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Acute Hypertriglyceridemia Promotes Intestinal Lymphatic Lipid and Drug Transport: A Positive Feedback Mechanism in Lipid and Drug Absorption

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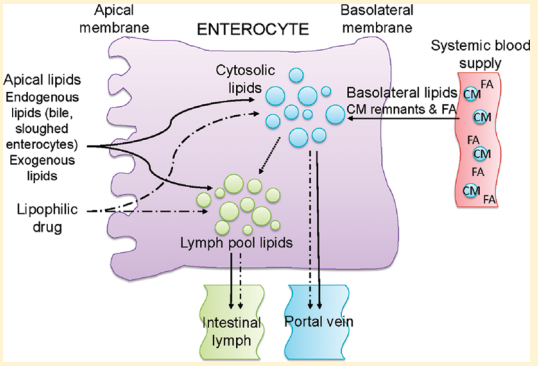
Supporting Information

ABSTRACT: Elevated systemic levels of triglyceride-rich lipoproteins (TRL) are a risk factor for the development of atherosclerosis. In patients with metabolic syndrome (MetS), intestinal TRL overproduction contributes to high systemic TRL levels, and recent studies suggest that systemic changes in MetS such as increases in plasma fatty acids and insulin resistance stimulate intestinal TRL production. The current study has examined whether increases in systemic TRL influence intestinal lipid transport and lipoprotein assembly pathways and evaluates the impact of these changes on the absorption and lymphatic transport of lipids and a model lipophilic drug (halofantrine). Mesenteric lymph-duct or bile-duct cannulated rats were administered IV saline or ^{14}C-labeled chylomicron (CM) (to increase systemic TRL) and intraduodenal ^{3}H lipids and drug. Changes to biliary lipid output and lymphatic lipid and drug transport were subsequently examined. Increasing systemic TRL concentrations stimulated a significant increase in lymphatic lipid and drug transport. The increased lipids in lymph were not derived from bile or the intestinal blood supply (fatty acid or IV infused ^{14}C-CM). Rather, an increase in lymphatic transport of duodenally sourced lipids was evident. Increasing plasma levels of TRL therefore stimulated lipid absorption and lymphatic transport via a positive feedback process. The data also suggest that the changes to intestinal TRL formation that result from raised systemic TRL levels may impact on the absorption of highly lipophilic drugs and therefore the reproducibility of drug treatments.



KEYWORDS: metabolic disease, dyslipidemia, hypertriglyceridemia, atherosclerosis, lipoprotein, triglyceride rich lipoprotein, lipid absorption, drug absorption, lymphatic drug transport

INTRODUCTION

Atherosclerotic cardiovascular disease (CVD) is a leading cause of death in developed countries, the risk of which is increased by systemic elevations in LDL-cholesterol[1] and plasma triglyceride (TG).[2−4] Hypertriglyceridemia results primarily from increases in TG in VLDL and chylomicron (CM) (collectively referred to as triglyceride rich lipoproteins (TRL)). Atherosclerotic patients also typically display at least one feature of metabolic syndrome (MetS), i.e., obesity, insulin resistance and/or dyslipidemia. Hypertriglyceridemia[5,6] is present in both the fed and fasted states in MetS and until recently has been attributed to overproduction of VLDL in the liver and impaired systemic TRL clearance. Recent studies, however, have demonstrated that alterations to intestinal TRL production in insulin resistant and/or obese humans[5,7,8] and animals[6,9−12] contribute to fasting and postprandial increases in systemic TRL and TG levels. Systemic changes in MetS such as increases in plasma fatty acids[7,8] and insulin resistance[11,13,14] have also been suggested to stimulate intestinal TRL production, although, to this point, the potential for hypertriglyceridemia to impact on intestinal TRL processing and intestinal lipid transport has not been examined specifically. This is a focus of the current studies.

Changes to systemic lipid and lipoprotein levels may also impact on the distribution and clearance of lipophilic drugs. Thus, increases in systemic lipoprotein concentrations generally reduce the volume of distribution and clearance of lipophilic drugs as lipoprotein association reduces the free fraction of drug available for renal or hepatic clearance and distribution to tissues.[15,16] The impact of raised systemic concentrations of TG and TRL on the intestinal absorption and trafficking of lipophilic drugs across the enterocyte, however, has not been examined previously. For some highly lipophilic drug molecules, association with lipid transport and lipoprotein assembly pathways in the enterocyte leads to drug transport to the systemic circulation via the intestinal lymph rather than the portal blood.[17,18] Where changes to enterocyte based lipoprotein production are evident in MetS, changes to intestinal lipid and drug transport via the intestinal lymphatic system may also be envisaged.

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The purpose of the current study was therefore to measure the impact of increasing systemic TG and TRL on the intestinal absorption and lymphatic transport of lipids and a model lipophilic drug, halofantrine. To achieve this, a rat model was developed to increase systemic concentrations of TG and TRL through continuous intravenous infusion of CM obtained from donor animals. The effect of intravenous infusion of CM on lymphatic lipid and drug transport was subsequently examined. To further explore the mechanism by which CM infusion affects lipoprotein formation and lymphatic lipid and drug transport, the source of the lipids recruited into intestinal lymph during infusion of TRL was also evaluated. The lipid sources examined were intraduodenally administered exogenous lipids, biliary lipids absorbed from the intestinal lumen and plasma fatty acids or chylomicron remnants from the intestinal blood supply. The data suggest that increases in systemic TG and TRL stimulate a positive feedback mechanism which increases absorption and lymphatic transport of lipids and may play a role in maintaining dyslipidemia; and that the increases in lymphatic lipid transport that result can support increases in the intestinal lymphatic transport of highly lipophilic drugs.

EXPERIMENTAL SECTION

Materials. Halofantrine base (GlaxoSmithKline, India), oleic acid [1-^{14}C] (Perkin-Elmer Life Sciences, Boston, MA), oleic acid (OA), sodium taurocholate and sodium chloride (Sigma Chemicals, Australia), Tween 80 (BDH Chemicals, Australia) and normal saline for injection (Baxter Healthcare, Australia) were used as received. Acetonitrile, sodium dodecyl sulfate and glacial acetic acid were HPLC grade. Water was obtained from a Milli-Q (Millipore, Milford, MA) purification system. Ketamine and xylazine (Ilium Ketamil and Xylazil, Troy laboratories, Australia) were used for anesthesia. Triglyceride kit (Roche diagnostics GmbH, Mannheim, Germany) Control serum II and Wako Phospholipid kit (Wako Chemicals, Richmond, VA) were used for analysis of triglyceride (TG) and phospholipid (PL) levels. Irgasafe plus (Packard Bioscience, Meriden, CT) liquid scintillation cocktail was used for liquid scintillation counting of radioactivity levels. All other chemicals were analytical reagent grade.

Animal Experiments. All experiments were approved by the local animal ethics committee and were conducted in accordance with the Australian and New Zealand Council for the Care of Animals in Research and Teaching guidelines. All studies were conducted using male Sprague−Dawley rats (280−320 g) which were maintained on a standard diet, acclimatized in the lab for at least 3 days prior to and then fasted overnight before experiments. Water was available ad libitum. Prior to surgery, rats were anesthetized by subcutaneous injection of a combination of ketamine, xylazine and acepromazine, and anesthesia was maintained throughout the experiments with top up doses of ketamine and acepromazine as described previously.[19] Surgery was performed to insert cannulas into the duodenum (for formulation administration and rehydration), jugular vein (for intravenous infusion of CM or saline), mesenteric lymph duct (for lymph collection) or bile duct (to collect bile but not pancreatic secretions) as required for particular experiments, and as described previously.[20,21] After surgery, rats were rehydrated for 0.5 h via intraduodenal infusion of normal saline at a rate of 2.8 mL/h. At the conclusion of the experiment, rats were killed via a lethal intraperitoneal dose of 1 mL of sodium pentobarbitone (100 mg/mL).

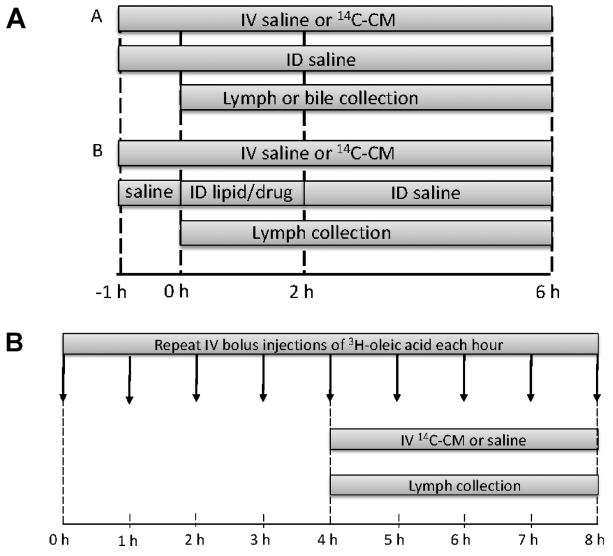

Figure 1. Schematic diagram. (A) The timing of intravenous (IV) administration of saline or ^{14}C-chylomicron (CM), intraduodenal (ID) administration of saline (panel A) or lipid and drug (panel B) and collection of lymph or bile in experiments to determine whether IV ^{14}C-CM enhances uptake of lipids into lymph or bile and to measure the transport of ^{14}C-CM lipids into lymph and bile. (B) The timing of bolus intravenous (IV) administration of fatty acids (^{3}H-oleic acid, as a marker of plasma fatty acids), IV administration of ^{14}C-chylomicron (CM) and collection of lymph in experiments to determine if ^{14}C-CM enhances lymph uptake of plasma fatty acids.

Collection of Chylomicron from Donor Rats. To collect CM for intravenous (IV) infusion to recipient rats, donor rats were infused via a duodenal cannula with 708 μmol (200 mg) of oleic acid (containing 10 μCi of ^{14}C-oleic acid) dispersed in 5.6 mL of 0.5% Tween 80 in saline over 2 h and for the remainder of the experiment with 2.8 mL/h normal saline. The oleic acid formulation was prepared and the stability assessed as described previously.[20] Mesenteric lymph was collected over 6 h into a tube containing 100 μL of heparin (1000 U/mL). To separate ^{14}C-containing CM, 6 mL of lymph was layered under 6 mL of normal saline in a polyallomer centrifuge tube (Beckman, CA) and the tube ultracentrifuged at 202048g for 1 h 35 min in a SW40Ti rotor (Beckman, CA).[22] The CM fraction formed a semisolid plug at the top of the centrifuge tube and was removed and placed in a 12 mL polypropylene tube, and the ^{14}C-CM fraction was resuspended via addition of normal saline. The TG concentration of the resuspended ^{14}C-CM was measured as described below and the TG concentration adjusted to 9.8 mg/mL with saline. The concentration of radiolabel (from infused oleic acid) in the resuspended ^{14}C-CM was measured by scintillation counting.

Protocol for IV Infusion of Recipient Rats. Lymph was collected the day before, and ^{14}C-CM separated from the lymph of donor rats on the day of infusion into recipient rats. ^{14}C-CM was infused into the jugular vein of recipient rats at a rate of 1 mL/h (containing a total of 9.8 mg/h of TG or 34.7 μmol/h of TG associated fatty acids), and control rats were infused with 1 mL/h normal saline via the jugular vein. ^{14}C-CM or saline was infused IV for 1 h before commencing the intraduodenal infusions and infusion of the ^{14}C-CM or saline was continued throughout the experiment (as depicted in Figure 1) unless otherwise stated.

Table 1. Cumulative Lymphatic Transport of Halofantrine (% of Dose), Total Fatty Acids (μmol), Exogenous ^{3}H-Oleic Acid (μmol), ^{14}C-Oleic Acid from IV Infused Chylomicron (CM, μmol) and Endogenous (Nonlabeled) Fatty Acid (μmol) and Cumulative Biliary Transport of Phospholipid Fatty Acids (μmol) over 6 h in Control or IV ^{14}C-CM Infused Lymph or Bile Duct Cannulated Rats Administered 2.8 mL/h Saline or 200 μg of Halofantrine Dispersed in 14.2 μmol ^{3}H-Oleic Acid in 5.6 mL of 0.2% Tween 80 over 2 h into the Intestine[a]

intestinal formulation	intravenous infusion	halofantrine in lymph (% of dose)	total fatty acids in lymph (μmol)	^{3}H oleic acid from intraduodenal lipids in lymph (μmol)	^{14}C oleic acid from IV CM in lymph (μmol)	endogenous fatty acids in lymph (μmol)	fatty acid in bile (μmol)	exogenous ^{3}H oleic acid in lymph (% of dose)
saline	saline		54.8 ± 6.8			54.8 ± 6.8	21.5 ± 2.4	
saline	^{14}C-CM		72.1 ± 4.7[b]		0.38 ± 0.14	71.7 ± 4.0[b]	22.0 ± 2.1	
14.2 μmol of ^{3}H-oleic acid	saline	5.1 ± 0.8	63.4 ± 5.9	6.2 ± 0.5		57.2 ± 5.8		43.4 ± 3.8
14.2 μmol of ^{3}H-oleic acid	^{14}C-CM	8.0 ± 1.0[b]	78.3 ± 7.7[b]	8.8 ± 0.1[b]	0.47 ± 0.01	69.0 ± 9.5		62.2 ± 0.60[b]

[a] Data represent mean ± SEM for n = 4 rats. [b] Significantly greater than in the group administered saline intravenously ($p < 0.05$).

Effect of IV Chylomicron Infusion on Lipid and Drug Transport into Lymph. Figure 1A shows the timing of IV and intraduodenal infusions and sample collection in these experiments. Rats which were continuously infused IV with either saline or ^{14}C-CM (as described above) also received an intraduodenal infusion of either 2.8 mL/h saline or 14.2 μmol (4 mg) of oleic acid (containing a trace quantity of 0.5 μCi of ^{3}H-oleic acid) and 200 μg of halofantrine in 5.6 mL of 0.2% Tween 80 in saline (prepared as described previously[20]). The intraduodenal infusion was initiated at time 0 (i.e., 1 h after commencing IV infusions of saline or ^{14}C-CM) and continued for 2 h. After 2 h the drug and lipid intraduodenal infusions were halted and 2.8 mL/h saline was infused into the duodenum of all rats for the remainder of the experiment.

Lymph was collected into tared tubes, which were changed every hour, and the volume of lymph collected determined gravimetrically. Lymph concentrations of halofantrine, radiolabels and lipid (TG and phospholipid (PL)) were measured as described below.

Effect of IV Chylomicron Infusion on Lipid Transport into Bile. Rats were infused continuously IV with saline or ^{14}C-CM (as described above and in Figure 1A) and received an intraduodenal infusion of 2.8 mL/h saline. Bile was collected into tared tubes, which were changed every hour, and the volume of bile collected determined gravimetrically. Bile concentrations of radiolabel and lipid (TG and PL) were measured as described below.

Effect of IV Chylomicron Infusion on Transport of Plasma Fatty Acids into Lymph. Figure 1B shows the timing of IV and intraduodenal infusions and sample collection in these experiments. Rats were given repeated short (1 min) bolus IV infusions of 0.354 μmol (0.1) mg of oleic acid (containing 0.5 μCi of ^{3}H-oleic acid) dispersed in 1 mL of 0.2% Tween 80 into the jugular vein every hour in order to attain steady state uptake of oleic acid into the lymph from the systemic circulation. Lymph was collected into tubes changed hourly throughout the experiment. Once steady state ^{3}H-oleic acid transport into the lymph was achieved (after 5 bolus infusions of ^{3}H-oleic acid), a continuous 1 mL/h IV infusion of either saline or ^{14}C-CM was commenced (Figure 1B). The continuous IV infusions were briefly interrupted each hour to allow bolus injection of the ^{3}H-oleic acid formulation. Lymph concentrations of radiolabels (^{3}H-oleic acid and ^{14}C-CM) and lipids (TG and PL) were measured as described below.

Analytical Methods. *Halofantrine Analysis.* The lymph concentrations of halofantrine were determined using a previously validated HPLC method.[20,23] Recovery of halofantrine spiked into blank lymph (at low, medium and high concentrations of 0.5, 1, and 2 μg/mL) was >95% (n = 5 analyses at each concentration), and the assay was accurate and precise (to within ±10% of nominal concentrations) at injected concentrations between 25 and 1000 ng/mL.

Lipid Analysis. Lymph concentrations of TG and PL and bile concentrations of PL were determined using commercial enzymatic colorimetric methods (Triglyceride kit (Roche diagnostics GmbH, Mannheim, Germany) Control serum II and Wako Phospholipid kit (Wako Chemicals, Richmond, VA)).

Scintillation Counting. The concentration of radiolabels (from ^{14}C-CM/oleic acid or ^{3}H-oleic acid) in lymph and CM samples was measured by scintillation counting following addition of 3 mL of Irgasafe plus to 200 μL of lymph or 20 μL of CM and a 20 s vortex. The scintillation method was validated by spiking blank lymph samples with low, medium and high

concentrations of ^{14}C-oleic acid or ^{3}H-oleic acid, and the measured concentrations were within 5% of the nominal concentration.

Bile samples were prepared for scintillation counting via addition of 100 μL of 30% hydrogen peroxide to 200 μL of bile in a 4 mL scintillation vial and incubation at 60 °C for 2 h to decolorize the bile. Three milliliters of Irgasafe plus was then added and the sample vortexed for 20 s. The scintillation method was validated by spiking blank bile samples with low, medium and high concentrations of ^{14}C-oleic acid or ^{3}H-oleic acid, and the measured concentrations were within 5% of the nominal concentration.

Calculations. The mass of TG, PL, exogenous ^{14}C or ^{3}H radiolabel or halofantrine transported in lymph or bile was calculated from the product of the measured concentration and the lymph or bile flow rate during each time period.

The transport of all endogenous and exogenous fatty acids in lymph and bile (expressed in terms of total fatty acid in moles) was calculated by assuming that each mole of TG and/or PL in lymph or bile contained 3 and 2 mol of fatty acid, respectively. Total fatty acid transport was thus equal to the addition of the number of moles of fatty acid transported in association with TG and/or PL. Endogenous fatty acid transport in intestinal lymph and bile was calculated by subtracting the mass of intraduodenal administered exogenous (radiolabeled) fatty acid recovered in each lymph and bile sample from the mass of total fatty acid recovered, as described previously.[20]

Statistical Analysis. Statistically significant differences were determined by ANOVA followed by Tukey's test for multiple comparisons at a significance level of $\alpha = 0.05$. Analyses were performed using SPSS for Windows versions 15.0. (SPSS Inc., Chicago, IL).

RESULTS

Effect of IV Infusion of Chylomicron on Intestinal Lymphatic Lipid and Drug Transport. IV infusion of CM significantly enhanced lymphatic transport of total fatty acids in animals administered either normal saline or oleic acid (14.2 μmmol) intraduodenally (Table 1 and Figure 2A,B). The rate of fatty acid appearance in the lymph was higher throughout the 0−6 h postdose period, and statistically significant differences were evident over the 0−1 h and 2−3 h period in the saline infused group and the 0−1 h and 3−4 h period in the group infused with oleic acid (Supplementary Figure 1 in the Supporting Information).

IV infusion of ^{14}C-CM also significantly ($\alpha < 0.05$) enhanced lymphatic transport of halofantrine more than 50% from a cumulative total of 5.1% to 8.0% of the dose over 6 h (Table 1 and Figure 2C). The increase in lymphatic halofantrine transport was most evident over the 4−6 h post dose period (data describing the rate of halofantrine lymphatic transport are given in Supplementary Figure 1C in the Supporting Information).

Impact of IV Infusion of Chylomicron on Biliary Lipid Output. The mean rate of fatty acid transport in bile was not significantly different in the groups receiving either intravenous CM or saline (4.6 ± 0.5 and 4.8 ± 0.8 μmol/h over the initial 3 h following bile duct cannulation, Figure 3A). IV infusion of CM therefore did not enhance PL fatty acid transport in bile (Table 1). In both the IV saline and CM infused groups, fatty acid transport in bile slowly declined over time (Figure 3B) following cannulation of the bile duct. This has been described previously and likely reflects depletion of the recirculating bile salt pool.[24]

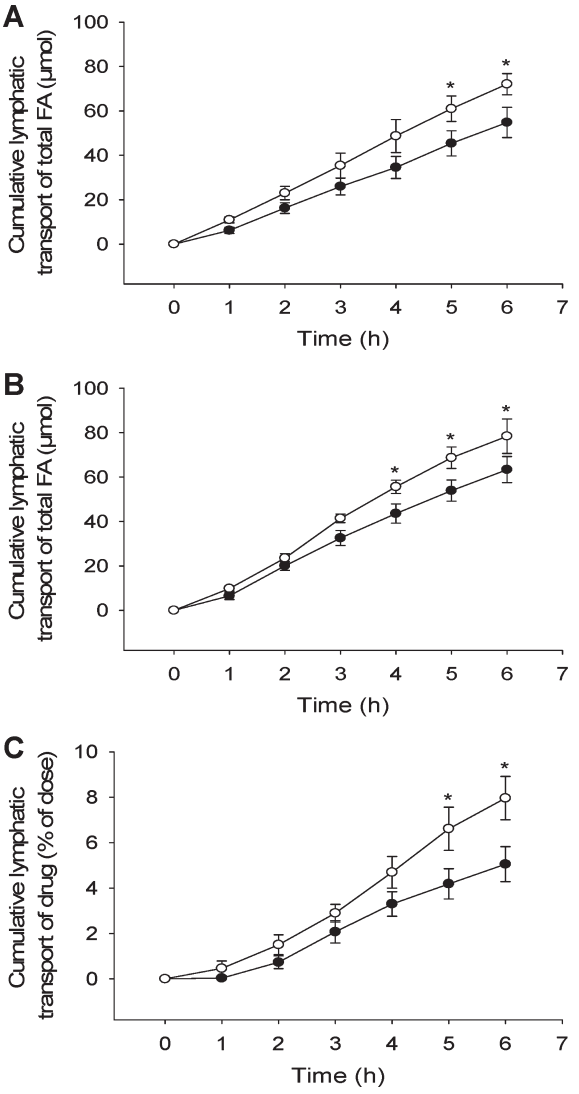

Figure 2. Cumulative lymphatic transport of total fatty acids (FA, μmol) versus time in control (●) or IV ^{14}C-CM (○) infused lymph-cannulated rats administered 2.8 mL/h saline into the intestine (panel A). Cumulative lymphatic transport of total FA (μmol) (panel B) and cumulative lymphatic transport of halofantrine (as a % of administered dose) (panel C) versus time in control (●) or IV ^{14}C-CM (○) infused lymph-cannulated rats administered 200 μg of halofantrine dispersed in 14.2 μmol of oleic acid in 5.6 mL of 0.2% Tween 80 over 2 h into the intestine. Data represent mean ± SEM for $n = 4$ rats. *Data significantly higher in IV ^{14}C-CM infused when compared to control rats at this time point ($\alpha < 0.05$).

Entry of Infused Chylomicron Lipids into Bile and Lymph Lipid Transport Pathways. Only a small proportion of intravenously infused ^{14}C-CM lipids (less than 0.3% of the dose) was recovered in the lymph regardless of whether animals were dosed intraduodenally with saline or oleic acid (Figure 4A). A negligible proportion (0.5%) of the fatty acids in the lymph were therefore derived from the IV infused CM (Figure 4B). Only small quantities (0.4% of the dose) of the IV infused ^{14}C-CM lipids were also recovered in the bile (Figure 4A), and <3% of the fatty acids in bile were derived from the infused ^{14}C-CM lipids

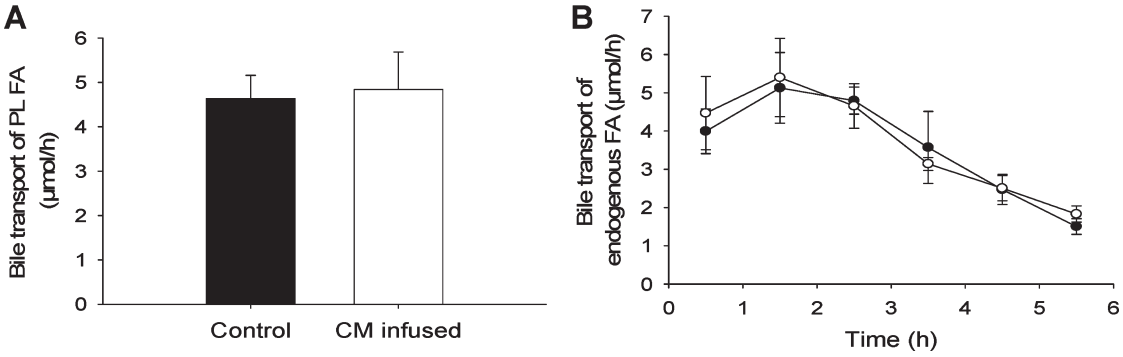

Figure 3. The mean rate of phospholipid (PL) fatty acid (FA) transport in bile over 0−3 h (μmol/h) (panel A) and the rate of PL FA transport in bile (μmol/h) versus time (panel B) in control (● or black) or IV ^{14}C-CM (○ or white) infused lymph-cannulated rats administered 2.8 mL/h saline into the intestine. Data represent mean ± SEM for n = 4 rats.

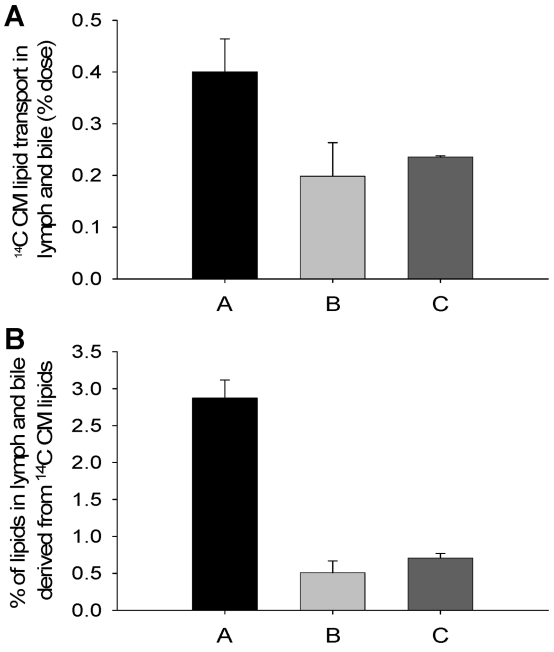

Figure 4. (Panel A) % of the administered dose of radiolabeled ^{14}C-CM lipids which is transported into the bile or lymph over 6 h and (panel B) % of the fatty acids (FA) in bile and lymph which are derived from IV infused ^{14}C-CM lipids over 6 h after administration of ^{14}C-CM via continuous intravenous infusion and 2.8 mL/h saline or 200 μg of halofantrine dispersed in 14.16 μmol of oleic acid (OA) in 5.6 mL of 0.2% Tween 80 (lymph only) over 2 h into the intestine of lymph or bile duct cannulated rats. Column A (black bar) shows data for bile, column B (light gray) shows data for lymph in rats administered saline, and column C (dark gray) shows data for lymph in rats administered OA. Data represent mean ± SEM for n = 4 rats.

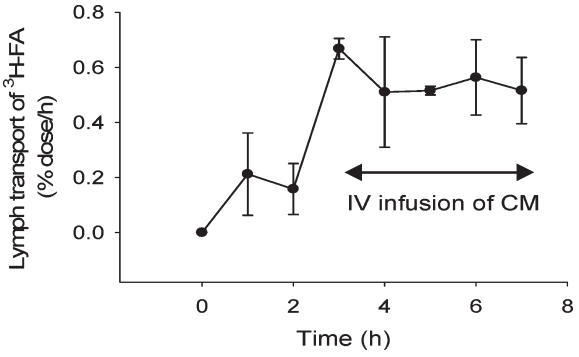

Figure 5. Rate of transport of IV administered ^{3}H-oleic acid (as a marker for plasma fatty acids (FA)) into the lymph (% of the mass administered each hour which is recovered in the lymph each hour) versus time in lymph-cannulated rats administered 2.8 mL/h saline into the intestine and an IV bolus of ^{3}H-oleic acid into the jugular vein every hour throughout the experiment. Rats were administered ^{14}C-CM via IV infusion after 4 h. Data represent mean ± SEM for n = 4 rats.

(Figure 4B). The IV infused ^{14}C-CM lipids therefore made little direct contribution to lipid transport in either lymph or bile.

Effect of IV Infusion of Chylomicron on Lymphatic Transport of Plasma Fatty Acids. Figure 5 depicts the proportion of an hourly administered IV dose of ^{3}H oleic acid (as a marker for plasma fatty acids) that was transported into the lymph each hour. Steady state fatty acid transport was reached 3−4 h into the period of IV dosing of ^{3}H oleic acid, and only a small proportion (0.5% of the dose at steady state) of the IV infused ^{3}H oleic acid was transported into lymph. After 4 h of IV dosing of ^{3}H oleic acid, a continuous IV infusion of ^{14}C-CM was initiated. IV infusion of the ^{14}C-CM did not increase transport of the IV infused ^{3}H-oleic acid into the lymph, and steady state lymphatic transport of ^{3}H-oleic acid remained unchanged. These results suggest that IV infusion of ^{14}C-CM did not enhance transport of fatty acids in the plasma into lymph.

Effect of IV Infusion of Chylomicron on Lymphatic Transport of Apical (Duodenal) Lipids. IV infusion of CM significantly increased the rate of exogenous (duodenal) ^{3}H-oleic acid transport into the lymph over the 1−4 h postdose period (Supplementary Figure 2 in the Supporting Information) and therefore increased the cumulative lymphatic recovery of exogenous ^{3}H-oleic acid (Figure 6A,B, Table 1). Since the total administered quantity of ^{3}H-oleic acid was relatively small, however, this increase was modest: from 6.2 to 8.8 μmol in rats given the IV infusion of ^{14}C-CM compared to the rats administered saline IV (Table 1). In contrast, endogenous fatty acid transport into the lymph of rats infused intraduodenally with either saline or oleic acid increased substantially (although not significantly due to the variability in endogenous lipid transport) in the groups administered the ^{14}C-CM rather than saline IV (Table 1). IV infusion of ^{14}C-CM therefore increased total fatty acid transport into the lymph (Table 1), and these increases appear

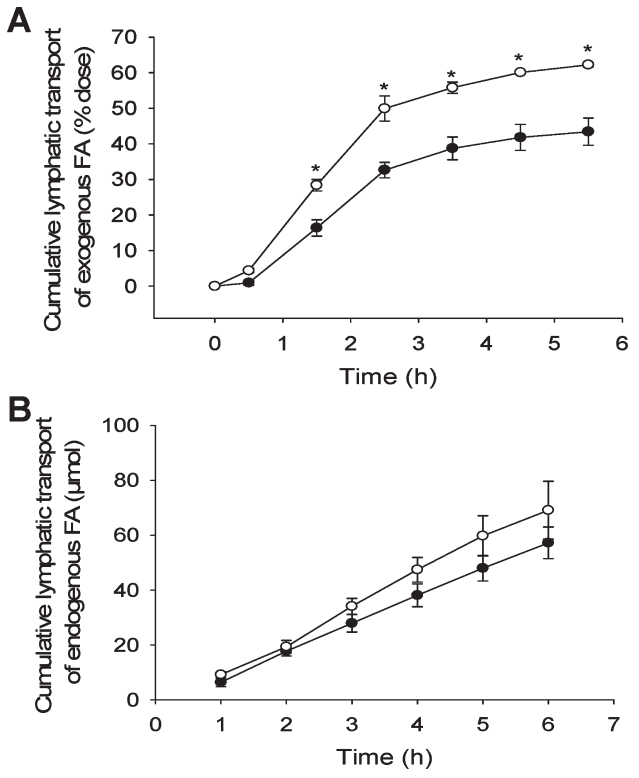

Figure 6. (Panel A) Cumulative lymphatic transport of exogenous ^{3}H-oleic acid (% of administered dose) and (panel B) cumulative lymphatic transport of endogenous fatty acid (FA) (μmol) versus time in control (●) or IV ^{14}C-CM (○) infused lymph-cannulated rats administered 200 μg of halofantrine dispersed in 14.2 μmol of ^{3}H-oleic acid in 5.6 mL of 0.2% Tween 80 over 2 h into the intestine. Data represent mean ± SEM for n = 4 rats. *Data significantly higher in IV ^{14}C-CM infused when compared to control rats at this time point (α < 0.05).

to result from enhanced lymphatic transport of both intraduodenally administered exogenous fatty acid and endogenous fatty acid.

DISCUSSION

Hypertriglyceridemia is a common dyslipidemia which contributes to an increased risk of death from atherosclerotic CVD.[2−4] Hypertriglyceridemia is present in both the fed and fasted state in MetS and appears to result from a combination of increased TRL assembly in the liver and intestine and reduced clearance of TRL.[5,6] Intestinal lipoprotein formation is stimulated by increases in plasma fatty acids suggesting that raised plasma fatty acid concentrations may contribute to alterations in intestinal lipid processing in MetS.[5,7,8,25] Here we examined whether increases in systemic TG and TRL concentrations (as occur in MetS) influence intestinal lipid processing and lipoprotein assembly. The data suggest the potential for a positive feedback mechanism in lipid absorption where increased plasma levels of TG and TRL stimulate enhanced incorporation of apical (luminal) lipids into intestinal TRL, promote intestinal lymphatic lipid transport and therefore potentially perpetuate hypertriglyceridemia. In the current studies these positive feedback mechanisms also appeared to impact on the lymphatic transport of a highly lipophilic drug and may have relevance for the absorption of other highly lipophilic, lymphatically transported drugs.

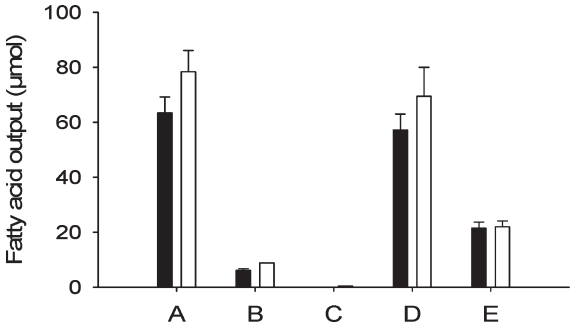

Figure 7. Cumulative lymphatic transport of total fatty acids (FA) (A), intraduodenally (ID) administered exogenous ^{3}H-oleic acid (OA) (B), ^{14}C-OA from chylomicron (C) and endogenous FA (D) and cumulative biliary transport of phospholipid FA (E) (all in μmol) over 6 h in saline control (black bars) or IV ^{14}C-OA labeled CM (white bars) infused lymph cannulated rats administered 200 μg of halofantrine dispersed in 14.16 μmol of ^{3}H-OA in 5.6 mL of 0.2% Tween 80 over 2 h ID or bile duct cannulated rats administered 2.8 mL/h saline ID. Data represent mean ± SEM for n = 4 rats.

Increasing systemic concentrations of TRL via IV infusion of donor chylomicron enhanced the intestinal lymphatic transport of both endogenous and intraduodenally administered exogenous lipids (Table 1, Figure 7). A series of studies were therefore conducted to explore the mechanism by which increasing systemic TG and TRL concentrations enhanced intestinal lipoprotein formation, in particular, whether the increased lipid levels in the lymph were derived from lipids taken up into the enterocyte across the apical membrane (such as intraduodenally administered exogenous lipids, endogenous biliary lipids or endogenous lipids derived from other sources such as sloughed enterocytes) or the basolateral membrane (such as plasma fatty acids or chylomicron remnants).[17,20,21,26] Surprisingly few of the IV infused ^{14}C CM lipids were recovered in the lymph directly (Figures 4 and 7). Uptake of plasma fatty acids into lymph was also not stimulated by CM infusion (Figures 5 and 7), suggesting that the increases in lymphatic lipid transport were not a result of basolateral uptake of CM remnants or plasma fatty acids into the enterocyte. CM infusion did, however, enhance the proportion of intraduodenally infused (exogenous ^{3}H) lipids that were transported into the lymph (Figures 6 and 7). The majority of the increase in the quantity of lipids in the lymph resulted from increases in lymphatic transport of endogenous and not exogenous lipids (Figure 7), presumably due to the relatively low quantities of ^{3}H exogenous lipid administered. Since there was no increase in lymphatic lipids from basolateral sources (i.e., CM remnants or fatty acids), the increase in endogenous lipids likely resulted from an increase in the efficiency of lipid absorption across the apical membrane of the enterocyte and transport into the intestinal lymph (Figure 8). Endogenous lipids stored within the enterocyte pools may also have been redirected into the lymph although the contribution from this source is expected to be minimal as animals were fasted overnight prior to experiments.

Consistent with the alterations in lymph lipid transport seen here in animals administered CM, previous studies have described differences in exogenous and endogenous lymphatic lipid transport in animal models of MetS, obesity and diabetes.[10,12,27] Elevation of plasma fatty acid concentrations in humans (and animals) by infusion of intralipid and heparin has also been shown

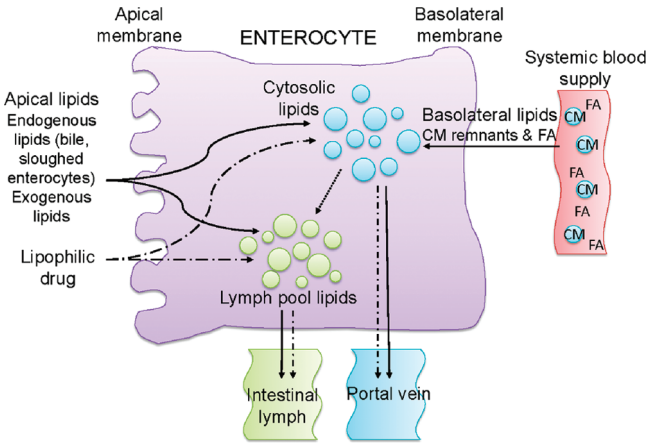

Figure 8. Positive feedback between systemic lipid levels and intestinal lipid processing. Lipids enter the intestinal absorptive cells (enterocytes) across either the basolateral or apical membrane. Apical lipids are sourced from the intestinal lumen and include endogenous lipids from bile and sloughed enterocytes as well as exogenous lipids such as those from the diet or lipid-based formulations. Basolateral lipids are sourced from the intestinal blood supply and include systemic lipids such as plasma fatty acids (FA) and chylomicron (CM) remnants. Apical lipids may be absorbed, transported across the cytosol and taken up into the systemic circulation via the portal vein or may be assembled into lipoproteins in the lymph lipid pool (green circles) and be transported to the systemic circulation via the intestinal lymph system. Basolateral lipids are incorporated into a cytosolic pool of lipids (blue circles) and are predominantly transported from the enterocyte via the portal vein (although some may also enter the lymph lipid pool). Highly lipophilic drugs may be incorporated into the process of apical and basolateral lipid transport and transported to the systemic circulation via either the portal vein or intestinal lymph (represented by dashed lines). Increasing systemic lipid concentrations via IV infusion of CM increases intestinal lipoprotein assembly and lymphatic lipid transport. However, IV infused CM are not incorporated into the lymph lipid pool, assembled into lipoproteins and transported via the lymph. Instead IV infusion of CM appears to cause a change in the processing of apical lipids such that an increased proportion of apical lipids are incorporated into the lymph lipid pool, assembled into lipoproteins and transported via the lymph. This leads to a coincident increase in drug incorporation into the lymph lipid pool and drug transport via the lymph. The mechanism by which IV infusion of CM can influence intestinal processing of apical lipids is as yet unidentified but may be a result of upregulation or mobilization of intestinal proteins involved in lipid transport, synthesis and lipoprotein assembly in the presence of increased systemic or intestinal concentrations of lipids.

to increase the levels of intestinally derived ApoB48 in the plasma.[5,7,8,25] Since plasma fatty acids are increased as a result of insulin resistance, increased plasma fatty acids have been hypothesized to contribute to the perturbation in intestinal lipoprotein processing in insulin resistant humans with MetS.[5] The current study is consistent with these previous suggestions (i.e., that MetS may lead to changes in intestinal lipoprotein production) but further suggests that increased systemic concentrations of TG and TRL stimulate increased lipoprotein formation via improvements in the efficiency of lymphatic transfer of endogenous and exogenous lipids from the gastrointestinal tract or enterocyte-based lipid stores.

In animal models of MetS, the levels of expression of a number of enzymes and transporters involved in intestinal lipid transport and lipoprotein formation are altered.[11,13] It is possible, therefore, that increases in systemic concentrations of TRL and TG stimulate changes to the levels of expression and/or mobilization of proteins involved in lipid absorption in turn leading to increases in efficiency of lymphatic transport of luminal lipids. Alternatively, increased systemic lipid levels may have led to changes in the levels of endogenous mediators of lipid metabolism (such as insulin[28,29] or incretins[30]) leading to an indirect alteration in intestinal lipid transport. Further studies will address these hypotheses.

Increasing plasma levels of TRL via IV infusion of chylomicrometers also increased the lymphatic transport of halofantrine (Figure 4). Many studies have shown an association between lymphatic transport of lipids and drugs.[20,31,32] The increase in lymphatic transport of halofantrine in the animals administered an IV infusion of CM, when compared to saline, was therefore likely a result of increased lymphatic lipid transport (Table 1, Figure 4). A previous study from this laboratory has also suggested that apical but not basolateral lipid sources may be more able to support lymphatic transport of halofantrine,[20] and this is consistent with the current finding that IV infusion of CM appears to increase the transport of apical but not basolateral lipids into the lymph (and coincidentally facilitate lymphatic drug transport).

Changes to intestinal lipid processing in animals with raised systemic concentrations of TRL and TG may therefore impact on the intestinal absorption of lymphatically transported, highly lipophilic drugs (typically drugs with a log $P > 5$ and long chain TG solubility >50 mg/g,[18] or high affinity for TRL[33]). This may be clinically important when, for example, enhanced lymphatic transport facilitates avoidance of first pass through the liver and therefore reduces the extent of first pass hepatic metabolism,[34,35] or where transport via the lymph within lipoproteins impacts on systemic pharmacokinetics and distribution to therapeutic and toxic sites.[15] Since absorption and bioavailability assessments for many new drug candidates are not conducted in patients with hypertriglyceridemia and/or MetS, yet the increasing prevalence of MetS suggests that almost all patient populations will include some patients with MetS, the current studies raise the possibility that, for some highly lipophilic drugs, the extent and mechanism of absorption (portal blood versus intestinal lymph) may differ in the broader patient population when compared with the clinical trial population.

■ ASSOCIATED CONTENT

Supporting Information. Supplementary Figure 1 showing rate of lymphatic transport of total fatty acids and halofantrine in control and IV CM infused rats. Supplementary Figure 2 showing rate of lymphatic transport of exogenous ^{3}H-oleic acid and endogenous fatty acids in control and IV CM infused rats. This material is available free of charge via the Internet at http://pubs.acs.org.

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