The Lymph Lipid Precursor Pool Is a Key Determinant of Intestinal Lymphatic Drug Transport

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ABSTRACT

The influence of the size and turnover kinetics of the enterocyte-based lymph lipid precursor pool (LLPP) on intestinal lymphatic drug transport has been examined. Mesenteric lymph duct-cannulated rats were infused intraduodenally with low (2–5 mg/h) or high (20 mg/h) lipid-dose formulations containing 100 μg/h halofantrine (Hf, a model drug) and 1 μCi/h 14C-oleic acid (OA) (as a marker for lipid transport) until steady-state rates of lipid (dX/dt)ss and drug (dD/dt)ss transport in lymph were obtained. After 5 h, the infusion was changed to formulations of the same composition but excluding 14C-OA and Hf, allowing calculation of the first order rate constants describing turnover of lipid (KX) and drug (KD) from the LLPP into the lymph from the washout kinetics. The mass of lipid (XLP) and drug (DLP) in the LLPP was also determined. Biliary-lipid output was determined in a separate group of rats that had been infused with the same formulations. The results indicate that after administration of high lipid doses, lymphatic drug transport is dependent on the mass of exogenous lipid available in the LLPP and the rate of lipid pool turnover into the lymph. In contrast, after administration of low lipid doses, biliary-derived endogenous lipids are most likely to be the primary drivers of drug incorporation into the LLPP and lymph. Therefore, the LLPP size and composition seem to be major determinants of lymphatic drug transport, and formulation components, which increase lipid pool size, may therefore enhance lymphatic drug transport.

After oral administration, drugs are typically absorbed by the enterocytes lining the small intestine and are subsequently transported to the systemic circulation via the portal blood or mesenteric lymph. For the majority of drugs, transport to the systemic circulation occurs via the portal vein because the flow rate of portal blood is approximately 500-fold higher than that of mesenteric lymph (Bollman et al., 1948; Reininger and Sapirstein, 1957; Porter and Charman, 2001). However, significant lymphatic transport may occur when highly lipophilic drugs (log P > 5 and triglyceride (TG) solubility >50 mg/ml) are intercalated into lipid transport and lipoprotein formation processes in the enterocyte (Charman and Stella, 1986b, 1992). Lymphatic lipid and drug transport are closely related, and historically, lymphatic drug transport was believed to be marginal except when lymph lipid flux was increased by the administration of relatively large quantities of lipid (such as that contained in a high-fat meal). However, a recent study in our laboratory has demonstrated that substantial lymphatic drug transport may occur after administration of small quantities of lipid (i.e., that contained in a single capsule formulation) to fasted greyhound dogs (Khoo et al., 2003). This study reported that administration of a small exogenous lipid dose led to an increase in endogenous fatty acid (FA) uptake into the enterocyte and transfer into lymph, thereby providing the necessary lipid flux to support lymphatic drug transport. We have subsequently reported that different sources of endogenous FA may be recruited into the lymph and that biliary-derived endogenous FA seem to most effectively support lymphatic drug transport (Trevaskis et al., 2005). Moreover, the difference in the propensities of the different sources of endogenous FA to enhance lymphatic drug transport were proposed to reflect the way in which the endogenous FA was pooled within the enterocyte (Trevaskis et al., 2005).

Two separate intracellular lipid pools are present in the intestinal mucosa (Mansbach and Parthasarathy, 1982; Mansbach and Arnold, 1986; Tipton et al., 1989; Mansbach...
and Dowell, 1992; Nevin et al., 1995; Mansbach and Nevin, 1998). One pool is located in the smooth endoplasmic reticulum and Golgi and consists primarily of lipids derived from exogenous (e.g., dietary) sources. Lipids from this pool are predominately transported to the systemic circulation via the mesenteric lymph, and as such, this pool is referred to throughout this publication as the lymph lipid precursor pool (LLPP). The second pool of lipids (the portal lipid precursor pool) is located within the cytosol and comprises mostly endogenous lipids that enter the enterocyte via basolateral uptake from the intestinal blood. The lipids from the portal lipid precursor pool are generally transported from the enterocyte to the systemic circulation via the portal vein.

Relatively little is known of the process(es) by which drugs associate with developing lipoproteins in the enterocyte and how they are subsequently directed into the lymphatic capillaries. However, it seems likely that the size and nature of the different lipid pools residing in the enterocyte will influence the partitioning of highly lipophilic drugs between the lipoproteins destined for absorption via the lymphatic system and the lipids absorbed directly via the portal vein.

The current study, therefore, has examined the influence of the size and dynamics of the LLPP on the lymphatic transport of FA and drug after administration of a series of lipid formulations. This is the first study to examine the role of intracellular lipid trafficking pathways on lymphatic drug transport and has relevance to both an improved understanding of the mechanisms by which lipophilic drugs gain access to the lymph and to the design of delivery systems to more efficiently promote lymphatic drug transport.

### Materials and Methods

#### Materials

Halofantrine (1,3-dichloro-alpha-[2-(dibutylamino)ethyl]-6-(trifluoromethyl)-9-phenanthrenemethanol) base (Hf) (GlaxoSmithKline, Uxbridge, Middlesex, UK), Hf internal standard (2,4-dichloro-6-trifluoromethyl-9-[1-(2-dibutylamino)ethyl]phenanthrenemethanol HCl) (GlaxoSmithKline), oleic acid (1-Octyl) (PerkinElmer Life and Analytical Sciences, Boston, MA), oleic acid (OA), l-α-lysophosphatidylcholine (LPC), taurocholate (Sigma Chemical, Melbourne, Australia), Tween 80 (BDH, Melbourne, Australia), and normal saline for injection (Baxter, Sydney, Australia) were used as received. Acetonitrile, sodium dodecyl sulfate, and glacial acetic acid were of high-performance liquid chromatography grade. Water was obtained from a Milli Q (Millipore Corporation, Milford, MA) purification system. Hypnoveil (1 mg/ml midazolam) (Sigma Chemical) and Hypnorm (0.15 mg/ml fentanyl citrate; 10 mg/ml fluanisone) (Vet Drug, York, UK) were used for anesthesia. TG and phospholipid (PL) kits, control for automated systems, and Precinorm U (Roche Diagnostics, Indianapolis, IN) were used for analysis of triglyceride and phospholipid levels. Starlsinct (Packard Bioscience, Meriden, CT) liquid scintillation cocktail was used for liquid scintillation counting. All other chemicals were of analytical reagent grade.

#### Choice of Animal Model

The rationale underpinning the choice of an anesthetized rat model for the current studies has been described recently (Trevaskis et al., 2005). In brief, a rat model was chosen because it allows for comparison with a substantial historical data base describing the biochemistry of lipid absorption and intracellular processing that is not available in other animal species. An anesthetized (rather than conscious) rat model was employed to improve the chance of surgical success because it is easier to maintain the patency of the lymph duct cannula in anesthetized animals. However, anesthesia may reduce gastric emptying and the efficiency of intestinal lipid processing, thus potentially the extent of lymphatic drug transport. As such, animals were dosed intraduodenally to avoid the problem of delayed gastric emptying. Previous studies from our laboratory have shown that, although the intestinal lymphatic transport of Hf may be reduced after administration of simple lipid solution formulations to anesthetized rather than conscious rats, these differences may be circumvented by the intraduodenal administration of highly dispersed mixed micellar formulations (Raub et al., 1992; Porter et al., 1996a). Therefore, in the current study, anesthetized rats were dosed with highly dispersed mixed micellar solutions containing the predigested lipids OA and LPC to prevent complications associated with reduced intestinal processing and digestion and to avoid the issues of particle size dependence in lymphatic drug transport in anesthetized animals.

#### Composition of Lipid Formulations

The compositions of all of the administered formulations are given in Table 1. The formulations were prepared and emulsified, and their stability was assessed as described previously (Trevaskis et al., 2005). Particle-size analysis of these formulations was performed in triplicate using a Zetasizer 3000 equipped with a 5-milliwatt He-Ne laser at 633 nm (Malvern Instruments Ltd., Malvern, UK). Lipid doses of 2, 5, or 20 mg of OA/h (or 7.0, 17.7, and 70.8 μmol/h, respectively) were administered, because these lipid doses are approximately equivalent to the administration of 0.5 to 5 g of lipid/h to humans on a milligram/kilogram basis. Therefore, these lipid doses are relevant to the expected amount of lipid in a pharmaceutical formulation or a small quantity of food. The concentration of LPC in the formulations (5.2 mg/h or 10 μmol/h) was similar to the concentration of PC previously shown to enhance lymphatic lipid transport (Tso et al., 1978; Mansbach and Dowell, 1993; Nevin et al., 1995). Formulations were either dispersed in bile salt (BS) solution comprising 5 mM sodium taurocholate in phosphate buffer (pH 6.9), or they were dispersed in 0.2% (v/v) Tween 80 in normal saline (pH 7.0), where administration in the absence of BS was required. The rate of BS infusion was chosen to reflect the physiological rate of BS secretion into the rat intestine in the fasted state (Kararli, 1995).

### Table 1

Composition of the lipid formulations used for intraduodenal infusion to rats either continuously to steady state (A–G) or over 2 h (H–I) A through G represent the lipid formulation doses administered per hour, whereas H through I represent total doses. Formulations A through G also contained 1 μCi/h 14C-OA and 100 μg/h Hf. Formulations H through I contained 5 μCi of 14C-OA and 200 μg of Hf in total. Two milligrams, 5 mg, and 20 mg/h OA are equivalent to 7.0, 17.7, and 70.8 μmol/h, respectively.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Lipid Component</th>
<th>Aqueous Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>No lipid (control)</td>
<td>0.2% Tween 80 in normal saline</td>
</tr>
<tr>
<td>B</td>
<td>5 mg of OA</td>
<td>5 mM Sodium taurocholate in phosphate buffer, pH 6.9</td>
</tr>
<tr>
<td>C</td>
<td>2 mg of OA/5.2 mg of LPC</td>
<td>5 mM Sodium taurocholate in phosphate buffer, pH 6.9</td>
</tr>
<tr>
<td>D</td>
<td>5 mg of OA/5.2 mg of LPC</td>
<td>5 mM Sodium taurocholate in phosphate buffer, pH 6.9</td>
</tr>
<tr>
<td>E</td>
<td>20 mg of OA</td>
<td>0.2% Tween 80 in normal saline</td>
</tr>
<tr>
<td>F</td>
<td>20 mg of OA</td>
<td>5 mM Sodium taurocholate in phosphate buffer, pH 6.9</td>
</tr>
<tr>
<td>G</td>
<td>20 mg of OA/5.2 mg of LPC</td>
<td>5 mM Sodium taurocholate in phosphate buffer, pH 6.9</td>
</tr>
<tr>
<td>H</td>
<td>40 mg of OA</td>
<td>5 mM Sodium taurocholate in phosphate buffer, pH 6.9</td>
</tr>
<tr>
<td>I</td>
<td>40 mg of OA/10.4 mg of LPC</td>
<td>5 mM Sodium taurocholate in phosphate buffer, pH 6.9</td>
</tr>
</tbody>
</table>
Experimental Overview

The aim of the experiments was to determine the influence of changes to the size and turnover kinetics of the LLPP (which were induced by administering different lipid-based formulations) on lymphatic drug transport. After bolus administration of a lipid-based formulation, the size of the LLPP and its turnover kinetics are dynamic and cannot be quantified and/or compared between formulations. Therefore, in these studies, lipid-based formulations (OA was used as an FA source) were continuously infused into the duodenum of lymph-cannulated rats until steady-state rates of lipid and drug transport into lymph were achieved. Under these steady-state conditions, both the size and the turnover kinetics of the LLPP can be accurately measured and compared with the rate and extent of lymphatic drug transport. Specifically, the current studies have examined the impact of infusion of different lipid doses in the presence and absence of BS and LPC.

In a separate group of rats, formulations were similarly infused to steady state, after which the bile duct was cannulated to assess the impact of different formulations on biliary lipid secretion. These experiments were performed to determine whether increases in the size of the LLPP (which were observed under certain experimental conditions) reflected an increase in biliary lipid output leading to transport of biliary-derived lipids via the lymphatic system.

The data show that, under certain experimental conditions, the addition of LPC to the lipid formulations may increase the size of the LLPP and enhance lymphatic drug transport under steady-state conditions. Therefore, the effect of adding LPC to 20 mg of OA/h formulation, which was administered as a bolus single dose (i.e., under nonsteady-state conditions), was also examined. This experiment was designed to probe whether the LLPP also acts as a driver of lymphatic drug transport under nonsteady-state conditions (realizing that the size and kinetics of the LLPP could not be accurately measured under these conditions).

Surgical Procedures

All surgical and experimental procedures were conducted in accordance with the Australian and New Zealand Council for the Care of Animals in Research and Teaching guidelines and approved by the local institutional Animal Ethics Committee. Male Sprague-Dawley rats (280–320 g) were employed and fasted overnight with free access to water before surgery. Anesthesia was induced and maintained using a combination of fentanyl, fluanisone, and midazolam, and the trachea, mesenteric lymph duct, and duodenum were cannulated as reported previously (Trevaskis et al., 2005). Surgery and subsequent lymph collection were performed with the rat placed on a heated pad (Ratek Instruments Pty., Victoria, Australia) at 37°C to maintain body temperature. After completion of the surgery, a continuous intraduodenal infusion of 2.8 ml/h normal saline was initiated and animals were allowed to stabilize for 0.5 h before initiation of infusion of the experimental lipid formulations.

Experimental Procedures

Determination of the Mass of Lipid and Drug in the Lymph Lipid Precursor Pool and Rate of Turnover into Lymph under Steady-State Conditions. The experimental protocol was based on that described by Mansbach and Arnold (1986). Lipid-based formulations (see Table 1) containing Hf and 14C-OA were infused continuously into the duodenum at 2.8 ml/h until steady-state rates of total FA (endogenous plus exogenous) and drug transport into intestinal lymph were achieved. The time required to reach steady state was validated by continuous intraduodenal infusion of the experimental lipid formulations. Therefore, in these studies, lipid-based formulations (OA was used as an FA source) were continuously infused into the duodenum of lymph-cannulated rats until steady-state rates of lipid and drug transport into lymph were achieved. Under these steady-state conditions, both the size and the turnover kinetics of the LLPP can be accurately measured and compared with the rate and extent of lymphatic drug transport. Specifically, the current studies have examined the impact of infusion of different lipid doses in the presence and absence of BS and LPC.

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Analysis of Samples

Halofantrine Transport in Lymph. Lymph concentrations of Hf were determined using a validated high-performance liquid chromatography assay as described previously (Trevaskis et al., 2005).

Exogenous and Endogenous FA Transport in Lymph and Endogenous Lipid Output in Bile. Lymph TG and lymph PL and bile PL concentrations were determined using commercial enzymatic colorimetric methods running on a Cobas Mira clinical chemistry analyzer (Roche Diagnostics). Total FA transport (endogenous plus exogenous) in lymph and endogenous FA output in bile was calculated as described previously (Trevaskis et al., 2005), based on the assumption that each mole of TG and PL comprised 3 and 2 mol of FA, respectively. Exogenous FA transport in lymph was determined from the 14C-OA label as described previously (Trevaskis et al., 2005). Endogenous FA transport in intestinal lymph was determined from the difference between the total FA transport and exogenous FA transport in lymph.

Data Analysis and Calculations for Steady-State Experiments. For each formulation, steady-state transport rates of total (exogenous plus endogenous) FA into lymph ([dX/dt]ss) (micromoles/hour) were calculated as the average of the individual values obtained during each hourly period after attainment of steady state (4–10 h). Likewise, the steady-state transport rates of Hf (micromoles/hour) ([dD/dt]ss) exogenous FA (micromoles/hour), and endogenous FA (micromoles/hour) into lymph were measured during hour 5 (i.e., 4–5 h postinitiation of infusion and once steady state had been attained but before ceasing infusion of the drug and 14C-OA) for each formulation.

After ceasing infusion of the trace quantity of 14C-OA label or drug, the washout kinetics of exogenous FA and drug into the lymph could be assessed. Semilog plots of the concentration of 14C-FA in lymph (expressed as micromoles of 14C-FA per micromoles of total FA) versus time (hour) after ceasing infusion of 14C-OA was linear,
sustaining that the washout kinetics were first order and could be described by eq. 1,
\[ C = C_0 e^{-Kt} \] (1)

where \( t \) represents time (in hours), \( K \) is the first order rate constant describing transfer of \(^{14}\)C-OA from the LLPP into the lymph (hour\(^{-1}\)), \( C_0 \) represents the \(^{14}\)C-OA concentration in total FA in the lymph at the start of the washout period, and \( C \) represents the \(^{14}\)C-OA concentration in total FA in the lymph at time \( t \). The decline in concentration of \(^{14}\)C-FA in the lymph was assumed to reflect the decline in concentration of \(^{14}\)C-FA in the LLPP and the turnover of FA from the LLPP into the lymph. The first order rate constant describing FA turnover into lymph \((K_2)\) (hour\(^{-1}\)) was therefore determined from the gradient of the semilog plots obtained. The washout kinetics were determined from the decline in the concentration of \(^{14}\)C-FA measured as a function of total FA in the lymph to correct for fluctuations in the total mass of FA transported into lymph at each hourly time point. The first order rate constant describing drug turnover into the lymph \((K_1)\) (hour\(^{-1}\)) was calculated in an analogous fashion to \( K_2 \).

Assuming first order kinetics, the \((dX/dt)_{ss}\) is a function of \( X_{LP} \) at steady state and \( K_2 \) (hour\(^{-1}\)) (this relationship is described in eq. 2 and is represented schematically in Fig. 1). Because \( X_{LP} \) and \( K_2 \) are constant after administration of a given formulation to steady state, \((dX/dt)_{ss}\) may also be represented by a pseudo-zero order rate constant, \( K_{X0} \). Therefore, \( X_{LP} \) was calculated using eq. 2 from the measured \((dX/dt)_{ss}\) and the \( K_2 \) from the washout plots.

\[ (dX_{LP}/dt)_{ss} = K_2 \times X_{LP} = K_{X0} \] (2)

An analogous set of equations was used to calculate the \( D_{LP} \) (see Fig. 1) as described in eq. 3,

\[ (dD_{LP}/dt)_{ss} = K_0 \times D_{LP} = K_{DO} \] (3)

where \( K_{DO} \) is the pseudo-zero order rate constant for drug transport into the lymph given that both \( D_{LP} \) and \( K_0 \) are constant at steady state.

**Statistical Analysis.** Statistically significant differences were determined by ANOVA followed by Tukey’s test for multiple comparisons at a significance level of \( \alpha = 0.05 \). All statistical analyses were performed using SPSS for Windows, version 11.5.0 (SPSS Inc., Chicago, Ill).

![Fig. 1. Schematic of FA and drug transport from the enterocyte-based lymph lipid precursor pool into the lymph at steady state. XLP is the mass of total FA in the lymph lipid precursor pool (in micromoles) at steady state and is removed from the lymph lipid precursor pool by a first order rate process (described by \( K_2 \), hour\(^{-1}\)). Therefore, \((dX/dt)_{ss}\) is dictated by the equation \((dX/dt)_{ss} = K_2 \times X_{LP}\). Likewise, \( D_{LP} \) is the mass of drug in the lipid pool (microgram) at steady state, and drug is removed from the lipid pool by a first order rate process described by \( K_0 \) (hour\(^{-1}\)). Therefore, \((dD/dt)_{ss}\) is dictated by the equation \((dD/dt)_{ss} = K_0 \times D_{LP}\).](attachment:fig1.png)

**Results**

**Formulation Characteristics.** Formulations B, C, and D comprised single populations of lipid droplets with mean diameters of 205, 26.7, and 55.4 nm, respectively. The mean droplet size of Formulation E was 172 nm, which was a composite of two different sized populations of lipid droplets with mean diameters of 90 to 100 and 280 to 310 nm, which occurred at 60 to 70 and 30 to 40% frequency, respectively. Formulation F (which was the same formulation as H) had a mean droplet size of 295 nm, which again reflected the mean of two different sized lipid droplet populations with mean diameters of 90 to 100 and 380 to 400 nm, occurring at 30 to 40 and 40 to 60% frequency, respectively. Formulation G (which was the same formulation as I) also comprised two different sized lipid droplet populations, with mean diameters of 50 to 70 and 340 to 360 nm at 20 to 30 and 70 to 80% frequency, that resulted in an overall mean droplet size of 273 nm. Therefore, it is apparent that all of the formulations were highly dispersed mixed micellar dispersions, with mean particle sizes of 300 nm. A small reduction in particle size was evident on the addition of LPC to the low-dose lipid-dose formulations; however, LPC had no impact on the particle size of the 20 mg of OA containing formulations. Because the particle size of the formulations was similar and submicron in all cases, differences in lymphatic drug transport were not expected to reflect differences in the efficiency of absorption of the drug from the small intestine lumen due to the degree of dispersion of the administered formulations.

**Steady-State Experiments**

**Endogenous and Exogenous Fatty Acid Levels in the Lymph Lipid Precursor Pool and Lymph.** Table 2 shows the mass of total (endogenous plus exogenous), endogenous, and exogenous FA in the LLPP at steady state after 5 h of intraduodenal infusion of the different lipid formulations. The corresponding rates of total (including the delineation of TG- and PL-associated FA), endogenous, and exogenous FA transport into the lymph and the proportion of the exogenous lipid dose transported into the lymph at steady state are given in Table 3, and the rates of endogenous FA output in bile are given in Table 4. All lipid-containing formulations enhanced the mass of total FA in the LLPP and the rate of total FA transport into lymph. These increases resulted from both recruitment of endogenous FA into the lymph and the absorption and lymphatic transport of exogenous FA. It should be noted that formulations C, D, and G included an additional quantity of exogenous lipid in the form of 10 \(\mu\)mol/h (5.2 mg/h) LPC, which could theoretically contribute up to an additional 10 \(\mu\)mol/h of exogenous FA to total intestinal lymphatic lipid transport rates but which is not taken into account using the calculation method employed (because the LPC was not radiolabeled). This in turn could lead to overestimation of endogenous FA transport rates in lymph in these groups. However, LPC is predominantly resynthesized to PC before transport to the systemic circulation via the intestinal lymph and does not provide a FA source for TG synthesis (Scow et al., 1967; Nilsson, 1968; Sato, 1970). Therefore, lymphatic FA that is derived from the infusion of exogenous LPC is expected to be present in the lymph as PL-associated FA. Because the increases in total (endogenous plus exogenous) PL-associated FA transport were low in...
formulations. The lipid formulation doses were administered at a rate of 100 μg/h, 1 μg/h 14C-OA and 0.2% Tween 80 in normal saline (A), 5 mg of OA in 5 mM BS solution (B), 2 mg of OA/5.2 mg of LPC in 5 mM BS solution (C), 5 mg of OA/5.2 mg of LPC in 5 mM BS solution (D), 20 mg of OA in 0.2% Tween 80 (E), 20 mg of OA in 5 mM BS solution (F), or 20 mg of OA/5.2 mg of LPC in 5 mM BS solution (G). The over- and underbars identify different pairs.

Data were obtained at steady state in mesenteric lymph duct-cannulated, anesthetized rats (n = 4, mean ± S.E.M.) after 5 h of continuous intraduodenal infusion of lipid formulations. The lipid formulation doses were administered at a rate of 100 μg/h, 1 μg/h 14C-OA, and 0.2% Tween 80 in normal saline (A), 5 mg of OA in 5 mM BS solution (B), 2 mg of OA/5.2 mg of LPC in 5 mM BS solution (C), 5 mg of OA/5.2 mg of LPC in 5 mM BS solution (D), 20 mg of OA in 0.2% Tween 80 (E), 20 mg of OA in 5 mM BS solution (F), or 20 mg of OA/5.2 mg of LPC in 5 mM BS solution (G). The over- and underbars identify different pairs.

**TABLE 2**

The mass of total (endogenous plus exogenous) FA, endogenous FA, and exogenous FA in the lymph lipid precursor and the proportion of the lymph lipid precursor pool comprising endogenous FA

Data obtained at steady state in mesenteric lymph duct-cannulated, anesthetized rats (n = 4, mean ± S.E.M.) after 5 h of continuous intraduodenal infusion of lipid formulations. The lipid formulation doses were administered at a rate of 100 μg/h. The lipid formulation doses were administered at a rate of 100 μg/h, 1 μg/h 14C-OA, and 0.2% Tween 80 in normal saline (A), 5 mg of OA in 5 mM BS solution (B), 2 mg of OA/5.2 mg of LPC in 5 mM BS solution (C), 5 mg of OA/5.2 mg of LPC in 5 mM BS solution (D), 20 mg of OA in 0.2% Tween 80 (E), 20 mg of OA in 5 mM BS solution (F), or 20 mg of OA/5.2 mg of LPC in 5 mM BS solution (G). The over- and underbars identify different pairs.

**TABLE 3**

The rate of total (endogenous plus exogenous) FA (including the contribution of TG-associated FA and PL-associated FA), endogenous FA, and exogenous FA transport into lymph and the proportion of the exogenous FA dose transported into the lymph

Data obtained at steady state in mesenteric lymph duct-cannulated, anesthetized rats (n = 4, mean ± S.E.M.) after 5 h of continuous intraduodenal infusion of lipid formulations. The lipid formulation doses were administered at a rate of 100 μg/h, 1 μg/h 14C-OA, and 0.2% Tween 80 in normal saline (A), 5 mg of OA in 5 mM BS solution (B), 2 mg of OA/5.2 mg of LPC in 5 mM BS solution (C), 5 mg of OA/5.2 mg of LPC in 5 mM BS solution (D), 20 mg of OA in 0.2% Tween 80 (E), 20 mg of OA in 5 mM BS solution (F), or 20 mg of OA/5.2 mg of LPC in 5 mM BS solution (G). The over- and underbars identify different pairs.

**TABLE 4**

Rate of endogenous FA output (as phospholipid) in bile at steady state

Rates (n = 4) were bile duct-cannulated after 5 h of continuous intraduodenal infusion of the lipid formulations, and bile was collected for 2 h. Data represent mean ± S.E.M.

<table>
<thead>
<tr>
<th>Rate of endogenous FA Output</th>
<th>ANOVA for Significant Differences*</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Rate of total FA transport in lymph (μmol/h)</td>
<td>13.5 ± 1.0</td>
<td>19.1 ± 0.6</td>
</tr>
<tr>
<td>TG-associated FA (μmol/h)</td>
<td>10.3 ± 0.8</td>
<td>16.7 ± 2.5</td>
</tr>
<tr>
<td>PL-associated FA (μmol/h)</td>
<td>3.2 ± 0.2**</td>
<td>2.5 ± 0.1*</td>
</tr>
<tr>
<td>Rate of endogenous FA transport in lymph (μmol/h)</td>
<td>13.5 ± 1.0</td>
<td>12.1 ± 0.6</td>
</tr>
<tr>
<td>Rate of exogenous FA transport in lymph (μmol/h)</td>
<td>N.A.</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td>Proportion (%) of the exogenous FA dose transported into the lymph</td>
<td>N.A.</td>
<td>39.6 ± 1.1**</td>
</tr>
</tbody>
</table>

* Formulations grouped by bars are not significantly different (P > 0.05).

For all cases (<4 μmol/h greater than control) (Table 3), it is apparent that the contribution of exogenous LPC to lymphatic FA output is sufficiently low to justify the approach taken. The importance of exogenous, endogenous, and biliary lipid to total lymphatic lipid flux varied as a function of exogenous lipid dose as described below.

**Low-Lipid Doses (2-5 mg/h).** On infusion of the low lipid-dose (2 or 5 mg) formulations (formulations B–D), the majority (>60%) of the FA in the LLPP and transported into the lymph was derived from endogenous sources (Tables 2 and 3) and, as such, increases to endogenous FA recruitment had the largest impact on total FA (and drug) transport in lymph. Infusion of the 5 mg of OA in BS formulation did not increase the mass of endogenous FA in the LLPP or the rate of endogenous FA transport into lymph compared with the control (lipid-free) formulation. However, a significant increase in endogenous FA output in bile (Table 4) was observed, suggesting that infusion of the 5 mg of OA in BS formulation resulted in a larger proportion of the endogenous FA in the LLPP being derived from biliary sources. Administration of 5 mg of OA in BS formulation also slightly increased the total mass of FA in the LLPP and lymph (Tables 2 and 3), presumably reflecting the absorption of exogenous FA.

In contrast, the addition of LPC to the 5 mg of OA in BS formulation led to a significant increase in endogenous FA recruitment into the LLPP (p < 0.05) (Table 2) and transport of endogenous FA into the lymph (p < 0.05) (Table 3). This in turn was reflected in an increase in total FA in the LLPP and lymph (p < 0.05) because endogenous FA was the primary
source of FA in the lymph at low exogenous lipid loads). After the addition of LPC to the 5 mg of OA infusion, there was also a small (4.2 \( \mu \text{mol/h} \)) but statistically significant (\( p < 0.05 \)) increase in endogenous FA output in bile at steady state (Table 4) compared with that observed in the absence of LPC, suggesting that at least part (~40%) of the increase in endogenous FA transport rate into lymph was a consequence of increased biliary lipid secretion. The addition of LPC to the 5 mg of OA in BS formulation did not change exogenous FA transfer into the lymph (Table 3).

In comparison to the endogenous FA transport rates obtained after administration of control or 5 mg of OA in BS formulations (Tables 2 and 3), LPC increased endogenous FA transport into the lymph, even when coadministered with a lower exogenous lipid dose (2 mg of OA). Surprisingly, however, even though the endogenous FA transport rates into the lymph were markedly different for the 2 mg of OA/LPC and 5 mg of OA formulations, both led to a similar increase in endogenous FA output in bile (Table 4). Therefore, although the addition of LPC to the lower (2 mg) OA dose increased endogenous FA transport into the lymph relative to administration of 5 mg of OA in BS alone, these lipids seemed to be derived from nonbiliary-related sources. Data for infusion of 2 mg of OA/h in the absence of BS or LPC or infusion of 5 mg of OA in the absence of BS were not collected, because infusion of 5 mg of OA, even in the presence of BS, failed to increase endogenous lymphatic FA transport compared with the control formulation (Table 2).

**High-Lipid Dose (20 mg/h).** After infusion of the higher lipid dose (20 mg of OA) formulations (formulations E–G), the majority of the FA in the LLPP and transported into the lymph was derived from endogenous (and not endogenous) sources (Tables 2 and 3) and, as such, changes to exogenous FA transport had the largest impact on total FA (and drug) transport in lymph. Indeed, all of the formulations containing 20 mg of OA/h led to recruitment of a similar mass of endogenous FA into the LLPP and transport of exogenous FA into the lymph (Tables 2 and 3).

**Relationship between Lymph Lipid Precursor Pool Size and Lipid Transport into the Lymph.** Figure 2A shows the relationship between the \( X_{\text{LP}} \) and the \( (dX_{\text{LP}}/dt)_{ss} \) after administration of the different lipid formulations. Increases in the \( X_{\text{LP}} \) (endogenous plus exogenous), which were observed after an increase in lipid dose (from 2 to 5 to 20 mg of OA/h) or upon addition of LPC to the formulations, were in general reflected in increases in the \( (dX_{\text{LP}}/dt)_{ss} \) (Fig. 2A). Interestingly, however, \( (dX_{\text{LP}}/dt)_{ss} \) did not increase in direct proportion with \( X_{\text{LP}} \). Because \( (dX_{\text{LP}}/dt)_{ss} \) is a product of \( X_{\text{LP}} \) and the \( K_{X} \) (eq. 2), the greater fractional increase in \( X_{\text{LP}} \) relative to \( (dX_{\text{LP}}/dt)_{ss} \) reflects a decline in \( K_{X} \) as \( X_{\text{LP}} \) expands. This decline in \( K_{X} \) with an increase in \( X_{\text{LP}} \) is depicted in Fig. 3. A similar (but less highly correlated) relationship was evident between the mass of either exogenous lipid or endogenous lipid [rather than the data for \( X_{\text{LP}} \) shown in Fig. 3 and \( K_{X} \) (data not shown)].

**Drug Levels in the Lymph Lipid Precursor Pool and Lymph.** Table 5 shows the mass of drug in the LLPP and the rate of drug transport into lymph after administration of all lipid formulations. In general, all of the lipidic formulations led to an increase in drug in the LLPP and an increase in lymphatic drug transport. However, in line with the observed changes to lipid levels in the LLPP, changes to the mass of drug in the LLPP and the rate of drug transport in lymph reflected both the lipid dose and the source of LLPP lipids.

**Low Lipid Doses (2–5 mg/h).** Administration of all of the low lipid-dose (2–5 mg of OA/h) formulations increased lymphatic drug transport compared with the control (lipid-free)
The rank order of lymphatic drug transport for the low lipid-dose formulations was 5 mg of OA/LPC in 5 mM BS solution > 5 mg of OA in 5 mM BS solution > 2 mg of OA/LPC in 5 mM BS solution > control. Figure 4A shows the relationship between the mass of lipid and drug in the LLPP, and Fig. 4B shows the relationship between the mass of lipid and drug transport into lymph. It is evident that, whereas a broad relationship between lipid and drug transport was observed across all formulations, at low lipid doses (and low-lipid pool sizes), the relationship between the mass of drug in the LLPP and lymph and the mass of FA available in the LLPP and lymph was less clear. In particular, the administration of 2 mg of OA/LPC substantially enhanced endogenous (and therefore total) FA transport in lymph compared with the 5 mg of OA/BS formulation but failed to support an increase in lymphatic drug transport. In contrast, Fig. 4C shows that, at low exogenous lipid doses, the rate of lymphatic drug transport was more directly related to the rate of endogenous FA output in bile (as opposed to total FA output in lymph).

**High Lipid Dose (20 mg/h).** Administration of a higher lipid dose (20 mg of OA/h) increased lymphatic drug transport and the mass of drug associated with the LLPP compared with the low lipid-dose formulations (Table 5). The addition of BS and LPC to the high lipid-dose formulations further increased the mass of drug associated with the LLPP and the rate of lymphatic drug transport (Table 5). Figure 4, A and B, indicates that, after administration of the high lipid-dose formulations, the mass of drug in the LLPP and lymph was closely related to the mass of FA [which is predominantly exogenous FA at the higher lipid doses (Tables 3 and 4)] available to solubilize drug.

**Relationship between Mass of Drug in the Lymph Lipid Precursor Pool and Drug Transport into the Lymph.** In a situation analogous to that described for lipid transport into the lymph, increases in the DLP were also reflected in increases in (dD/dt)ss (Fig. 2B). Unlike the lipid transport data, however, the fractional increases in (dD/dt)ss and DLP were similar after administration of the different formulations and $K_D$ (the first order rate constant describing drug turnover from the LLPP into the lymph (eq. 3)) was relatively constant at all LLPP sizes (Fig. 3). This is in contrast to the lipid transport data where $K_D$ decreased significantly as the lipid pool expanded (Fig. 3). $K_D$ was also significantly less than $K_X$ after administration of all formulations ($p < 0.05$) (Fig. 3).

**Nonsteady-State Experiments.** Because the addition of LPC to the 20 mg of OA/h formulation significantly enhanced the size of the LLPP and the rate of lymphatic drug transport under steady-state conditions, the effect of adding LPC to the 20 mg of OA/h formulation administered over 2 h was also examined. Figure 5 shows the effect of adding LPC to the high lipid-dose formulation on the cumulative lymphatic transport of total FA and HF. In line with the steady-state data, the addition of LPC to this nonsteady-state formulation significantly enhanced both lymphatic transport of total FA (A) and HF (B). The differences in lymphatic lipid transport were particularly evident over 2 to 4 h postdose (1.5–2-fold differences), a period coincident with the timescale of maximum drug absorption.

**Discussion**

Recent data has suggested that the manner in which endogenous and exogenous lipids are trafficked through and pool within the enterocyte may influence their ability to support lymphatic drug transport (Trevaskis et al., 2005). The current study was conducted to examine this hypothesis directly and more specifically to determine whether the size and turnover dynamics of the LLPP influence the rate and extent of lymphatic drug transport.

**Lipid and Drug Transport from the Lymph Lipid Precursor Pool into the Lymph.** In general, increases in the XLP led to increases in the rate of total FA transport into the lymph at steady state (Fig. 2A). Interestingly, however, the $K_{X}$ declined as the total mass of FA in the LLPP ex-

**Table 5**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass of drug in the lipid pool (µg)</td>
<td>9.6 ± 1.2</td>
<td>23.7 ± 1.7</td>
<td>15.4 ± 0.7</td>
<td>44.4 ± 6.0</td>
<td>51.2 ± 3.6</td>
<td>68.7 ± 3.5</td>
<td>113.4 ± 4.3</td>
</tr>
<tr>
<td>Rate of drug transport into lymph (µg/h)</td>
<td>3.6 ± 0.3</td>
<td>9.9 ± 0.6</td>
<td>7.2 ± 0.4</td>
<td>15.9 ± 0.2</td>
<td>22.0 ± 2.0</td>
<td>25.6 ± 0.8</td>
<td>33.7 ± 1.0</td>
</tr>
</tbody>
</table>

* Formulations grouped by bars are not significantly different ($P > 0.05$).
This suggests that the process of FA transport from the LLPP into the lymph has a finite capacity and may saturate as the LLPP expands. This is consistent with a previous study that has suggested that lipid transport through the enterocyte into the lymph may be limited by the rate at which transport vesicles, which carry premature lipoproteins from the endoplasmic reticulum to the Golgi, bud off from the endoplasmic reticulum membrane (Mansbach and Dowell, 2000).

Increases in the $D_{LP}$ also led to increases in the rate of lymphatic drug transport (Fig. 2B), but in contrast to the lipid transport data, the $K_D$ was relatively constant at all LLPP sizes, indicating that drug transport capacity was not expanded (Fig. 3). This suggests that the process of FA transport from the LLPP into the lymph has a finite capacity and may saturate as the LLPP expands. This is consistent with a previous study that has suggested that lipid transport through the enterocyte into the lymph may be limited by the rate at which transport vesicles, which carry premature lipoproteins from the endoplasmic reticulum to the Golgi, bud off from the endoplasmic reticulum membrane (Mansbach and Dowell, 2000).

Fig. 5. A, the cumulative mass of total FA (endogenous plus exogenous) transported into the lymph. B, the cumulative transport of Hf (% dose) into the lymph versus time (in hours) in mesenteric lymph duct-cannulated anesthetized rats ($n = 4$, mean ± S.E.M.) after intraduodenal infusion over 0 to 2 h of 200 $\mu$g of Hf with either 40 mg of OA in 5 mM bile salt solution (○) or 40 mg of OA/10.4 mg of LPC in 5 mM bile salt solution (○).

Increases in the $D_{LP}$ also led to increases in the rate of lymphatic drug transport (Fig. 2B), but in contrast to the lipid transport data, the $K_D$ was relatively constant at all LLPP sizes, indicating that drug transport capacity was not saturable.
limited by increasing mass of lipid (Fig. 3) or drug (Table 2) in the LLPP. $K_D$ was also significantly lower than $K_X$, especially at low LLPP sizes. Therefore, at least for Hf, drug transport into the lymph does not seem to occur simply by drug association with the lipoproteins in the LLPP and subsequent turnover of drug-loaded lipoproteins into the lymph. Rather, it seems that some other process(es) is/are reducing the proportional transfer of drug into the lymph. The reasons underlying this difference in transport are not known at this time but might reflect 1) a preference for drug to remain associated with the LLPP, 2) metabolism of the drug either within the LLPP or during transport into the lymph, and/or 3) passive diffusion of the drug out of the LLPP followed by absorption into the portal vein.

Drug Solubilization in the Lymph Lipid Precursor Pool. It is apparent from Fig. 2B that increases in drug solubilization in the LLPP lead to increases in lymphatic drug transport and is also apparent from Fig. 4 that, in general, an increase in the mass of total FA in the LLPP increases solubilization of drug in the LLPP and ultimately increases lymphatic drug transport. This is consistent with previous studies that have shown that drug transport into the lymph is typically related to the mass of triglyceride transported into lymph (Noguchi et al., 1985; Charman and Stella, 1986a; Caliph et al., 2000; Porter and Charman, 2001) but extends these previous observations to an understanding that it is the size and turnover kinetics of the LLPP that are key to dictating lymphatic drug transport profiles. In general, formulation components that swell the LLPP and enhance lymphatic lipid transport, therefore, may stimulate lymphatic drug transport. However, closer examination of the current data (Fig. 4; Tables 2 and 3) suggests that this relationship is more complex and, in part, a function of the contribution of endogenous and exogenous lipids to the LLPP.

The differential roles of endogenous lipids, exogenous lipids, and the LLPP in dictating lymphatic drug transport profiles were explored using BS and LPC (the digestion product of PC), because these materials have previously been suggested to have an impact on lymphatic lipid and drug transport (Tso et al., 1977; Mansbach and Dowell, 1993; Nevin et al., 1995). In this regard, a recent study from our laboratory, conducted under nonsteady-state conditions, showed that BS infusion can stimulate endogenous lipid uptake into the lymph and coincidently increase lymphatic drug transport (Trevaskis et al., 2005). However, in the steady-state experiments described here, BS failed to increase the mass of endogenous lipid in the LLPP or the rate of lymphatic transport of endogenous lipids, although BS infusion in the presence of low exogenous lipid doses resulted in a larger proportion of the endogenous lipids in the lymph derived from biliary sources (and this occurred in tandem with an increase in lymphatic drug transport). At this stage, it is not clear why acute infusion of BS seems to be capable of recruiting endogenous lipids into the lymph, whereas under...
dependent on the size of the LLPP, increases in lymphatic nrous lipids in the LLPP. Therefore, it is apparent that, al-
important compared with the dominant exogenous lipids. As such, whereas endogenous lipid levels were enhanced relative to control, they were relatively un-
tributed only 22% of the lipids in the LLPP. Finally, the
increase in lymphatic drug transport mediated by the addition of LPC to the 20 mg of OA/h formulation reflected the ability of LPC to expand the LLPP. However, this capacity was markedly de-
the importance of the LLPP in determining the rate and extent of lymphatic drug transport and enhance the current understanding of the mechanisms by which lipophilic drug molecules access the lymph during transport through the enterocyte. The data further suggest that formulation com-
products (such as LPC), which expand the LLPP and stimu-
may be usedfully incorporated into formulations to enhance lymphatic drug transport.

In summary, these findings provide the first evidence of the importance of the LLPP in determining the rate and extent of lymphatic drug transport and enhance the current understanding of the mechanisms by which lipophilic drug molecules access the lymph during transport through the enterocyte. The data further suggest that formulation components (such as LPC), which expand the LLPP and stimulate biliary lipid secretion, may enhance lymphatic drug transport.

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