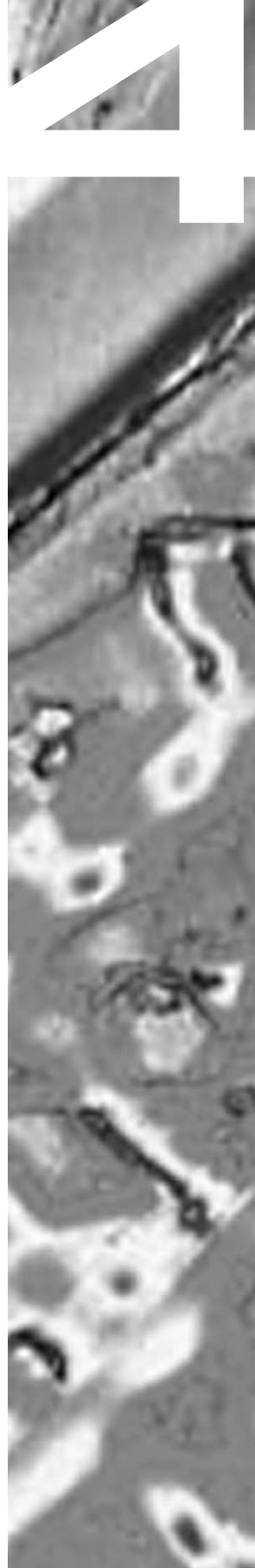


Lymphatic chylomicron size is inversely related to biliary phospholipid secretion in mice

Conditionally accepted for publication in Am J Physiol 2005

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ABSTRACT

Background: Biliary phospholipids (PL) stimulate dietary fat absorption by facilitating intraluminal lipid solubilization and by providing surface components for chylomicron assembly. Impaired hepatic PL availability induces secretion of large VLDL, but it is unclear whether chylomicron size depends on biliary PL availability. Biliary PL secretion is absent in *Mdr2*^{-/-} mice, whereas it is strongly increased in essential fatty acid (EFA) deficient mice. We investigated lymphatic chylomicron size and composition in mice with absent (*Mdr2*^{-/-}) or enhanced (EFA deficiency) biliary PL secretion and in their respective controls, under basal conditions and during intraduodenal lipid administration.

Methods: EFA deficiency was induced by feeding mice a high-fat EFA-deficient diet for eight weeks. Lymph was collected by mesenteric lymph duct cannulation, with or without intraduodenal lipid administration. Lymph was collected in 30-minute fractions for 4 hours, and lymphatic lipoprotein size was determined by dynamic light scattering techniques. Lymph lipoprotein subfractions were isolated by ultracentrifugation and lipid composition was measured.

Results: Lymphatic lipoproteins were significantly larger in *Mdr2*^{-/-} mice than in *Mdr2*^{+/+} controls, both without (+50%) and with (+25%) intraduodenal lipid administration. In contrast, EFA-deficient mice secreted significantly smaller lipoproteins into lymph than EFA-sufficient controls (164±28 nm vs. 234±49 nm; p<0.001). Chylomicron size increased during fat absorption in both EFA-deficient and EFA-sufficient mice, but the difference between the groups persisted.

Conclusions: Present results strongly suggest that the availability of biliary PL is a major determinant of the size of intestinally produced lipoproteins, both under basal conditions and during lipid absorption. Altered chylomicron size may have physiological consequences for postprandial chylomicron processing.

INTRODUCTION

Biliary phospholipids (PL) have a well-documented function in transport of dietary lipids from the intestinal lumen into lymph. Apart from their role in intraluminal lipid solubilization, biliary phospholipids have been implicated as crucial components for adequate intestinal chylomicron (CM) assembly and secretion into lymph⁽²³⁾. Biliary phosphatidylcholines (PC), which compose ~95% of biliary phospholipids, provide the main source for chylomicron surface coating^(13; 17; 20), and the supply of bile PC to the intestine increases the synthesis of apoB48, the apolipoprotein needed for chylomicron formation^(6; 14). Additionally, the high essential fatty acid (EFA) content of biliary PC may be required for maintenance of normal intestinal mucosal membrane composition and function^(7; 22). Reduced availability of PC for hepatic VLDL assembly in rats has been associated with decreased VLDL secretion and with assembly of relatively large VLDL particles⁽²⁴⁾. It is well-established that fat absorption and intestinal lipoprotein secretion are strongly impaired in situations of disturbed bile formation, such as cholestasis. Studies in rats with interrupted enterohepatic circulation by means of permanent bile diversion⁽²³⁾ demonstrated a substantial lipid accumulation in intestinal mucosal cells. Administration of bile salts to these bile-diverted rats partially restored lymphatic lipid transfer, but only when both bile salts and biliary phospholipids were supplemented, lymphatic lipid transport was fully reinstated. Yet, a direct relationship between biliary phospholipid availability and intestinal lipoprotein size has not been established.

We recently characterized fat absorption in two mouse models with altered biliary phospholipid secretion⁽³⁰⁾. EFA deficiency increases biliary phospholipid secretion by ~80%, in conjunction with a decrease by 30-40% in dietary lipid absorption. In *Mdr2*^{-/-} mice, in which biliary phospholipid secretion is absent⁽²¹⁾, plasma appearance of enterally administered lipid is delayed and lipid accumulates in enterocytes. Quantitatively, however, lipid absorption is unaffected in these mice⁽²⁶⁾.

In the present study, we investigated whether and to what extent quantitative or qualitative alterations in biliary phospholipid secretion affect chylomicron secretion into lymph. Chylomicron size and composition were measured after mesenteric lymph duct cannulation, using the aforementioned mouse models with altered intraluminal biliary phospholipid availability, i.e., *Mdr2*^{-/-} mice (no biliary PL secretion), EFA-deficient mice (increased biliary PL secretion) and corresponding control mice. Our results show that the absence of biliary phospholipid secretion in mice is accompanied by production of intestinal lipoproteins of increased size and decreased phospholipid content, whereas EFA deficiency, associated with increased biliary phospholipid secretion, has the opposite effect

MATERIALS AND METHODS

Animals

Mice homozygous for disruption of the multidrug resistance gene-2 (*Mdr2*^{-/-}) and wildtype (*Mdr2*^{+/+}) mice with a free virus breed (FVB) background were obtained from the breeding colony at the Central Animal Facility, Academic Medical Center, Amsterdam, the Netherlands⁽⁶⁾. For dietary induction of EFA deficiency in mice, male wildtype FVB mice were obtained from Harlan (Horst, the Netherlands). All mice were 8 weeks old, weighed 25 to 35 gram and were housed in a light-controlled (lights on 6 AM - 6 PM) and temperature-controlled (21°C) facility. Mice were allowed tap water and chow *ad libitum*. The experimental protocols were approved by the Ethics Committee for Animal Experiments, University of Groningen, the Netherlands.

Experimental diets

The standard laboratory low-fat chow (RMH-B, Arie Blok BV, Woerden, the Netherlands) contained 14 energy% fat. The high-fat EFA-deficient (EFAD) diet contained 34 energy% fat, and had the following fatty acid composition: 41.4 mol% palmitic acid (C16:0), 47.9 mol% stearic acid (C18:0), 7.7 mol% oleic acid (C18:1n-9) and 3 mol% linoleic acid (C18:2n-6). An isocaloric EFA-sufficient (EFAS) diet was used as control diet, containing 37 energy% fat with 32.1 mol% C16:0, 5.5 mol% C18:0, 32.2 mol% C18:1n-9 and 30.2 mol% C18:2n-6 (diet numbers 4141.08 (EFAD) and 4141.07 (EFAS), Arie Blok BV, Woerden, the Netherlands).

Experimental procedures

Induction of EFA deficiency in mice

All mice were fed standard laboratory chow from weaning. For induction of EFA deficiency, wildtype FVB mice were fed the EFA-deficient (EFAD) diet for eight weeks. A control group of FVB mice was fed the isocaloric EFA-sufficient (EFAS) diet for eight weeks. This method for induction of EFA deficiency was previously applied in mice and characterized by our group^(15; 29; 30).

Mesenteric lymph duct cannulation in mice

Cannulation of the mesenteric lymph duct was performed according to procedures described by Wang *et al.*^(27; 28) Non-fasted mice were anesthetized with halothane/NO₂ and dorsally arched over a cotton cylinder for optimal visualization of the mesenteric lymph duct. After extra-abdominal displacement of the intestine, the common mesenteric lymph duct was exposed by removal of surrounding tissues and membranes using a blunt mini-kocher. A 0.305 x 0.635 mm (id x od) silicone catheter was

introduced through the abdominal wall and positioned parallel to the lymph duct. Subsequently, the catheter was carefully inserted into a small incision in the lymphatic duct and fixated by drops of tissue glue at the junction of lymph duct and catheter. A subgroup of mice subsequently received an intraduodenal 200 μ l bolus of a parenteral lipid emulsion (Intralipid® 20%, Fresenius Kabi, 's Hertogenbosch, the Netherlands), mixed with glucose 3.3% and NaCl 0.3% (50:50), after collection of a 30-minute baseline lymph sample. After repositioning the intestine, the abdominal incision was closed with 8-10 sutures and mice were placed in restrainer cages in a 37°C incubator. Analgesia was maintained with intraperitoneal injection of buprenorfine (Temgesic®) 0.1 mg/kg. Lymph was collected by gravity into EDTA-containing microtubes, in 30-minute fractions for 4 hours after cannulation of the mesenteric lymph duct.

Determination of lymphatic lipoprotein size and composition

Lymphatic lipoprotein size and volume distribution profiles were analyzed within 6 hours after lymph collection by dynamic light scattering techniques, using a Nicomp model 370 submicron particle analyzer (Nicomp Particle sizing Systems, Santa Barbara, CA, USA). Particle diameters were calculated from the volume distribution patterns provided by the analyzer. The lymphatic chylomicron (CM) fraction ($d < 1.006$) was isolated after complementing collected lymph with a 1.006 g/ml NaCl solution containing 0.02% NaN_3 to a final volume of 1 ml, and subsequent centrifugation at 40000 rpm at 4°C in an Optima TM LX table top centrifuge (Beckman Instruments, Inc., Palo Alto, CA, USA) for 15 minutes. The top layer containing the chylomicron fraction was isolated by tube slicing and the volume was recorded by weight. The remaining lymph solution was again complemented with 1.006 g/ml NaCl to a final volume of 1 ml and centrifuged at 120000 rpm for 1 hour and 40 minutes for isolation of the very low density lipoprotein (VLDL) fraction.

Analytical techniques

Plasma lipids were measured using commercially available assay kits from Roche (Mannheim, Germany) for triglycerides and total cholesterol, and from WAKO chemicals GmbH (Neuss, Germany) for phospholipids.

Calculations and statistics

All results are presented as means \pm S.D. for the number of animals indicated. Data were statistically analyzed using Student's t-test or ANOVA test with post-hoc Bonferroni correction. Level of significance was set at $p < 0.05$. Analyses were performed using SPSS for Windows software (SPSS, Chicago, IL).

RESULTS

Lymph flow was highly variable between and within individual mice, ranging between 1.0 and 15.8 $\mu\text{l}/\text{min}$ per 100 g body weight, but no significant differences in lymph flow were observed between experimental groups and their respective controls (data not shown).

In *Mdr2*-Pgp-deficient (*Mdr2*^{-/-}) mice, biliary phospholipid secretion into the intestine is virtually absent (<0.5 nmol/min per 100 g)⁽²¹⁾. Voshol *et al.* described that post-prandial plasma appearance of chylomicrons is impaired in *Mdr2*^{-/-} mice⁽²⁶⁾. Figure 1 shows that non-fed *Mdr2*^{-/-} mice secreted chylomicrons of significantly greater size (+51%) into lymph than *Mdr2*^{+/+} controls during the first 2 hours of lymph collection (131 ± 23 vs. 87 ± 26 for *Mdr2*^{-/-} and *Mdr2*^{+/+} mice, respectively, $p < 0.001$).

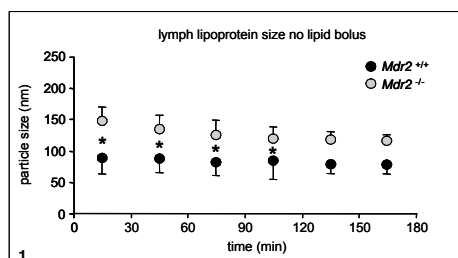


Figure 1: Lymphatic lipoprotein size (nm), measured by dynamic light scattering, in lymph of non-fasted *Mdr2*-Pgp-deficient (*Mdr2*^{-/-}) mice (grey circles) and *Mdr2*^{+/+} mice (black circles). Lipoprotein size was measured in 30-minute fractions of collected mesenteric lymph, and lymph was collected for 3 hours. Data represent means ± SD of 6-10 mice per group. * $p < 0.05$ for differences between *Mdr2*^{+/+} and *Mdr2*^{-/-} mice.

Concentrations of triglycerides (TG), phospholipids (PL) and cholesterol were significantly lower in lymph of *Mdr2*^{-/-} mice than of controls (Figure 2a). The decrease in PL and cholesterol content (-57% and -93%, respectively) was considerably more pronounced than that of triglyceride (-26%). In the isolated lymphatic chylomicron (Figure 2b) and very low density lipoprotein (VLDL) fractions (Figure 2c), similar differences in lipid content were detected: in the first hour of lymph collection, concentrations of phospholipid, triglyceride and cholesterol were decreased in lipoproteins of *Mdr2*^{-/-} mice compared to controls, but the relative triglyceride concentration slightly increased (CM fraction: 86 ± 3% vs. 78 ± 7%; VLDL fraction: 85 ± 1% vs. 80 ± 1% for *Mdr2*^{-/-} and *Mdr2*^{+/+} mice, respectively, $p < 0.005$). The core-to-surface ratio of lymphatic lipoproteins (i.e., [TG]/[PL], Figure 2d) was increased in total lymph as well as in the isolated CM and VLDL fractions of *Mdr2*^{-/-} mice during the first hour of lymph collection, indicating secretion of larger lipoproteins in *Mdr2*^{-/-} mice than in controls. In the later fractions, a similar trend was observed, but the increased core-surface ratio only reached significance in the VLDL fraction.

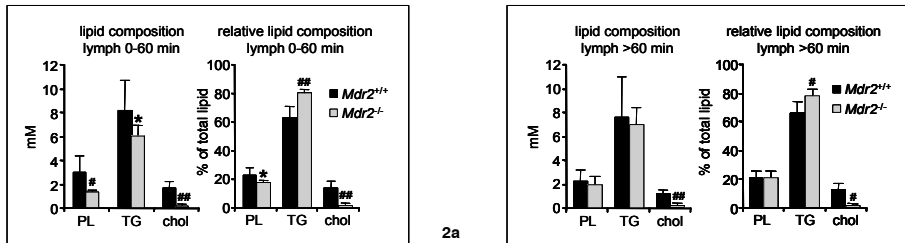


Figure 2a: Absolute (mM) and relative (%) concentrations of phospholipid (PL), triglyceride (TG) and cholesterol (chol) in lymph of *Mdr2*^{+/+} mice (grey bars) and *Mdr2*^{-/-} mice (black bars) in the first hour of lymph collection and after the first hour. Data represent means \pm SD of 6-10 mice per group. **p*<0.05, #*p*<0.005, ##*p*<0.001 for differences between *Mdr2*^{+/+} and *Mdr2*^{-/-} mice.

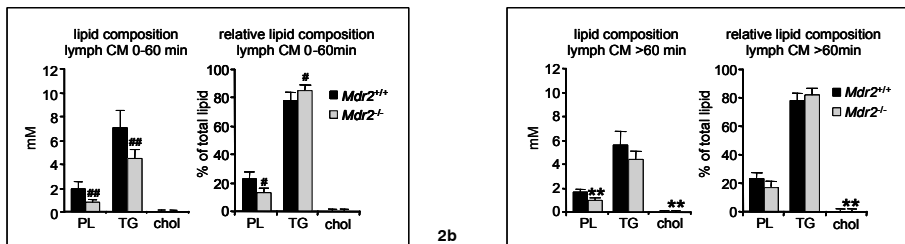


Figure 2b: Absolute (mM) and relative (%) concentrations of phospholipid (PL), triglyceride (TG) and cholesterol (chol) in the isolated lymphatic chylomicron (CM) fraction of *Mdr2*^{+/+} mice (grey bars) and *Mdr2*^{-/-} controls (black bars) in the first hour of lymph collection and after the first hour. Data represent means \pm SD of 6-10 mice per group. **p*<0.01, #*p*<0.005, ##*p*<0.001 for differences between *Mdr2*^{+/+} and *Mdr2*^{-/-} mice.

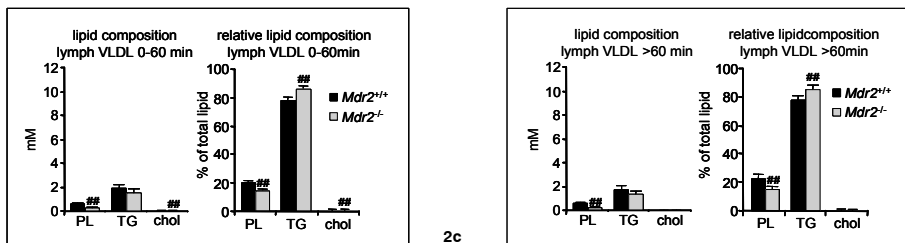


Figure 2c: Absolute (mM) and relative (%) concentrations of phospholipid (PL), triglyceride (TG) and cholesterol (chol) in the isolated lymphatic very low density lipoprotein (VLDL) fraction of *Mdr2*^{+/+} mice (grey bars) and *Mdr2*^{-/-} controls (black bars) in the first hour of lymph collection and after the first hour. Data represent means \pm SD of 6-10 mice per group. **p*<0.01, #*p*<0.005, ##*p*<0.001 for differences between *Mdr2*^{+/+} and *Mdr2*^{-/-} mice.

We also assessed the effects of absence of biliary phospholipids on chylomicron formation during the active phase of lipid absorption. After collection of a 30-minute baseline sample, a 200 μ l lipid bolus was administered intraduodenally to *Mdr2*^{-/-} and *Mdr2*^{+/+} mice. Similar to the non-fed state, the average diameter of lymph lipoproteins during lipid absorption was significantly larger in *Mdr2*^{-/-} mice than in *Mdr2*^{+/+} controls (183 \pm 41 nm vs. 152 \pm 44 nm; *p*<0.001). The increase in lipoprotein size after lipid administration occurred more rapidly in *Mdr2*^{-/-} mice (Figure 3).

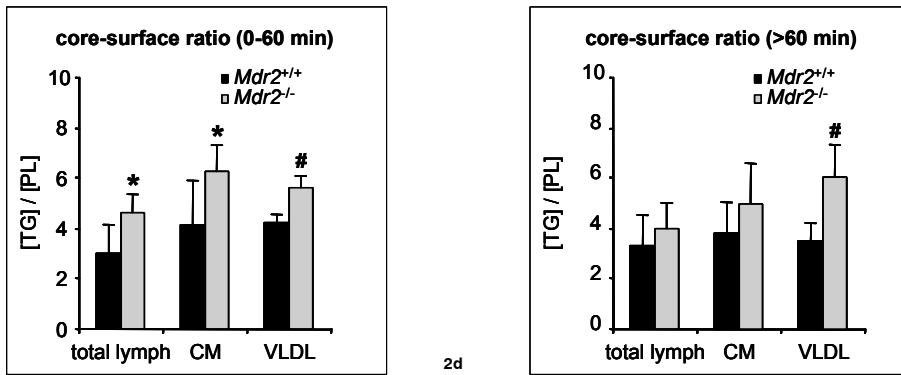


Figure 2d: Core-to-surface ratio, estimated by the ratio of triglyceride (TG) and phospholipid (PL) concentration (mM) in total lymph and in the isolated lymphatic chylomicron (CM) and very low density lipoprotein (VLDL) fractions of *Mdr2*^{+/+} mice (grey bars) and *Mdr2*^{-/-} mice (black bars) in the first hour of lymph collection and after the first hour. Data represent means \pm SD of 6-10 mice per group. * $p < 0.05$, # $p < 0.005$ for differences between *Mdr2*^{+/+} and *Mdr2*^{-/-} mice.

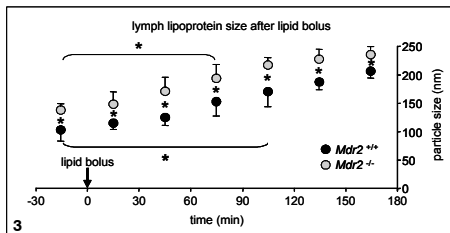


Figure 3: Lymphatic lipoprotein size (nm), determined by dynamic light scattering, in lymph of *Mdr2*^{+/+} mice (grey circles) and *Mdr2*^{-/-} controls (black circles) during active lipid absorption. Mesenteric lymph was collected and lipoprotein size was measured in 30-minute fractions, prior to, and for 3 hours after intraduodenal lipid bolus administration. Data represent means \pm SD of 5-9 mice per group. * $p < 0.05$ for differences between *Mdr2*^{+/+} and *Mdr2*^{-/-} mice

Figure 4 shows the absolute and relative lipid concentrations in lymph, at baseline and 1 and 2 hours after intraduodenal lipid administration. Absolute phospholipid, triglyceride and cholesterol concentrations (Figure 4a) were significantly lower in *Mdr2*^{-/-} mice than in controls, and did not increase over time. Relatively, however, lymph of *Mdr2*^{-/-} mice contained more triglyceride and less cholesterol and phospholipid than that of *Mdr2*^{+/+} controls (Figure 4b), suggesting the presence of larger particles. Indeed, core-to-surface ratio's were increased in *Mdr2*^{-/-} mice compared to controls (4.1 ± 1.4 vs. 2.7 ± 0.9 , $p < 0.001$; Figure 4c). Thus, a quantitative decrease in biliary phospholipid secretion in *Mdr2*^{-/-} mice was associated with secretion of larger lymphatic lipoproteins.

To assess the effects of increased biliary phospholipid secretion, we measured lymphatic lipoprotein size and composition in mice after dietary induction of EFA deficiency. Previously, we characterized the effects of EFA deficiency on fat absorption and biliary phospholipid secretion⁽³⁰⁾. EFA-deficient (EFAD) mice have a dietary lipid malabsorption ranging between 60-70% of the amount ingested, combined with a 70% increased bile flow and an 83% increased biliary phospholipid excretion. Biliary

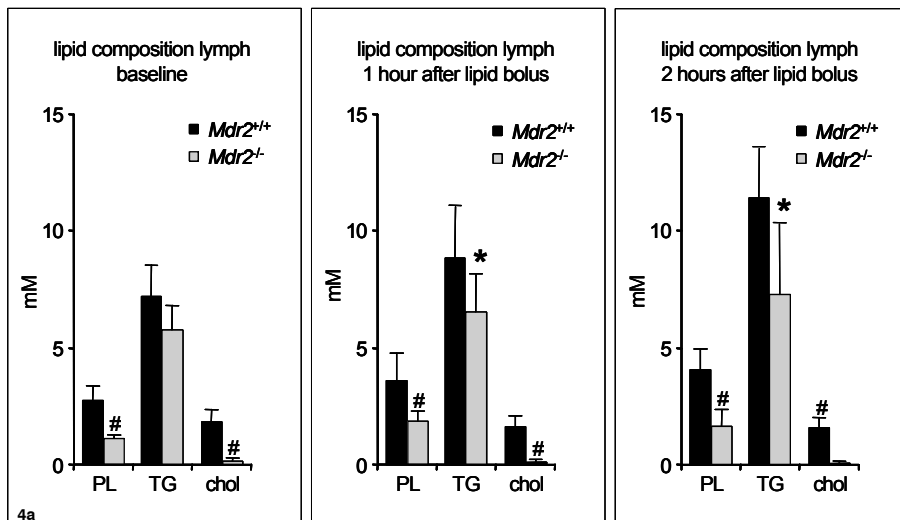


Figure 4a: Concentrations of PL, TG and cholesterol (chol) in mM, in lymph of *Mdr2*^{-/-} mice (grey bars) and *Mdr2*^{+/+} controls (black bars) at baseline, and at 1 and 2 hours after intraduodenal lipid administration. Data represent means ± SD of 5-9 mice per group. *p < 0.05, #p < 0.005 for differences between *Mdr2*^{-/-} and *Mdr2*^{+/+} mice.

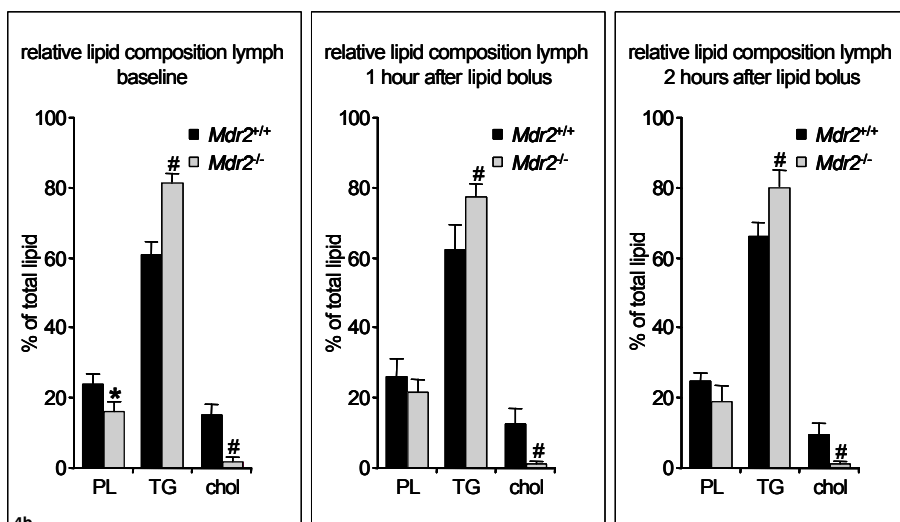


Figure 4b: Relative concentrations of PL, TG and cholesterol, expressed as % of total lipid, in lymph of *Mdr2*^{-/-} mice (grey bars) and *Mdr2*^{+/+} controls (black bars) at baseline, and at one and two hours after intraduodenal lipid administration. Data represent means ± SD of 5-9 mice per group. *p < 0.05, #p < 0.005 for differences between *Mdr2*^{-/-} and *Mdr2*^{+/+} mice.

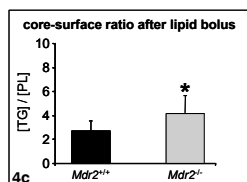


Figure 4c: Core-to-surface ratio, estimated by the ratio of triglyceride (TG) and phospholipid (PL) concentrations (mM) in lymph of *Mdr2*^{-/-} mice (grey bars) and *Mdr2*^{+/+} controls (black bars). Data represent means ± SD of 5-9 mice per group. *p < 0.001 for differences between *Mdr2*^{-/-} and *Mdr2*^{+/+} mice.

phospholipid acyl chains of EFA-deficient mice contained significantly less essential fatty acids and their long-chain metabolites (i.e., C18:2n-6, C18:3n-6 and C20:4n-6), and more non-essential fatty acids (C16:1n-7, C18:1n-7, C18:1n-9) than EFA-sufficient controls (Figure 5a). Figure 5b shows that at baseline, before enteral lipid administration, lymphatic chylomicrons of EFA-deficient mice were significantly smaller (-32%) than those of EFA-sufficient controls. This decreased particle size in EFA-deficient mice persisted during the active phase of fat absorption, i.e., for two hours after lipid bolus administration (164 ± 28 nm vs. 234 ± 49 nm; $p < 0.001$). Lymph of EFA-deficient mice contained, both absolutely and relatively, more phospholipid and less triglyceride and cholesterol than lymph of EFA-sufficient controls (Figure 6a and 6b). This was associated with a lower core-to-surface ratio in EFA-deficient mice (3.4 ± 0.9 vs. 5.7 ± 1.6 for EFA-deficient and EFA-sufficient mice, respectively; $p < 0.001$, Figure 6c), indicating secretion of smaller lymphatic lipoproteins.

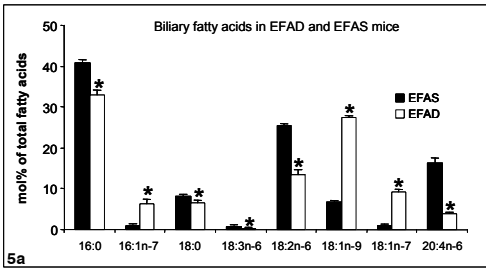


Figure 5a: Relative fatty acid composition of biliary phospholipids from EFA-deficient (EFAD, open bars) and EFA-sufficient (EFAS, black bars) mice. Individual concentrations of palmitic acid (C16:0), palmitoleic acid (C16:1n-7), stearic acid (C18:0), dihomo-gamma-linolenic acid (C18:3n-6), linoleic acid (C18:2n-6), oleic acid (C18:1n-9) and arachidonic acid (C20:4n-6) are expressed as molar percentages of total fatty acids. Data represent means \pm SD of 7 mice per group. * $p < 0.001$ for differences between EFAD and EFAS mice.

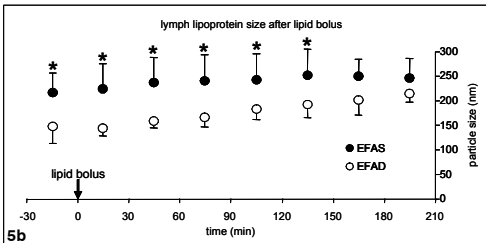


Figure 5b: Lymphatic lipoprotein size (nm), determined by dynamic light scattering, in lymph of EFA-deficient (EFAD) mice (open circles) and EFA-sufficient (EFAS) controls (black circles), during active lipid absorption. Mesenteric lymph was collected and lipoprotein size was measured in 30-minute fractions, prior to, and for 3.5 hours after intraduodenal lipid bolus administration. Data represent means \pm SD of 6-9 mice per group. * $p < 0.05$ for differences between EFAD and EFAS mice.

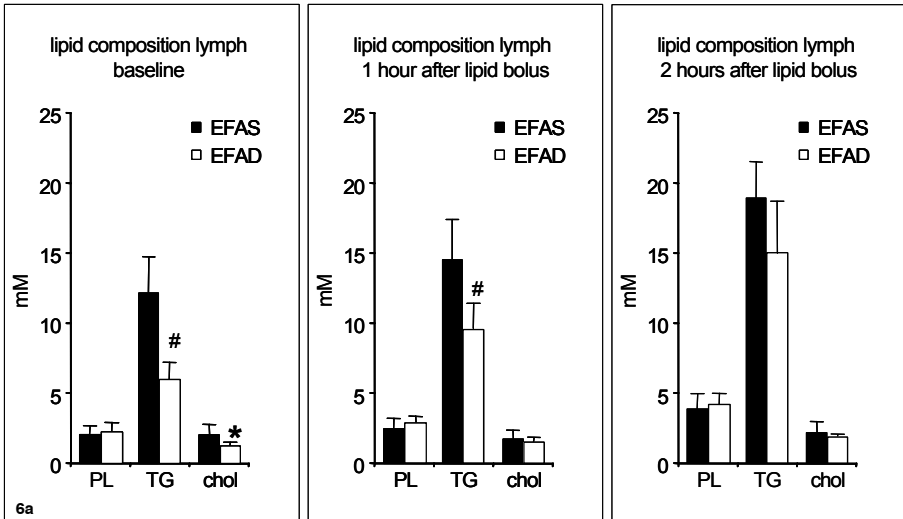


Figure 6a: Concentrations of PL, TG and cholesterol (chol) in mM, in lymph of EFA-deficient (EFAD, white bars) mice and EFA-sufficient (EFAS, black bars) controls at baseline, and at one and two hours after intraduodenal lipid administration. Data represent means \pm SD of 6-9 mice per group. * $p < 0.05$, # $p < 0.005$ for differences between EFAD and EFAS mice.

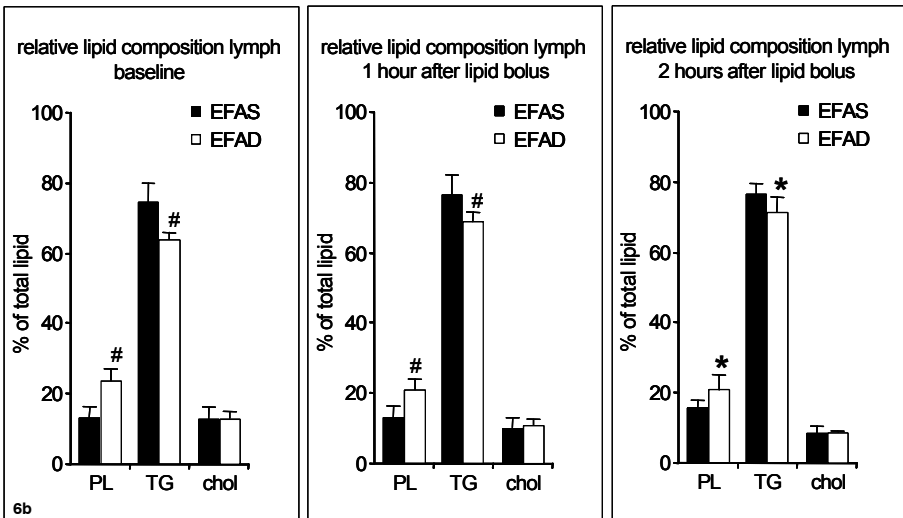


Figure 6b: Relative concentrations of PL, TG and cholesterol, expressed as % of total lipid, in lymph of EFAD (white bars) mice and EFAS (black bars) controls at baseline, and at 1 and 2 hours after intraduodenal lipid administration. Data represent means \pm SD of 6-9 mice per group. * $p < 0.05$, # $p < 0.005$ for differences between EFAD and EFAS mice.

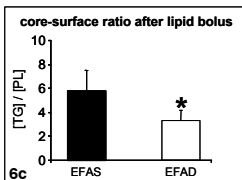


Figure 6c: Core-to-surface ratio, estimated by the ratio of triglyceride (TG) concentration (mM) and phospholipid (PL) concentration (mM) in lymph of EFA-deficient (EFAD, white bars) mice and EFA-sufficient (EFAS, black bars) controls. Data represent means \pm SD of 6-9 mice per group. * $p < 0.001$ for differences between EFAD and EFAS mice.

DISCUSSION

Biliary phospholipids (PL) facilitate efficient transport of dietary lipids from the intestinal lumen into lymph, primarily by providing the surface coat for chylomicrons, but also by stimulating apoB48 synthesis and maintaining adequate enterocyte membrane composition.

Several conditions can alter biliary phospholipid secretion; essential fatty acid (EFA) deficiency in mice is associated with decreased EFA contents of biliary phospholipid and profoundly increased bile flow, whereas *Mdr2*-Pgp-deficiency is associated with virtual absence of biliary phospholipid secretion. We previously demonstrated that in both of these murine models, postprandial plasma appearance of enterally administered lipids is decreased. In EFA-deficient mice, this is combined with decreased net intestinal fat absorption compared to EFA-sufficient controls, as determined by fecal fat balance. In *Mdr2*^{-/-} mice, however, net intestinal fat absorption is only marginally affected, indicating that despite postprandial hypolipidemia, dietary fat is eventually almost quantitatively absorbed during biliary phospholipid deficiency. In the present study, we applied these two *in vivo* models to investigate the relationship between biliary phospholipid secretion rate and the size and composition of lipoproteins produced by the intestine.

Our data indicate that in the absence of biliary phospholipid secretion, significantly larger lipoproteins are secreted into lymph. The secretion of large lymphatic lipoproteins could be deduced from several independent observations: from particle size determination by dynamic light scattering techniques, from the relatively increased triglyceride and decreased phospholipid and cholesterol concentrations in lymph of *Mdr2*^{-/-} mice, and from the calculated lymphatic lipoprotein core-to-surface ratio. Our results on altered intestinal chylomicron formation during biliary phospholipid scarcity in mice are in line with those of Ahn *et al.*, who reported on decreased lymphatic PL and TG output and an increased lymphatic TG-to-PL ratio in zinc-deficient rats, possibly due to limited supply of biliary phospholipids to the enterocytes during zinc deficiency⁽¹⁾. The lipoproteins secreted by *Mdr2*^{-/-} mice were continuously larger during active fat absorption, but strikingly, in non-fasted mice, the size difference was only significant during the first two hours of lymph cannulation. Since mice had access to chow *ad libitum* in the night prior to the lymph cannulation experiments, this phenomenon could refer to a delay in intestinal chylomicron formation or transport into lymph (and subsequently into the plasma compartment) in *Mdr2*^{-/-} mice, as previously postulated⁽²⁶⁾. Possibly, the large chylomicrons assembled during intraluminal phospholipid deficiency enter the lymph more slowly than during sufficient intestinal phospholipid availability. This speculation, combined

with the fact that there is no quantitative lipid malabsorption in *Mdr2*^{-/-} mice, supports the concept that the amount of intraluminal bile phospholipid is important for the rate of, but not for net intestinal absorption of dietary lipid.

Remarkably, cholesterol was virtually absent in intestinal lipoproteins secreted by *Mdr2*^{-/-} mice, possibly related to the fact that biliary cholesterol secretion is strongly reduced in these animals. Voshol *et al.* previously reported on reduced intestinal cholesterol absorption in *Mdr2*^{-/-} mice^(25; 26), which was postulated to result from increased intestinal *de novo* cholesterol synthesis combined with accelerated enterocyte desquamation due to exposure to detergent lipid-free bile. However, Kruit *et al.* recently reported that fractional cholesterol absorption is unimpaired in *Mdr2*^{-/-} mice⁽¹²⁾. The application of different methods for quantifying cholesterol absorption, i.e., the plasma dual isotope method and the fecal dual isotope method, respectively, probably explains the discrepancy between these studies. The reduction in plasma high-density lipoprotein (HDL) cholesterol levels noted in both reports is conceivably related to altered chylomicron composition and secretion in *Mdr2*^{-/-} mice, since a major part of HDL is thought to be derived from excess chylomicron surface material (phospholipid and cholesterol), shed during the lipolytic process.

The intestinal requirement of phospholipid for production of chylomicrons may be comparable to that of the liver for the assembly and secretion of VLDL. Verkade *et al.* demonstrated in choline-deprived rats that during hepatic phosphatidylcholine scarcity, fewer but larger VLDL particles are secreted from the liver^(24; 32). We recently observed that EFA-deficient mice secreted larger VLDL particles from the liver than controls⁽²⁹⁾, possibly secondary to hepatic phosphatidylcholine depletion due to increased biliary phospholipid secretion rates.

Animal models for increased biliary phospholipid secretion are relatively rare. Since the profoundly augmented biliary phospholipid secretion rate in EFA-deficient mice affects hepatic lipoprotein size and is associated with dietary fat malabsorption, we considered the EFA-deficient mouse an intriguing model to further study the potentially organ-specific effects of phospholipid availability on intestinal lipoprotein production. Our data demonstrate that EFA deficiency in mice is associated with lymphatic secretion of considerably smaller chylomicrons compared to EFA-sufficient controls, as determined by three different particle size estimation techniques. Since EFA-deficient mice secrete larger hepatic VLDL particles than EFA-sufficient controls, secretion of smaller lipoproteins apparently is not an intrinsic feature of EFA deficiency. Rather, EFA deficiency differentially affects lipoprotein size in liver and intestine, with phospholipid availability as the major determinant of lipoprotein size.

Our data are in accordance with studies by Amate *et al.*, who demonstrated that dietary EFA-rich phospholipid administration to piglets resulted in production of lymphatic lipoproteins with significantly smaller diameters compared to piglets fed EFA-rich triglycerides⁽²⁾.

Our present data do not allow conclusions on quantitative lipid absorption due to the highly variable lymph flow rates in these studies. Possibly, continuous enteral lipid administration could overcome this limitation of our murine lymph cannulation model in future experiments.

Although the *Mdr2*^{-/-} and EFA-deficient mouse models correspond well regarding postprandial hypolipidemia, they profoundly differ with respect to overall intestinal lipid absorption as determined by fat balance. Whilst EFA deficiency in our mouse model profoundly decreased EFA levels in acyl chains of biliary phospholipid, as described by Bennett-Clark^(3; 4) in EFA-deficient rats, we previously demonstrated that EFA-deficient *Mdr2*^{-/-} mice have similar fat malabsorption as EFA-deficient *Mdr2*^{+/+} mice⁽³⁰⁾. Since EFA-sufficient *Mdr2*^{-/-} mice do not malabsorb dietary fat, this indicates that neither absence of biliary PL secretion, nor EFA depletion of biliary phospholipid quantitatively affect intestinal fat absorption. In the present study we chose not to investigate EFA-deficient *Mdr2*^{-/-} mice, since these animals do not differ in their absence of biliary phospholipid secretion compared with EFA-sufficient *Mdr2*^{-/-} mice.

Baseline lymphatic chylomicrons from EFA-sufficient were significantly larger than those from *Mdr2*^{+/+} mice (Figures 1, 3 and 5b), which can be attributed to the fact that the EFAD and EFAS diets were high-fat diets (34 energy% fat) and the *Mdr2*^{+/+} and *Mdr2*^{-/-} mice were fed standard chow (14 energy% fat).

Hayashi and Tso demonstrated that the number of secreted intestinal lipoproteins remains relatively constant during active lipid absorption, while lipoprotein size increases⁽⁹⁾. Obviously, in *Mdr2*-deficiency, an increased TG-to-PL ratio is a highly favorable means to maintain triglyceride packaging into chylomicrons during lack of surface coat material. In addition, up to 20% of lipoprotein phospholipid is thought to be derived from *de novo* synthesis, and it could be speculated that in biliary phospholipid deficiency, intestinal phospholipids also partially compensates for biliary phospholipids for chylomicron surface coating.

Not only biliary but also enterocytic phospholipids are markedly EFA-depleted during EFA deficiency^(10; 11; 16). The rapid intestinal cellular turnover rate of enterocytes, which is even faster during EFA deficiency⁽⁵⁾, renders the membranes of the intestinal mucosa particularly sensitive to altered intraluminal fatty acid availability. Structural membrane modifications, such as an increased degree of phospholipid fatty acid

saturation, affect the fluidity of intestinal membranes and may subsequently result in functional alterations of membrane enzymes and transporters, thus impairing intracellular events involved in chylomicron formation or secretion.

The deviant size and composition of lipoproteins secreted during altered biliary phospholipid secretion may not only affect the rate of plasma appearance of dietary triglycerides, but also intravascular chylomicron metabolism. Smaller chylomicrons have less affinity for lipoprotein lipase (LPL) than large ones, and there is competition between small chylomicrons and VLDL for available LPL on the capillary endothelium⁽³¹⁾. Plasma clearance of large chylomicrons is substantially more rapid than that of small particles⁽¹⁸⁾. Additionally, altered acyl chain composition of EFA-depleted biliary phospholipids on the chylomicron surface affects chylomicron clearance⁽¹⁹⁾; chylomicrons with saturated phospholipids are cleared more slowly than chylomicrons with a high content of PUFA in surface phospholipids. Thus, by affecting lipoprotein size and clearance rates, biliary phospholipids may affect plasma and hepatic lipid levels⁽²⁹⁾.

Our data are compatible with the concept that the size of intestinal chylomicrons, secreted into lymph under basal conditions or during active lipid absorption, is inversely related to the quantity of biliary phospholipid secretion, and that altered lymphatic lipoprotein size is not necessarily related to net dietary fat absorption as determined by fat balance.

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