that were infused into the intestine and reutilized for intestinal lipoprotein formation. Changes are shown as a percentage of the infusate specific activities (± SE) during fasting (in VLDL and HDL) and after feeding triolein (in VLDL, HDL, and chylomicrons (CM)). (Reproduced from *The Journal of Clinical Investigation,* 1984, **73:** 231-240, Fig. 3, by copyright permission of The American Society for Clinical Investigation.)

Downloaded from www.jlr.org by guest, on June 19, 2012

the plasma or the intestine) for intestinal lipoprotein formation. For each of the major lecithin species shown in Fig. 3, a clear pattern of specific activity change was observed. That is, the specific activity of lecithins in lymph HDL was consistently less than in VLDL in fasted animals and, with one exception (18:0–18:1 lecithin), in animals fed triglycerides. Moreover, the change in specific activity with feeding was appreciably different for HDL lecithins than for lecithins in VLDL or chylomicrons, which closely resembled VLDL. We further demonstrated, by a comparison of the composition of individual lecithins in plasma lipoproteins with newly formed lymph HDL, that the differences in specific activities observed between lymph HDL and VLDL could not be attributed to simple filtration of intact (non-radiolabeled) plasma HDL into lymph. Thus, with the turnover data provided by molecular species analysis, we believe we have been able to securely demonstrate that different intestinal lipoproteins originate by different mechanisms and/or from different intracellular sites, which is consistent with the demonstrated differences in apoprotein composition of these intestinal lipoproteins. Using HPLC analysis in this same or allied areas of research, the opportunity exists for more elaborate studies in which it may be possible to determine, with different portions of the lecithin molecule radiolabeled in conjunction with different lecithin precursors, the overall contribution of remodelling and de novo synthesis to lecithin formation in different lipoproteins.

In studies of phospholipid transport. A second major application of phospholipid molecular species analysis relates to studies of transport. Individual molecular species that have different acyl chain lengths and degrees of unsaturation will have different water solubilities and might, therefore, be anticipated to be transported between lipid particles in an aqueous medium or between lipid particles and tissues at different rates in certain circumstances. This appears to be especially relevant in cases of passive transport or in exchange reactions that are not protein-mediated. We have recently demonstrated (21) that, while the exchange of lecithins between two native lipoproteins is a biphasic process and similar to the exchange of lecithins between vesicles or between vesicles and lipoproteins, individual molecular species of lecithin not only

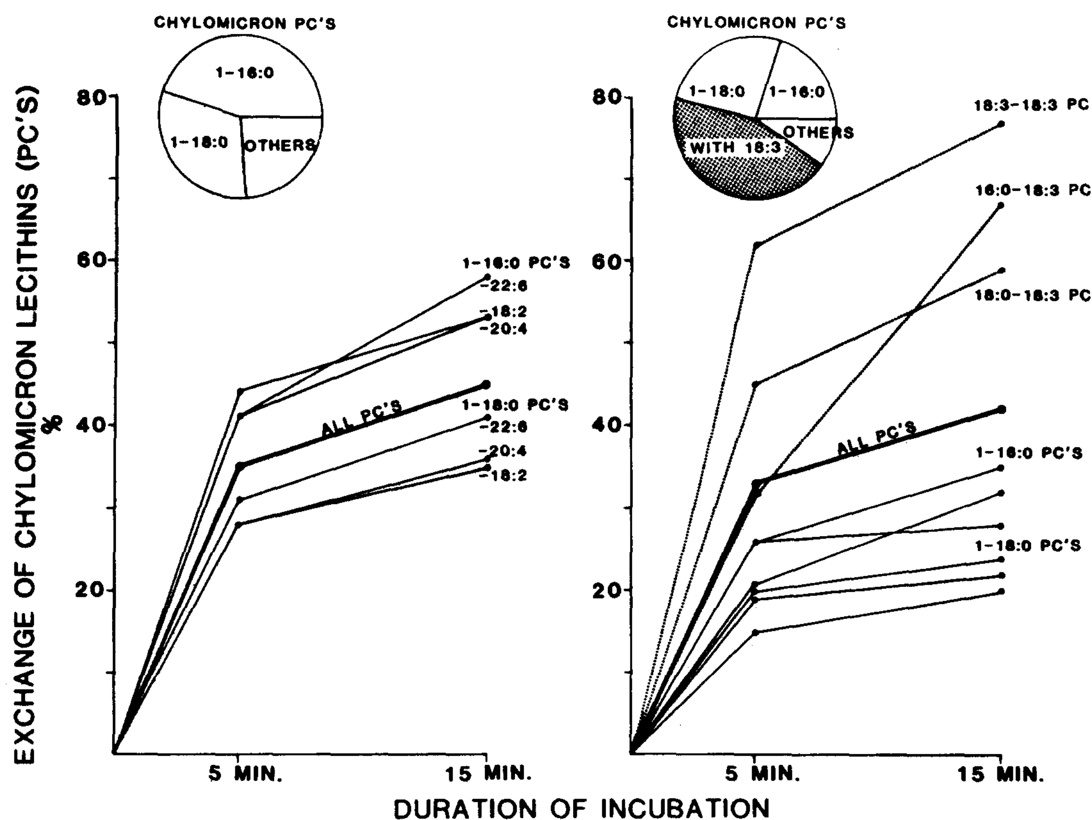

Fig. 4. Exchange of major molecular lecithins (PCs) between chylomicrons and HDL, in the absence of exchange proteins. Chylomicrons, with the compositions depicted in panels A and B, were obtained from lymph-cannulated rats fed specific triglycerides and then incubated with rat HDL. The percent equilibration is shown for individual lecithins and the total lecithin pool. Major *sn*-1 16:0 and *sn*-1 18:0 lecithins are numbered as follows: 1, 16:0(18:0)–22:6; 2, 16:0(18:0)–18:2; and 3, 16:0(18:0)–20:4. (Reprinted with modifications from reference (21), with permission of the Journal of Lipid Research.)

Downloaded from www.jlr.org by guest, on June 19, 2012

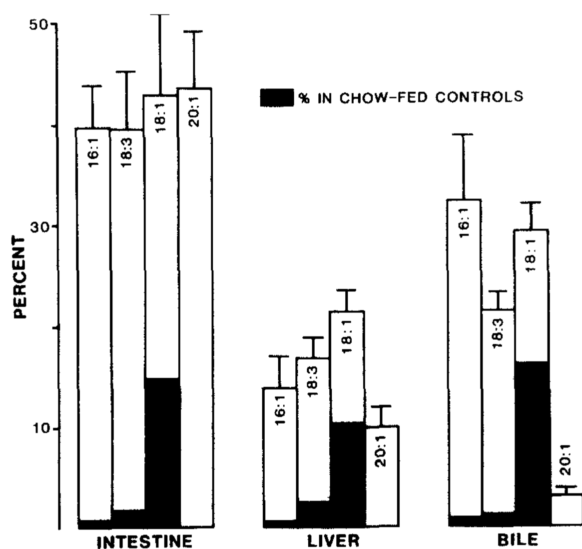

Fig. 5. Extent of incorporation of dietary fatty acids with different hydrophilic properties into the lecithins of the rat. Pure, symmetrical triglycerides, containing either 16:1, 18:3, 18:1, or 20:1, were fed as a 1-g bolus by gastric tube to individual animals for 5 days. During this period, all animals were allowed Purina lab chow ad libitum. Three to 5 hr after the last feeding, bile was collected for one-half hour and the animals were then killed. Lecithins were isolated by HPLC from the mucosa of the proximal intestine, liver, and bile. The percent (± SE) of newly evolved lecithins is shown for three-five animals in each group.

exchange at different rates but exchange at rates that may be modified by changes in the composition of the co-existing lecithin population. Thus, for chylomicrons incubated with HDL in the absence of exchange proteins, we found that the exchange of *sn*-1 16:0-lecithins was more rapid than the corresponding *sn*-1 18:0-lecithins, but that the introduction of more hydrophilic 18:3-containing lecithins into chylomicrons slowed the exchange of other chylomicron lecithins—most notably, the *sn*-1 16:0-lecithins, which were the next most hydrophilic (**Fig. 4**). At present we have no explanation for the effect of this particular molecular interaction on the rate of exchange of individual molecular species. However, in studies of biliary lecithin secretion in the live animal, we have observed what appears to be analogous behavior of molecular species with different hydrophilic strengths. We undertook these particular studies with the presumption that selective secretion of lecithins in bile might reflect a predominantly "physical process" in which intrahepatic lecithins which were the most hydrophilic were the most readily solubilized by bile salts and, thus, the most readily secreted into bile.

Ordinarily bile contains lecithins that have a relatively high content of 16:0 and a low content of 18:0 (23). In contrast, liver contains a more general mix of lecithins, with a prominent array of 18:0 species (as shown in Fig. 2). Feeding studies were undertaken to radically change the acyl group composition of lecithins in the rat and to produce a range of lecithins with different hydrophilic strengths. As a result of feeding a series of pure triglycerides, lecithins were evolved that were more hydrophilic (containing 16:1 and 18:3 acyl groups), equally hydrophilic (containing 18:1), and less hydrophilic (containing 20:1) than the usual predominant biliary (16:0-containing) lecithins (Fig. 1). Molecular species analysis showed that, whereas increased amounts of the lecithins that contained the fatty acid fed were similarly formed in the intestine

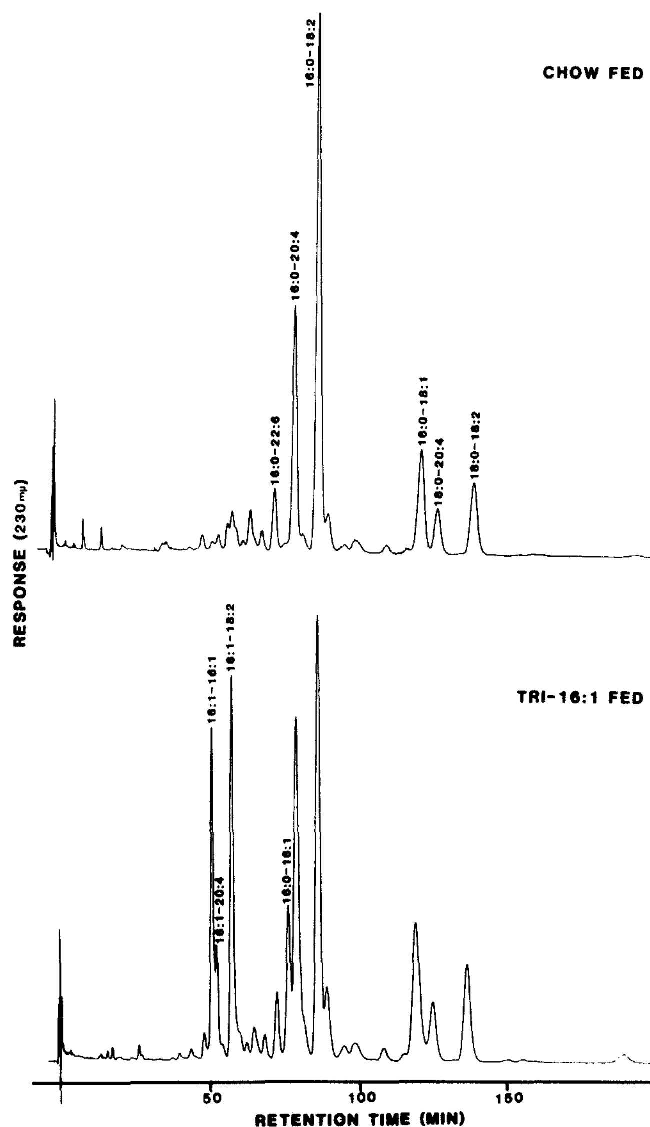

Fig. 6. HPLC of the lecithins (separated as diacylglycerobenzoates) of bile from a chow-fed rat and of bile from a rat fed triglyceride containing 16:1 (from the studies shown in Fig. 5). Chromatography was performed as indicated for Fig. 1. In the upper panel, the major *sn*-1 16:0 lecithins and *sn*-1 18:0 lecithins are identified, while in the lower panel the new lecithins containing 16:1 are identified. The specific position of acyl groups was not determined for the 16:1 lecithins but these are similarly shown with the more unsaturated fatty acid in the *sn*-2 position.

Downloaded from www.jlr.org by guest, on June 19, 2012

Fig. 7. Reciprocal changes between the secretion of bile lecithins containing the usually predominant *sn*-1 16:0 species (consisting largely of 16:0–18:2, 16:0–20:4, 16:0–18:1, and 16:0–22:6 lecithins) and novel, more hydrophilic lecithins containing 16:1 or 18:3 species. Data are from the studies shown in Fig. 5.

and taken up by the liver, only those lecithins that were more (or equally) hydrophilic than the *sn*-1 16:0 bile lecithins were secreted in bile to an appreciable extent (**Fig. 5**). A chromatogram illustrating the novel biliary lecithins generated after feeding 16:1 is contrasted with a chromatogram of bile from a chow-fed rat (**Fig. 6**). In association with increased biliary secretion of novel hydrophilic lecithins, there was suppressed secretion of the usual biliary *sn*-1 16:0 lecithins (**Fig. 7**). As indicated above, this pattern of molecular transport is analogous to the pattern of exchange that was observed between lipoproteins in vitro (and is similarly unexplained). It is apparent that, without molecular species analysis and with just a determination of the fatty acid composition of lecithins, we would have very limited insight into these particular characteristics of transport. It is our expectation that the distinct attributes of molecular transport, which we have already defined, will make it possible to trace, in isolated organelles, the pathway of intrahepatic transport of those species of lecithin that are preferentially secreted into bile.

In identification of natural phospholipid substrates and products in enzymatic reactions. A third application of phospholipid molecular species analysis pertains to the definition of the natural substrates and products of enzymatic reactions. Here, as in studies of turnover and transport, HPLC analysis of molecular species should enable identification of particular phospholipid molecules that are reacting in their natural environment. This may be especially relevant when a substrate is normally membrane-bound and it is not only desirable to avoid adding to a reaction mixture an exogenous, arbitrarily defined substrate but a vehicle, often with detergent properties, in which that substrate can be solubilized. Studies to date in which molecular species analyses have been performed have focused on the substrate requirements in remodeling reactions catalyzed by specific transacylases. As illustrated by three different studies (19, 20, 24), unique kinetic information can be obtained utilizing HPLC for molecular species separations.

First, studies have been conducted (20) to define the substrate specificity of plasma lysolecithin acyltransferase in a system in which plasma LDL, the known physiologic cofactor for this particular acyltransferase, is present and wherein the natural lecithins of LDL serve as the source of the new acyl groups used for lysolecithin esterification. Each of four major lysolecithins (containing 16:0, 18:0, 18:1, or 18:2), which were radiolabeled and incubated with plasma LDL, were esterified at similar rates and a number of new lecithin molecular species were evolved. However, with each lysolecithin studied, the specific activity of new lecithins generated was highest when 16:0 from an endogenous lecithin was utilized for acylation.

ASBMB
JOURNAL OF LIPID RESEARCH
Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 2. Percentage distribution of molecular species of hexadecylacylglycerophosphocholine formed by acylation of [^{3}H]hexadecyllysoglycerophosphocholine by macrophage membranes[a]

	Additions				
				CoA + ATP + Mg^{2+}	
Molecular Species	None	CoA	CoA + ATP + Mg^{2+}	− 18:2	+ 18:2
16:0–22:6	3.8	4.1	4.3	3.2	2.8
16:0–20:4	67.7	50.7	26.8	52.4	47.2
16:0–18:2[b]	17.7	27.3	24.4	13.6	31.9
16:0–18:1	7.9	10.9	16.3	12.5	7.1
16:0–16:0	2.5	5.6	27.0	16.6	10.1
16:0–18:0	0.3	1.4	1.2	1.7	0.9
Total product formed (pmol)	316	513	781	694	634

[a]Modified from Tables III and IV from Robinson, Blank, and Snyder (24).
[b]Also includes 16:0–20:3 and 16:0–22:4.

Preferential utilization of 16:0 in this reaction resulted in a shift in the lecithin molecular species composition in LDL and was most notable for an accumulation of 16:0–16:0 lecithin. Although lysolecithin acyltransferase activity produced no net change in lecithin mass, the change in overall lecithin composition, which was identified by an analysis of molecular species, might well have major implications for the surface properties and function of plasma LDL.

Two additional and related studies (19, 24) further demonstrate the utility of molecular species in this area of research. Considerable recent attention has been focused upon the reaction sequence by which platelet-activating factor, a potent vasoactive mediator, is formed and inactivated. Kramer et al. (19) have demonstrated that platelet membranes, in the absence of cofactors, will acylate a lysoglycerophosphocholine intermediate specifically with 20:4 to form the precursor of platelet-activating factor. Using HPLC to separate molecular species of phospholipids, these authors have further demonstrated that the substrates for this specific transacylation are the major endogenous 20:4 lecithins (i.e., 16:0–20:4 and 18:0–20:4 lecithins) in the platelet membrane. Similar cofactor-independent transacylases, in which an endogenous source of 20:4 is preferentially used for acylation, have also been described in intact neutrophils (25) and alveolar macrophages (24). In the study by Robinson, Blank, and Snyder (24), in which the specific molecular reaction products of macrophage transacylation were identified, the authors demonstrated that, depending upon the additions to macrophage membranes, transacylation produced different molecular species (of either alkylacyl or diacylglycerophosphorocholine). Shown in **Table 2** are the results obtained using hexadecyllysoglycerophosphocholine as the added (radiolabeled) substrate. As demonstrated, in the absence of any cofactor addition, transacylation predominantly resulted in the formation of a 20:4–containing species. However, with additions of cofactors or a specific fatty acid, the marked predilection for endogenous 20:4 in this reaction was lost and either a more general distribution of reaction products was obtained or a greater amount of the single exogenous fatty acid was utilized in product formation. The kinetics for the evolution of the 20:4 species in this reaction were distinctly different than for other molecular species. Thus, it is not surprising that both the K_m and V_{max} for the overall reaction were different, depending upon the particular additions to this system and the extent of formation of the 20:4 species.

Where there is a predilection for specific substrates or products, HPLC is a powerful tool for characterizing the kinetic behavior of different phospholipid molecular species. In this brief review, we have pointed out selected areas in biology where this technique has already had use. HPLC is much less cumbersome than the older procedures used to separate molecular species. Since HPLC permits for the first time isolation of intact molecules, we anticipate that studies characterizing the metabolism of single phospholipid molecules may become more commonplace.

The authors gratefully acknowledge the contributions of Margaret Collins and Joan Fasulo. The authors' research was supported by The General Medical Research Service of the Veterans Administration and NIH grant AM 28640.
Manuscript received 5 November 1985.

REFERENCES

1. Holub, B. J., and A. Kuksis. 1978. Metabolism of molecular species of diacylglycerophospholipids. *Adv. Lipid Res.* **16:** 1–125.
2. Cristie, W. W. 1982. Lipid Analysis, 2nd editon. Pergamon Press, Oxford.
3. Hosegawa, K., and T. Suzuki. 1975. Examination of acetolysis products of phosphatidylcholine by gas chromatography-mass spectrometry. *Lipids.* **10:** 667–672.
4. Curstedt, T., and J. Sjövall. 1974. Analysis of molecular species of ^{2}H-labelled phosphatidylcholines by liquid-gel chromatography and gas chromatography-mass spectrometry. *Biochim. Biophys. Acta.* **360:** 24–37.
5. Myher, J. J., A. Kuksis, L. Marai, and S. K. F. Young. 1978. Microdetermination of molecular species of oligo-

Downloaded from www.jlr.org by guest, on June 19, 2012

and poly-unsaturated diacylglycerols by gas chromatography–mass spectrometry of their *tert*-butyldimethylsilyl ethers. *Anal. Chem.* **50:** 557–561.
6. Renkonen, O. 1967. The analysis of individual molecular species of polar lipids. *Adv. Lipid Res.* **5:** 329–351.
7. Kuksis, A., and L. Marai. 1967. Determination of the complete structure of natural lecithins. *Lipids.* **2:** 217–224.
8. Patton, G. M., J. M. Fasulo, and S. J. Robins. 1982. Separation of phospholipids and individual molecular species of phospholipids by high-performance liquid chromatography. *J. Lipid Res.* **23:** 190–196.
9. Smith, M., and F. B. Jungalwala. 1981. Reversed-phase high performance liquid chromatography of phosphatidylcholine: a simple method for determining relative hydrophobic interaction of various molecular species. *J. Lipid Res.* **22:** 697–704.
10. Jungalwala, F. B., V. Hayssen, M. Pasquini, and R. H. McCluer. 1979. Separation of molecular species of sphingomyelin by reversed-phase high performance liquid chromatography. *J. Lipid Res.* **20:** 579–587.
11. Creer, M. H., and R. W. Gross. 1985. Reversed-phase high performance liquid chromatographic separation of molecular species of alkyl ether, vinyl ether, and monoacyl lysophospholipids. *J. Chromatogr.* **338:** 61–69.
12. Jungalwala, F. B., J. E. Evans, and R. H. McCluer. 1976. High performance liquid chromatography of phosphatidylcholine and sphingomyelin with direct detection in the region of 200 nm. *Biochem. J.* **155:** 55–60.
13. Blank, M. L., M. Robinson, V. Fitzgerald, and F. Snyder. 1984. Novel quantitative method for determination of molecular species of phospholipids and diglycerides. *J. Chromatogr.* **298:**473–482.
14. Rytter, D., J. E. Miller, and W. E. Cornatzer. 1968. Specificity for incorporation of choline and ethanolamine into rat-liver microsomal lecithins. *Biochim. Biophys. Acta.* **152:** 418–421.
15. Arvidson, G. A. E. 1968. Biosynthesis of phosphatidylcholines in rat liver. *Eur. J. Biochem.* **5:** 415–421.
16. Batenburg, J. J., W. J. Longmore, W. Klazinga, and L. M. G. VanGolde. 1979. Lysolecithin acytransferase and lysolecithin:lysolecithin acyltransferase in adult rat lung alveolar type II epithelial cells. *Biochim. Biophys. Acta.* **573:** 136–144.
17. Crecelius, C. A., and W. J. Longmore. 1984. Acyltransferase activities in adult rat type II pneumocyte-derived subcellular fractions., *Biochim. Biophys. Acta.* **795:** 238–246.
18. Angelico, M., D. E. Cohen, and M. C. Carey. 1985. Subselection of phospholipids during formation of native bile: physical–chemical coupling of bile salts (BS) and palmitoyl lecithins. *Clin. Res.* **33:** 538A.
19. Kramer, R. M., G. M. Patton, C. R. Pritzker, and D. Deykin. 1984. Metabolism of platelet-activating factor in human platelets. Transacylase-mediated synthesis of 1-O-alkyl-2-arachidonyl-*sn*-glycero-3-phosphocholine. *J. Biol. Chem.* **259:** 13316–13320.
20. Subbaiah, P. V., C. H. Chen, J. D. Bagdade, and J. J. Albers. 1985. Substrate specificity of plasma lysolecithin acyltransferase and the molecular species of lecithin formed by the reaction. *J. Biol. Chem.* **260:** 5308–5314.
21. Patton, G. M., S. J. Robins, J. M. Fasulo, and S. Bennett Clark. 1985. Influence of lecithin acyl chain composition on the kinetics of exchange between chylomicrons and high density lipoproteins. *J. Lipid Res.* **26:** 1285–1293.
22. Patton, G. M., S. Bennett Clark, J. M. Fasulo, and S. J. Robins. 1984. Utilization of individual lecithins in intestinal lipoprotein formation in the rat. *J. Clin. Invest.* **73:** 231–240.
23. Balint, J. A., E. C. Kyriakides, H. L. Spitzer, and E. S. Morrison. 1965. Lecithin fatty acid composition in bile and plasma of man, dogs, rats, and oxen. *J. Lipid Res.* **6:** 96–99.
24. Robinson, M., M. L. Blank, and F. Snyder. 1985. Acylation of lysophospholipids by rabbit alveolar macrophages. Specificities of CoA-dependent and CoA-independent reactions. *J. Biol. Chem.* **260:** 7889–7895.
25. Chilton, F. H., J. T. O'Flaherty, J. M. Ellis, C. L. Swendson, and R. L. Wykle. 1983. Selective acylation of lyso platelet activating factor by arachidonate in human neutrophils. *J. Biol. Chem.* **258:** 7268–7271.

Downloaded from www.jlr.org by guest, on June 19, 2012

ASBMB JOURNAL OF LIPID RESEARCH