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### Research Article

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## Utilization of Individual Lecithins in Intestinal Lipoprotein Formation in the Rat

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**A**bstract. To determine the molecular species composition of lecithins of different nascent lipoproteins, high density lipoproteins (HDL), very low density lipoproteins (VLDL), and chylomicrons (CM) were isolated from the mesenteric lymph of rats. Lymph was collected at 0°C with 5,5'-dithiobis-2-dinitrobenzoic acid added to inhibit lecithin-cholesterol acyl transferase. CM were separated by ultracentrifugation and HDL from VLDL by dextran  $\text{SO}_4\text{-Mg}^{+2}$  precipitation. Molecular species of lecithin were directly isolated by reverse phase high performance liquid chromatography. In fasted animals, the lecithin compositions of lymph HDL and VLDL were virtually the same and closely resembled the lecithin composition of intestinal mucosa. When bile lecithin was eliminated (by bile diversion), there was a marked change in lecithin composition of all lipoprotein and mucosal samples, which was most notable for a reduction in 16:0-species (which are predominant in bile) and a relative increase in the corresponding 18:0-species. Feeding unsaturated triglycerides (triolein, trilinolein, or a combination of triolein and trilinolein) also resulted in a change in HDL and VLDL lecithin composition. The effect was similar whether bile lecithin was present or eliminated and was notable for a reduction in 16:0-species, an increase in 18:0-species, and the emergence of large amounts of diunsaturated lecithins that corresponded to the fatty acid composition of the triglycerides fed (i.e., 18:1-18:1, 18:2-18:2, and 18:1-18:2 lecithins). When bile-diverted rats

were infused via the duodenum with a mix of [ $^{14}\text{C}$ ]choline-labeled lecithins (isolated from the bile of other rats), the incorporation of infused lecithins into different lymph lipoproteins was distinctly different. Individual lecithins were incorporated to a variable extent into each lipoprotein. In fasted rats the specific activities of all major molecular species of lecithin were relatively greater in VLDL than HDL, indicating that HDL derived proportionately more of its lecithins from an endogenous pool than did VLDL. Feeding triolein changed the specific activities of more of the lecithin species of VLDL than of HDL. The specific activities of lecithins in CM were more similar to VLDL than to HDL after triolein feeding. Results thus indicate that, although the lecithins of different mesenteric lymph lipoproteins are similar and may be derived from membrane sites with the same lecithin composition, lecithins incorporated into different lipoproteins originate from different metabolic pools and/or by different mechanisms.

### Introduction

During the multistep process of dietary lipid absorption, the phospholipid, lecithin, plays a prominent role: first in the intestinal lumen as a solubilizer of less polar lipids and, thereafter, within the intestinal cell as a major structural component of newly synthesized lipoproteins. Ordinarily, bile provides the bulk of the lecithin that is used within the intestinal lumen. However, the lecithin that is used within the cell for lipoprotein formation may be derived from a variety of sources including (a) bile lecithin, which is absorbed as lysolecithin and then reacylated to lecithin with the mucosal cell; (b) lecithin that is synthesized *de novo* by the intestine; and (c) preformed intestinal lecithin, which may be transferred from intracellular membranes or from the blood that is perfusing the intestine.

It has now been well established that the intestine synthesizes apoproteins and secretes into the mesenteric lymph newly formed high density lipoproteins (HDL), very low density lipoproteins

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(VLDL), and, after a lipid meal, chylomicrons (CM)<sup>1</sup> (1). Thus far, attempts to define the sources of lecithin in lipoproteins derived from the intestine have focused exclusively on CM. Evidence to date suggests that lecithin absorbed from the lumen of the intestine is preferentially used for CM-lecithin formation rather than lecithin that is synthesized *de novo* (2), and that the fatty acid composition of the lecithin of CM can be changed to resemble more closely the fatty acid composition of any absorbed lipid, be it bile lecithin or some specific triglyceride (3–5). However, even when a single triglyceride species is absorbed, a multiplicity of fatty acids (from, in addition, mucosal lipids) may be generated and combine in a vast number of permutations to form different molecular species of lecithin. Different molecular species of lecithin are synthesized by different mechanisms (6, 7) and have different rates of turnover (7, 8). Since different molecular species may have differing capacities to solubilize other lipids (9, 10), the particular molecular species composition of the lecithins that are components of lipoproteins may influence the overall biologic and physical properties of these lipoproteins.

To our knowledge, the molecular species composition of the lecithin (or any other phospholipid) component of newly synthesized lipoproteins has never been determined. In the present study, using newly developed high-performance liquid chromatography (HPLC) methods (11), we have determined (a) the composition of the lecithins of mesenteric lymph HDL, VLDL, and CM that represent nascent lipoproteins formed by the intestine; (b) the effect of bile lecithin on the molecular species of lecithin of all intestinal lymph lipoproteins; (c) the effect of specific triglyceride feeding on the molecular species composition of lymph lipoprotein lecithins; and (d) the relative rates of use of specific lecithins that are absorbed by the intestine in the formation of different nascent intestinal lipoproteins.

## Methods

**Study design.** Male Sprague-Dawley rats were used in all experiments. Rats were fed Purina chow (Ralston Purina Co., St. Louis, MO) ad lib. and weighed 250–300 g at the time of study. Four different series of experiments were performed in which the molecular species of lecithins of intestinal lymph lipoproteins were determined: (a) when rats were fasting and when bile lecithin was either present or eliminated by biliary diversion, (b) when rats were fed triglyceride (triolein) and when bile lecithin was either present or eliminated, (c) when rats with bile lecithin present were fed three different triglycerides (triolein, trilinolein, or a mixture of triolein and trilinolein), (d) when lecithins, radiolabeled in the choline moiety, were continuously infused into the duodenum of rats that were initially fasted and were then fed triolein.

Using pentobarbital for anesthesia (45 mg/kg, i.p.), surgery was performed to cannulate the mesenteric lymph duct (12), to cannulate the duodenum for infusions, and when indicated, to cannulate the stomach for triglyceride instillation and the common bile duct for elimination

of biliary lecithin from the intestinal lumen. After surgery, the animals were restrained in individual cages and allowed to recover for 24 h. During the recovery period food was withheld, but a continuous duodenal infusion of 0.85% NaCl-0.03% KCl was administered at 2.5 ml/h to ensure adequate lymph output. Lymph was then collected for the next 16–20 h while the animals continued to fast, and for 6 h (experiments 2 and 4) or for 4 h (experiment 3) after triglyceride was administered. It has previously been shown that prolonged fasting (here 40–44 h) does not adversely affect lipid transport into intestinal lymph (13), and is indeed desirable in order to avoid lymph collections during the first 24 h after bile diversion, when lipid transport is depressed (14).

All lymph was collected at 0°C in tubes that contained (final concentration) EDTA (2.5 mM), Na azide (7.7 mM), and, to inhibit lecithin-cholesterol acyl transferase, 5,5'-dithiobis-2-dinitrobenzoic acid (DTNB) (2.5 mM). When radiolabeled lecithin was administered (experiment 4), this was given as a continuous infusion into the duodenum. The radiolabeled infusate was prepared by infusing into the portal vein of bile duct-cannulated rats ~70  $\mu$ Ci of [1,2-<sup>14</sup>C]choline chloride (14.6  $\mu$ Ci/ $\mu$ mol) and sodium taurocholate (12.5 mM) at 2.4 ml/h for 48 h. Bile was collected during the entire period of isotopic choline administration while bile lecithin output was sustained by the constant infusion of taurocholate. The radiolabeled bile lecithins were isolated (together with bile cholesterol) by extraction with chloroform-methanol (2:1). The extract was dried and stored under N<sub>2</sub> until it was resolubilized in taurocholate for subsequent infusions.

Triglycerides, in amounts of 1.0 ml in experiments 2 and 4 (triolein) and 0.4 ml in experiment 3 (triolein, trilinolein, or an equal mixture of triolein and trilinolein) were given as a bolus directly into the stomach.

At the conclusion of lymph collections, animals were killed by an overdose of pentobarbital (intraperitoneal) and the intestinal lumen was thoroughly washed. The mucosa of the proximal half of the intestine was removed by scraping and the lipids were extracted.

**Isolation of lymph lipoproteins.** CM were separated from the rest of the lymph lipoproteins by flotation at  $3 \times 10^6$  g-min at 4°C in a swinging bucket rotor (SW 41) (Beckman Instruments, Inc., Fullerton, CA). The top creamy layer was obtained by tube slicing and was immediately extracted with chloroform-methanol (2:1). VLDL and/or small CM in the CM infranate were separated from HDL by precipitation with dextran sulfate 500-Mg<sup>+</sup>2 (2 M) at 0°C (15). The polyanions were added to the entire sample of whole lymph from fasting animals and to the remaining lymph after CM removal from fed animals. HDL, recovered in the supernate after polyanion precipitation, were concentrated by lyophilization and extracted in chloroform-methanol (2:1) while VLDL, in the precipitate, were extracted directly.

**Analytical procedures.** Lipids were extracted in chloroform-methanol (2:1) and partitioned according to Folch et al. (16). The lecithin fraction was first separated from neutral lipids and other phospholipids and then separated into individual molecular species by previously published HPLC procedures (11). Phospholipids and individual lecithin species were quantitated by phosphorus measurement, as previously described (11). When radiolabeled lecithins were present, each collected fraction was divided in two for liquid scintillation counting and for phosphorus determination.

**Presentation of data.** In all lipoprotein and mucosal samples, 25–30 molecular species of lecithin were separately quantitated with recoveries of  $101.6 \pm 3.4\%$  (SD) for mucosa ( $n = 13$ ) and  $102.3 \pm 4.9\%$  for lipoprotein samples ( $n = 31$ ). Lecithin compositions are presented as percentages and, for simplicity, only those molecular species are shown that regularly constituted  $\geq 1.5\%$  of the samples. Means were compared for statistical significance only for data not expressed as percentages, by

1. **Abbreviations used in this paper:** CM, chylomicrons; DTNB, 5,5'-dithiobis-2-dinitrobenzoic acid; HDL, high density lipoprotein; HPLC, high-performance liquid chromatography; VLDL, very low density lipoprotein.

Table I. Total Phospholipid Output in Mesenteric Lymph of Fasted and Fed Rats with Intact Bile Circulation or Bile Diversion

|                                  | Bile phospholipid present |           |          | Bile phospholipid absent |           |          |
|----------------------------------|---------------------------|-----------|----------|--------------------------|-----------|----------|
|                                  | HDL                       | VLDL      | CM       | HDL                      | VLDL      | CM       |
| <i>μmol phospholipid in 24 h</i> |                           |           |          |                          |           |          |
| Fasted                           | 1.0±0.2                   | 45.3±2.7  | —        | 1.5±0.4                  | 23.2±1.6* | —        |
| Fed triolein                     | 5.3±1.2‡                  | 24.4±1.2‡ | 49.1±6.0 | 6.0±1.0‡                 | 22.6±1.0  | 41.5±1.0 |

In fasted rats, lymph was collected for 16–20 h, beginning 24 h after lymph (and bile) cannulation. Triolein was administered as a gastric bolus and lymph was then collected for an additional 6 h. Data shown as mean±SE for 3–6 rats, normalized for a 24-h collection period.

\* Significantly different from Bile phospholipid present,  $P < 0.001$ .

‡ Significantly different from Fasted,  $P < 0.01$ .

either Student's *t* test for unpaired variables (Table I) or by analysis of variance, using Newman-Keuls post hoc *t* tests (Fig. 3).

## Results

The output in lymph of total lipoprotein phospholipids is shown in Table I. In fasting animals, phospholipid output was significantly reduced by biliary diversion because of an almost 50% reduction in the phospholipids of VLDL. The output of HDL phospholipids, which ranged from 1.0% (bile phospholipid present) to 1.5% (bile phospholipid eliminated) of the total lymph phospholipids, was unchanged by biliary diversion. After triglyceride administration, total lymph phospholipid output was increased which, in great part, was due to the appearance of CM but also to at least a fivefold elevation in HDL phospholipid secretion. In contrast to fasting, in triglyceride-fed rats, biliary diversion resulted in no changes in phospholipid secretion in any lipoprotein class.

The predominant phospholipid in all lipoproteins was lecithin, which accounted for the same percentage of total phospholipids in each lipoprotein fraction in fasted and fed rats, whether bile phospholipid was present or was eliminated. Lecithin accounted for 82.8±2.8% (SD;  $n = 12$ ) of the total phospholipid in HDL, 81.9±1.7% ( $n = 12$ ) in VLDL, and 83.4±0.6% ( $n = 6$ ) in CM.

*Molecular species of lecithins in bile, intestinal mucosa, and lymph lipoproteins in fasted rats.* In Table II, the molecular species composition of lecithins of bile, intestinal mucosa, and intestinal lymph lipoproteins is shown for fasting rats. Lecithins, which regularly comprise more than 96% of the total phospholipids in rat bile, are distinct in bile for the marked predominance of 16:0-species. Indeed, four 16:0-species comprise ~70% of the total lecithins present in bile. The effect of bile lecithins present in the lumen on the lecithin composition of the intestine and intestinal lipoproteins is clearly evident when bile lecithins are eliminated. As a result of biliary diversion, there was a marked decrease in the relative amounts of the two major 16:0-species (16:0-20:4 and 16:0-18:2) in conjunction with a relative increase in most of the 18:0-species, especially 18:0-18:2 lecithin.

However, whether bile lecithins were present or absent, the lecithin composition of HDL and VLDL from the same samples of lymph were extremely similar, both to each other and to the intestinal mucosa.

*Molecular species composition of lecithins in lymph lipoproteins after triglyceride feeding.* The effect of triolein administration on the lecithin composition of lipoproteins is shown in Table III. Again, the composition of all lipoproteins that were isolated from the same samples, now including a CM fraction, contained similar proportions of individual lecithins (with one exception, as discussed below). However, in contrast to the results in fasting animals (Table II), after triglyceride feeding the distributions of lecithins in lipoproteins obtained with and without bile lecithins present were similar. As expected, there was an increase in the major 18:1-containing lecithins after feeding the pure 18:1 triglyceride. However, an unanticipated finding was the formation of a large amount of 18:1-18:1 lecithin, which constituted <0.1% of the lecithins in bile and only 0.2–0.5% of the lecithins in the lymph and mucosa of fasting animals. In addition, for this single lecithin alone, there was a distinct difference in amounts between lipoproteins. CM were much more enriched in 18:1-18:1 lecithin than either HDL or VLDL. Mucosa, which generally resembled HDL and VLDL, contained 11.7±4.7% (SD) 18:1-18:1 lecithin with bile lecithins present and 15.6±2.1% with bile lecithins eliminated.

*Effect of absorption of different triglycerides on lipoprotein lecithin composition.* Studies were next performed to determine if absorption of other unsaturated triglycerides would also result in the formation of a relatively large percentage of lecithins containing the unsaturated acyl groups of the triglycerides in both position 1 and 2. Rats were thus fed equal volumes of triolein, trilinolein, or a 50–50 mixture of triolein and trilinolein and the lecithin composition of lymph lipoproteins was determined. Fig. 1 shows the change from fasting in the composition of the lecithins of VLDL after triglyceride administration. (Results are shown for VLDL only. Changes in HDL closely paralleled those of VLDL. Since CM were not present in the lymph of fasted animals, comparisons could not be made for CM.)

Table II. Composition of Lecithins in Bile, Intestinal Mucosa, and Mesenteric Lymph of Fasted Rats (Percent)

| Lecithin species | Lecithins in bile | Bile lecithin present |          |          | Bile lecithin absent |          |          |
|------------------|-------------------|-----------------------|----------|----------|----------------------|----------|----------|
|                  |                   | Mucosa                | VLDL     | HDL      | Mucosa               | VLDL     | HDL      |
| 16:0-22:6        | 4.3±0.6           | 1.2±0.2               | 1.5±0.1  | 1.6±0.2  | 1.6±0.3              | 1.8±0.3  | 2.9±0.6  |
| 16:0-20:4        | 16.1±0.6          | 22.6±1.0              | 20.1±0.9 | 17.5±0.7 | 6.1±0.2              | 6.4±0.2  | 7.6±0.5  |
| 16:0-18:2        | 40.9±1.9          | 21.5±0.6              | 26.3±0.5 | 25.2±0.4 | 15.8±0.1             | 13.5±0.8 | 15.8±1.1 |
| 16:0-18:1        | 8.8±0.3           | 6.0±0.3               | 3.4±0.2  | 4.5±0.3  | 9.3±0.4              | 5.7±0.4  | 5.9±0.3  |
| 18:0-22:6        | 1.3±0.2           | 1.8±0.2               | 1.9±0.2  | 2.2±0.2  | 3.5±0.2              | 3.6±0.1  | 4.8±0.3  |
| 18:0-20:4        | 5.5±0.5           | 14.0±1.1              | 12.3±0.5 | 11.3±0.7 | 10.5±0.3             | 15.0±1.0 | 14.7±1.1 |
| 18:0-18:2        | 7.9±0.4           | 11.5±1.1              | 14.7±0.6 | 13.8±0.7 | 22.0±1.2             | 22.6±1.0 | 18.2±1.6 |
| 18:0-18:1        | 0.4±0.1           | 1.7±0.4               | 1.8±0.1  | 1.5±0.2  | 3.6±0.1              | 3.6±0.4  | 2.2±0.5  |
| 18:1-18:2        | 2.4±0.3           | 3.5±0.1               | 3.9±0.2  | 5.5±0.4  | 6.9±0.2              | 7.0±0.5  | 6.5±0.7  |
| (18:2-18:1)*     |                   |                       |          |          |                      |          |          |
| Total‡           | 87.6±1.0          | 83.8±0.6              | 85.9±0.7 | 83.1±1.2 | 79.3±0.3             | 79.2±1.2 | 78.6±2.7 |

Bile lecithin was eliminated by bile diversion for the period of lymph collections. Bile lecithin composition was determined in samples taken during the first 24 h of bile diversion. Lymph was collected in EDTA, Na azide, and DTNB at 0°C for 16–20 h, beginning 24 h after lymph duct cannulation. Lipoproteins were separated by polyanion precipitation (see Methods). Mucosa was obtained from the proximal half of the small intestine at the conclusion of lymph collections. Data are the mean±SE for three to six animals. Only molecular species that comprised ≥1.5% of the lymph samples are listed.

\* The specific position of the fatty acids in this lecithin was not determined.

‡ Represents the total of the molecular species that are listed.

Table III. Effect of Triglyceride Absorption on the Composition of Lecithins in Intestinal Lymph Lipoproteins (Percent)

| Lecithin species | Bile lecithin present |          |          | Bile lecithin absent |          |          |
|------------------|-----------------------|----------|----------|----------------------|----------|----------|
|                  | CM                    | VLDL     | HDL      | CM                   | VLDL     | HDL      |
| 16:0-22:6        | 1.7±0.2               | 2.5±0.4  | 3.3±0.4  | 1.4±0.1              | 2.4±0.1  | 4.2±0.2  |
| 16:0-20:4        | 5.3±0.5               | 7.8±0.3  | 7.7±0.4  | 3.4±0.2              | 4.8±0.2  | 6.2±0.3  |
| 16:0-18:2        | 11.8±0.4              | 16.1±1.1 | 14.8±0.5 | 9.8±0.4              | 12.2±0.4 | 13.3±0.6 |
| 16:0-18:1        | 6.2±0.6               | 6.4±0.3  | 6.6±0.1  | 6.0±0.3              | 6.1±0.4  | 5.9±0.2  |
| 18:0-20:4        | 10.5±0.9              | 12.3±0.2 | 12.4±0.4 | 10.4±0.9             | 12.4±0.2 | 14.1±0.7 |
| 18:0-18:2        | 14.1±0.8              | 15.7±1.3 | 13.2±1.1 | 17.7±0.6             | 18.2±0.9 | 14.2±0.7 |
| 18:0-18:1        | 4.8±0.6               | 3.1±0.5  | 3.1±0.2  | 5.1±0.2              | 3.4±0.6  | 2.4±0.5  |
| 18:1-18:2        | 8.1±0.2               | 6.8±0.2  | 7.3±0.3  | 9.5±0.2              | 8.0±0.3  | 7.7±0.1  |
| (18:2-18:1)      |                       |          |          |                      |          |          |
| 18:1-18:1        | 22.9±1.4              | 13.3±0.9 | 15.8±1.8 | 22.8±0.8             | 15.6±0.7 | 14.9±0.6 |
| Total*           | 85.4±0.5              | 84.0±2.0 | 84.2±1.4 | 86.1±1.0             | 83.1±1.8 | 82.9±1.5 |

Lymph was collected in EDTA, Na azide, and DTNB at 0°C for 6 h after triolein was administered, and lipoproteins were isolated as described (Methods). Data are the mean±SE for three to four rats. Only molecular species that comprise ≥1.5% of the samples are listed.

\* See footnote to Table II.

It is apparent that feeding triolein (containing only 18:1 fatty acids) produced an increase in all 18:1-containing lecithins, feeding trilinolein (containing only 18:2 fatty acids) produced an increase in all 18:2-containing lecithins, and feeding triglycerides with both 18:1 and 18:2 fatty acids resulted in an increase in both 18:1- and 18:2-lecithins. In all cases, after triglyceride feeding 16:0-20:4 lecithin was again more prominently decreased than any other lecithin species. The most notable change, however, was in the magnitude of the increase in the diunsaturated lecithins that contained the same component fatty acids as the triglycerides that were fed. The increase in diunsaturates far exceeded any increase in monounsaturates that contained the same unsaturated fatty acid. This preferential increase in diunsaturated lecithins was particularly notable when a combination of the two triglycerides was fed. In this case, not only were both 18:1-18:1 and 18:2-18:2 lecithins increased in relatively large amounts, but 18:1-18:2 (or 18:2-18:1) lecithin was increased in amounts that clearly exceeded the marginal changes in any of the monounsaturated lecithins (containing either 18:1 or 18:2).

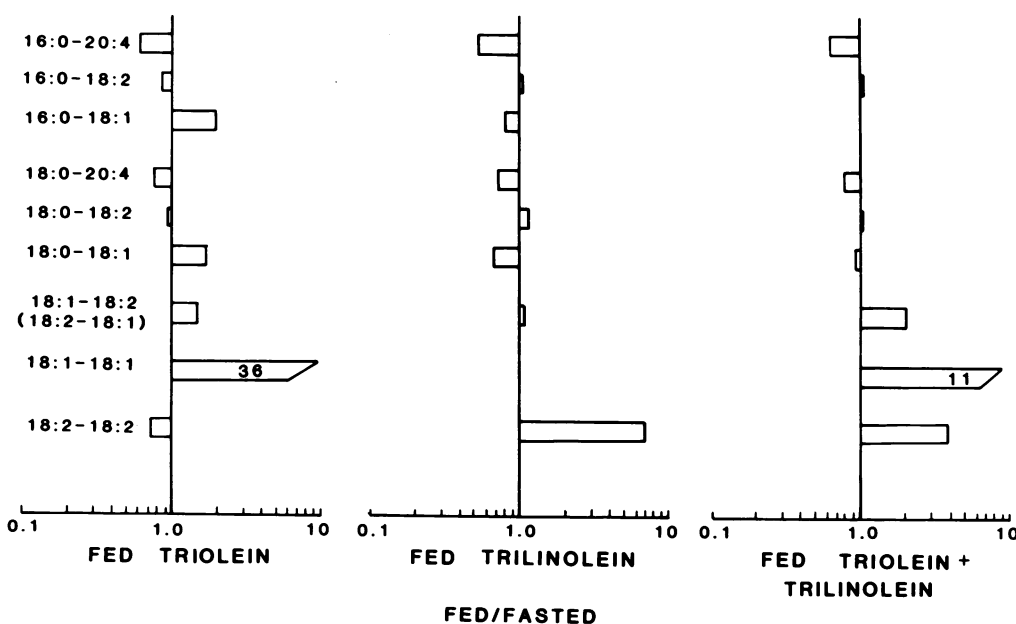
**Lecithin turnover in intestinal lipoproteins.** To compare the relative rates of utilization of individual bile lecithins in the synthesis of intestinal lipoproteins, bile lipids containing [ $^{14}$ C]choline-labeled lecithins were continuously infused into the duodenum of bile-diverted rats with mesenteric lymph cannula. Lymph was first collected when rats were fasting and subsequently after gastric administration of triolein. Recovery of infused radioactivity in lymph during the period of fasting averaged  $16.5 \pm 3.7\%$  (SD) and rose to  $28.9 \pm 4.2\%$  after triolein.

As illustrated in Fig. 2, there was an extensive change in the distribution of radioactivity in the lecithins of the infusate after these lecithins were absorbed and incorporated into lymph

lipoproteins. Lecithin radioactivity was 1.0% or greater in seven molecular species of the infusate which together accounted for 88.9% of the total infusate radioactivity. After lecithin absorption (which involves hydrolysis) and lecithin resynthesis, radioactivity recovered in lymph lipoproteins was distributed differently among lecithin species, but was still 1.0% or greater in only seven or eight fractions, which together accounted for 87.4–92.5% of the total radioactivity in any lipoprotein sample.

Compared with infusate (Fig. 2), the percentage of radioactivity in 16:0-18:2 and 16:0-18:1 lecithins (which together comprised nearly 70% of the infusate radioactivity) was markedly decreased in all lymph lipoproteins, and the percentage of radioactivity in all 18:0-lecithins was increased. For each molecular species of lecithin, the percentage of radioactivity was essentially the same in all lipoproteins that were isolated from the same lymph sample. However, in some lecithin fractions, the percent distribution of radioactivity was changed after fasted animals were fed triolein. This was especially true for 18:1-18:1 lecithin, which was not radiolabeled in fasted rats but accounted for 15–19% of the lecithin radioactivity after rats were fed.

In Fig. 3, specific activities of six of these individual lecithin species that were incorporated into lymph lipoproteins are shown relative to infusate specific activities for fasted and triolein-fed rats. The specific activities of all lecithins in all lipoprotein classes decreased after infusate lecithin absorption and lecithin reutilization for lipoprotein synthesis. The decline in specific activity was highly variable for individual molecular species and reflects variable utilization of nonradioactive choline-containing molecules for lipoprotein-lecithin resynthesis. The decrease in specific activity was greatest, both in HDL and VLDL, for 16:0-18:1 (to 4 and 10% of infusate specific activity) and least for 16:0-20:4 (to 43 and 79% of infusate activity). Assuming that each



**Figure 1.** Changes in lecithin composition of lymph VLDL as a result of feeding different triglycerides. Triolein, trilinolein, or a 50–50 mixture of triolein and trilinolein were administered as a gastric bolus in a 0.4 ml vol to two rats. Lymph was collected for 4 h and lipoproteins were isolated as described in Methods. The change from fasting in the composition of the major lecithins of VLDL is shown on a log scale, using for this calculation the VLDL data from fasting animals with bile lecithin present (shown in Table II). Numbers within the bars represent values too large to be shown on scale.

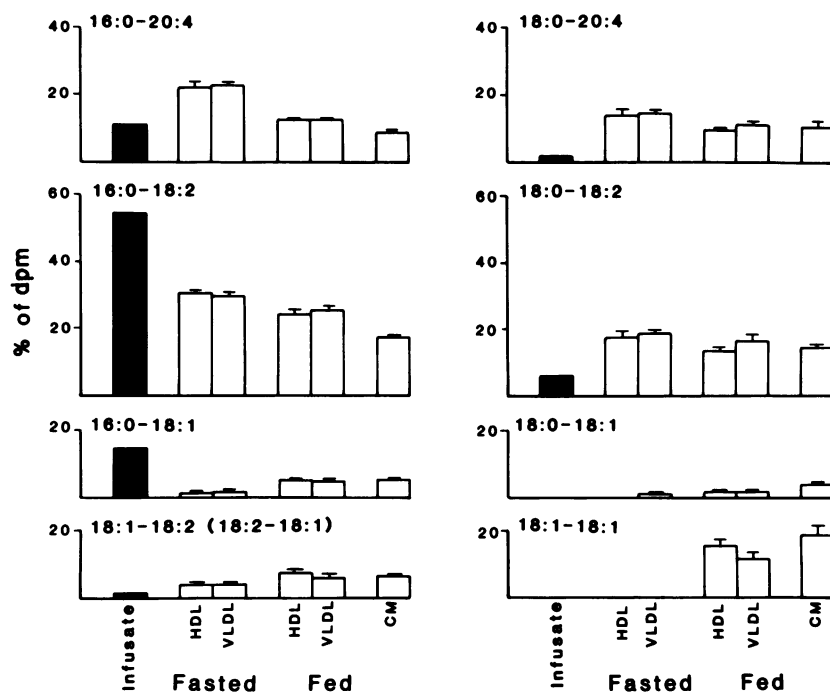


Figure 2. The distribution of radioactivity in major lecithins of the infusate and lymph lipoproteins. Radiolabeled lecithins were solubilized in sodium taurocholate and continuously infused into the duodenum of rats with bile duct and mesenteric lymph duct cannulas. Lecithins were infused at  $2.6 \mu\text{mol/h}$  and taurocholate at  $36.6 \mu\text{mol/h}$  while lymph was collected, first when rats were fasting and then after a gastric bolus of triolein. Lymph lipoproteins and molecular species of lecithin were isolated as described in Methods. Data shown as mean  $\pm$  SE for three to four rats.

molecular species of infusate lecithin was hydrolyzed to the same extent (partially to lysolecithin or more completely to glycerophosphorylcholine, phosphorylcholine, or choline) before resynthesis and incorporation into HDL and VLDL, it is notable that the relative utilization of nonradioactive choline for every molecular species of lecithin synthesized was considerably greater for HDL than VLDL (isolated from fasted as well as fed rats).

As a result of triolein absorption, there was a further decrease in the specific activity of lecithin molecular species that did not contain 18:1 and an increase or no change in the species that contained 18:1. Where a change in specific activity occurred with triolein feeding, the change was significantly greater for VLDL than for HDL in four (of six) species, whereas in only one species (18:0-18:1) was the change in HDL greater than in VLDL. After triolein, the specific activities of the lecithins of CM were very similar to VLDL, except in one instance (18:0-18:1). In contrast, the specific activities of the lecithins in HDL after triolein were significantly less than in CM in four instances (16:0-18:1, 18:0-20:4, 18:0-18:2, and 18:0-18:1 lecithins). Thus, while the decrease in specific activity from infusate was initially greatest for the lecithins of HDL, when animals were fasting, specific activities of HDL-lecithins were changed least by feeding and were, in the main, distinct from VLDL and CM, which were similar to each other.

## Discussion

The phospholipid composition of all plasma lipoproteins consists predominantly of lecithin (17). The fatty acid composition of the lecithins of all lipoproteins is virtually identical when li-

poproteins are isolated from the same samples of plasma (18). Furthermore, in a single study (19), when the molecular species composition of lecithins were analyzed, these too were found to be essentially the same in all plasma lipoproteins. In view of the well-known propensity for phospholipids to be both transferred and exchanged between lipoproteins in the plasma, we have undertaken the present study to determine, for the first time, the composition of lecithins of nascent lipoproteins before they enter the systemic circulation. This study was made possible by our recent development of HPLC methods which permit direct separation, with quantitative recoveries, of a multiplicity of naturally occurring molecular species of phospholipids (11).

Our studies show that (a) the molecular species composition of the lecithins of mesenteric lymph lipoproteins are similar to each other and to the mucosa from which these lipoproteins derive; (b) the composition of molecular species of lecithin of all intestinal lipoproteins can be greatly changed by changing the fatty acid composition of the glycerides that are being absorbed by the intestine; (c) the extent to which specific lecithins are newly synthesized depends largely on the available pool size of fatty acids (and, consequently, lecithins with unsaturated fatty acids in both 1 and 2 positions are preferentially synthesized rather than lecithins containing one unsaturated fatty acid when a triglyceride consisting exclusively of unsaturated fatty acids is fed); (d) the utilization of absorbed lecithins for lipoprotein formation differs for different molecular species of lecithin, the utilization of all major molecular species of lecithin differs for HDL and VLDL and, after triglyceride feeding, the utilization of specific lecithins for VLDL and CM is similar but differs from HDL.

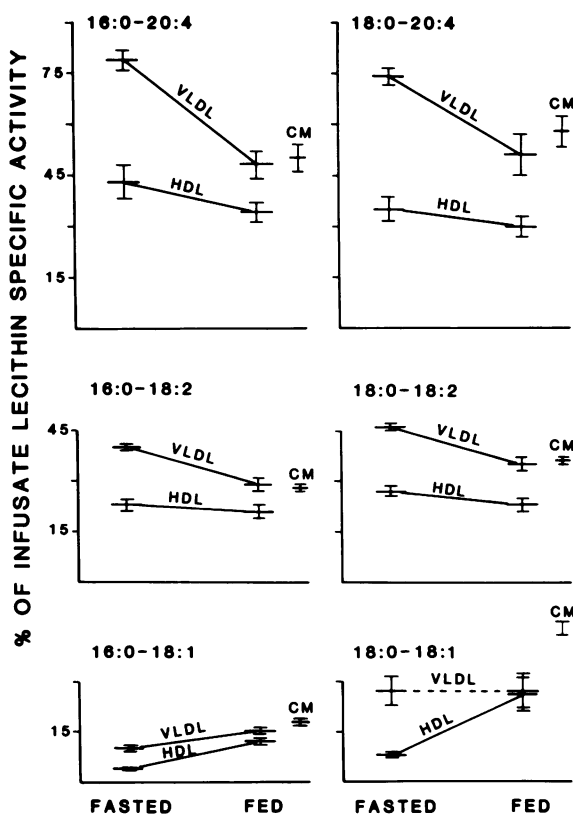


Figure 3. Changes in specific activities of six major molecular species of lecithin that were absorbed from the intestine and reutilized for intestinal lipoprotein formation. The changes are shown as a percentage of the infusate specific activities ( $\pm$ SE), first during fasting and then after feeding triolein. Infusate lecithins were radiolabeled with choline (see Methods) and continuously infused for the period of lymph collection. HDL and VLDL were isolated by polyanion precipitation and CM by ultracentrifugation (see Methods). Differences between lipoproteins were significant for (a) fasted vs. fasted conditions: HDL vs. VLDL—all lecithins; (b) fasted vs. fed conditions: HDL vs. HDL—16:0-18:1, 18:0-18:1 lecithins; VLDL vs. VLDL—16:0-20:4, 16:0-18:2, 16:0-18:1, 18:0-20:4, 18:0-18:2 lecithins; (c) fed vs. fed conditions: HDL vs. VLDL—16:0-18:1, 18:0-20:4, 18:0-18:2 lecithins; HDL vs. CM—16:0-18:1, 18:0-20:4, 18:0-18:2, 18:0-18:1 lecithins; VLDL vs. CM—16:0-18:1 lecithin.

We know of no data to suggest the presence of phospholipid exchange proteins in intestinal lymph. Furthermore, in other experiments we have incubated lymph CM with HDL at various temperatures and have found no significant exchange at 0°C (unpublished data). Therefore, lymph was collected at 0°C and lipoproteins were separated as rapidly as possible using a polyanion precipitation method. It is of course possible that phospholipids were exchanged between lipoproteins during their assembly and transport within the intestinal cell, before secretion into lymph. Although our data cannot exclude this possibility, significant exchange would appear to be unlikely in view of the

observed differences in the specific activities of individual lecithins of the lipoproteins isolated from lymph (experiment 4).

Our first series of experiments, which demonstrate that in fasting rats lymph, VLDL, HDL, and intestinal mucosa have the same lecithin composition, suggest that different intestinal lipoproteins derive their lecithins from pools of similar lecithin composition (whether from the same or different intracellular membranes) and that bile lecithin makes a major impact on the lecithin composition of intestinal lipoproteins. Very clearly, the elimination of bile lecithin that is exceedingly rich in 16:0-species results in a profound decrease in the 16:0-species of mucosa and lymph lipoproteins. In part, it seems probable that the change in lecithin composition is simply secondary to a change in the relative amount of available lecithins with particular acyl groups. Nevertheless, an explanation for the specific composition observed in these or any other experiment in this study is undoubtedly more complex. Lecithin that is absorbed from the intestinal lumen is extensively hydrolyzed (20–22). Lecithin hydrolysis as well as lecithin resynthesis may be preferentially more active or complete for particular lecithin species. Both reactions may be additionally subject to change when triglycerides are also being absorbed. However, such an explanation must, at this time, be purely speculative, since we know of no study in which rates of hydrolysis or synthesis of specific lecithins have been compared.

As a result of triolein feeding, CM were generated with lecithin molecular species that were especially rich in 18:1 fatty acid and that were very similar to the lecithins of VLDL and HDL isolated from the same lymph sample (with the single exception of 18:1-18:1 lecithin, as indicated earlier). For as yet some unexplained reason, even with bile lecithins present, triolein feeding was associated with a profound decrease in 16:0-20:4 lecithin, compared with fasting animals. This same change occurred when either trilinolein or the mixture of triglycerides was fed.

Triolein feeding also resulted in the formation of a relatively large amount of 18:1-18:1 lecithin, confirming an observation previously made by Arvidson and Nilsson (5). Since this lecithin was present in exceedingly small amounts in fasted animals, its formation after feeding was probably the result of *de novo* synthesis in which 18:1-18:1 diglyceride was used as a substrate (i.e., in the CDP-choline pathway). Furthermore, since we have also demonstrated (experiment 4) that this particular lecithin was newly radiolabeled after choline-labeled lecithin was infused in conjunction with triolein feeding, it is clear that to at least some extent the choline that was used for lecithin synthesis was derived from absorbed lecithin that had to be almost completely degraded (to choline or phosphorylcholine). This finding is consistent with previous work (23) which demonstrated that bile lecithin can be extensively hydrolyzed during absorption with loss of a fatty acid at not only the 2-position (predominantly within the intestinal lumen) but also at the more stable 1-position, within the intestinal cell. Feeding trilinolein or the mixture of triglycerides produced results entirely analogous to feeding triolein.



If we assume that all (or nearly all) lecithins with 16:0 and 18:0 in position 1 were formed by reacylation of 1-lysoclecithin after its absorption from the intestinal lumen, it is clear that the bulk of the lecithins in lipoproteins were synthesized by simple reacylation (Table III). This would conform with a previous observation (2), in which the total CM lecithin formed by reacylation of absorbed, radiolabeled lysoclecithin was measured. It is also clear from Table III, assuming that the increment in diunsaturated lecithins represents lecithins that are synthesized *de novo*, that *de novo* synthesis can be responsible for at least 20% of the lecithins of intestinal lipoproteins and is responsible for the greatest percentage change in lipoprotein lecithin composition after triglyceride feeding (Fig. 1).

In a fourth series of experiments, we determined the extent to which absorbed, choline-labeled bile lecithins were used for intestinal lipoprotein formation. We observed large initial differences in the specific activity of individual lecithins in lipoproteins as well as large and variable changes in specific activities as a result of triglyceride absorption. Obviously, different lecithins are metabolized to different extents by the intestine, possibly because of differing rates of hydrolysis and/or synthesis of individual lecithins. Since these processes were not specifically examined in our present study, we cannot provide a full explanation for the specific activity changes that were observed. However, it is very clear that individual lecithins of the same composition have different specific activities in different lymph lipoproteins that have been isolated from the same animals. Therefore, although the overall lecithin composition of different lipoproteins may be quite uniform, utilization of absorbed, individual lecithins for the formation of specific nascent intestinal lipoproteins is distinctly different.

A clear pattern of specific activity changes was observed: (a) The specific activity of lecithins in lymph HDL was consistently less than in VLDL when animals were fasted. This would indicate that proportionately less of the lecithin that was absorbed was incorporated into HDL than into VLDL. Alternatively, specific activity differences could reflect greater "dilution" of HDL lecithins than VLDL lecithins by the transfer from the plasma into lymph of nonradiolabeled lecithins, either as hydrolyzed portions of plasma lecithins or as single whole plasma lecithin molecules. Although some apoproteins (24, 25) and cholesterol (26) of plasma lipoproteins may be directly transferred into lymph, there is no evidence that plasma lecithin molecules are similarly transferred. However, even if this were the case, and the differences in specific activities of lymph HDL and VLDL could be attributed to disproportionately greater transfer of single lecithins from plasma HDL than from VLDL, the mechanism of formation of different intestinal lipoproteins would still be different. Only if whole, intact plasma HDL (containing nonradiolabeled lecithins) were directly filtered into lymph could the observed differences in specific activities between lymph HDL and VLDL be considered spurious or, at least, less compatible with our conception of different pools and/or different mechanisms of assembly for HDL and other nascent intestinal lipoproteins. We know of no data which would

Table IV. Comparison of Lecithin Composition of Plasma HDL and Lymph Lipoproteins of Fasted Rats

| Lecithin species | Plasma HDL | Lymph HDL | Lymph VLDL |
|------------------|------------|-----------|------------|
| 16:0-22:6        | 8.7±1.3    | 1.6±0.2   | 1.5±0.1    |
| 16:0-20:4        | 10.0±0.6   | 17.5±0.7  | 20.1±0.9   |
| 16:0-18:2        | 16.6±1.0   | 25.2±0.4  | 26.3±0.5   |
| 16:0-18:1        | 6.9±0.7    | 4.5±0.3   | 3.4±0.2    |
| 18:0-22:6        | 8.0±1.7    | 2.2±0.2   | 1.9±0.2    |
| 18:0-20:4        | 16.8±0.5   | 11.3±0.7  | 12.3±0.5   |
| 18:0-18:2        | 11.5±2.0   | 13.8±0.7  | 14.7±0.6   |
| 18:0-18:1        | 2.3±0.3    | 1.5±0.2   | 1.8±0.1    |
| 18:1-18:2        | 2.8±0.4    | 5.5±0.4   | 3.9±0.2    |
| Total*           | 83.6±1.0   | 83.1±1.2  | 85.9±0.7   |

Plasma samples were obtained from three lymph-diverted rats with bile lecithin present and analyzed as described for lymph samples. For comparison, the lecithin composition of lymph HDL and VLDL from these same rats are shown (data taken from Table II). Results are shown as mean percent±SE for the major lecithin species.

\* See footnote to Table II.

suggest that intact lipoproteins are filtered from plasma into lymph. To specifically examine this issue would require that several different components of a plasma lipoprotein be radiolabeled and isotope ratios be determined before and after re-isolation of this same lipoprotein from lymph. However, although these experiments have not been performed, we can calculate from the distribution of lecithins in lymph HDL and plasma HDL (obtained from lymph-diverted animals at the conclusion of several of our experiments) (Table IV) and the magnitude of the differences in specific activities between lymph HDL and VLDL lecithins that plasma HDL is almost certainly not transferred as an intact particle directly into lymph.<sup>2</sup>

(b) The specific activities of lecithins in HDL were less changed by feeding triglycerides than were the specific activities of lecithins in VLDL, which, after triglyceride, closely resembled

2. The percent composition of the lecithins of plasma HDL is shown in Table IV in conjunction with the composition of lymph HDL and VLDL (data taken from Table II). It is apparent that the compositions of lymph HDL and VLDL are extremely similar to each other, while the composition of plasma HDL is very different than either lymph lipoprotein. (The composition of plasma VLDL was very similar to plasma HDL and is not shown.) If intact plasma HDL were filtered into lymph to dilute lymph HDL and to account for the significant differences in specific activities of the lecithins of lymph HDL and VLDL (from 40 to 60% differences as shown in Fig. 1), the lecithin composition of lymph HDL should not so closely approximate lymph VLDL. Instead, with significant filtration of plasma HDL into lymph, the composition of lymph HDL would be expected to be intermediate between plasma HDL and lymph VLDL, and this was certainly not the case.

the specific activities of the lecithins in CM. That the utilization of absorbed lecithins for VLDL and CM should be similar, but different from HDL, is not surprising since both VLDL and CM are involved in bulk triglyceride transport, whereas HDL is not. (Indeed, lymph VLDL may be considered merely small CM that function primarily to carry the relatively small amounts of triglyceride synthesized by the intestine of fasting animals. On the other hand, the difference in the percentage of 18:1-18:1 lecithin in CM and VLDL (or "small CM") suggests that these lipoproteins may not have identical origins in the intestinal mucosa. This suggestion is supported by observed differences in the fatty acid compositions of the triglycerides of mesenteric lymph CM and VLDL after feeding fatty acids (27) and the differential effect of the surfactant, Pluronic L-81 (P-L Biochemicals, Inc., Milwaukee, WI), on the secretion of CM and VLDL into mesenteric lymph [28].)

We believe that the demonstrated differences in the specific activities of lymph lipoprotein lecithins provide unequivocal evidence that HDL are assembled by a different mechanism or at a different membrane site in the intestine than CM and VLDL. Although to some extent differences in apoprotein patterns of lymph HDL and CM (or VLDL) also suggest different synthetic origins of these lipoproteins, large scale transfer of apoproteins between lipoproteins occurs which can obscure differences (29). Thus, although apoprotein B is a major component of rat lymph CM, it is probably also present in lymph HDL (30). Furthermore, all of the major apoproteins of rat lymph HDL (A-I, A-IV, and C), including some not synthesized by the mucosa (C apoproteins), are clearly also present in rat lymph CM (1).

Finally, in agreement with our studies of rat lymph HDL secretion in which the output of A apoproteins was measured (31-33), we found no change in HDL phospholipid output after bile diversion and an increase in HDL phospholipid output after triglyceride feeding. The most notable effect of bile diversion that we observed was an almost 50% reduction in VLDL phospholipid secretion in fasted animals. VLDL in fasted animals is responsible for the bulk of lymph triglyceride transport. It is therefore likely that the reduction in VLDL phospholipid when bile was eliminated simply reflects a decrease in the amount of fatty acids that derive from bile lecithin and that serve as substrates for intestinal triglyceride synthesis in the absence of dietary fatty acids.

In contrast to fasting, when triglyceride was fed we observed no decrease in the lymph output of either VLDL or CM phospholipid as a result of bile diversion. Although others have found lymph triglyceride output reduced as a result of biliary lecithin elimination, this has only been observed at sustained and maximum rates of intestinal lipid absorption and lymph triglyceride secretion (34, 35). With more variable, and sub-maximal, triglyceride transport which occurs when triglyceride is administered as a gastric bolus, the absence of bile lecithin probably is compensated for by an increase in *de novo* intestinal lecithin synthesis.

In future studies, using HPLC to separate intact molecular species, it will be possible to assess directly the contribution of

*de novo* synthesis of individual lecithins (with isotopic choline as a precursor) and the extent of hydrolysis and reacylation (with lecithins radiolabeled at several positions) of individual lecithins during the formation of intestinal lipoproteins.

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