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Intestinal lymphatic vessels and their role in chylomicron absorption and lipid homeostasis

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ABSTRACT

Purpose of review: In this review, we describe novel findings related to intestinal cholesterol transport in lymphatic vessels.

Recent findings: Studies have shown that chylomicron entry to lacteals and lymph movement in intestinal lymphatic capillaries is an active process. Regulators of this intestinal chylomicron transport include among others the autonomous nervous system, transcription factors like PLAGL2, and molecular regulators such as VEGF-A/Nrp1/VEGFR1, VEGF-C/VEGFR3, DLL4, CALCRL and GLP-2. Chylomicron transport in intestinal lymphatics is now emerging not only as an option for drug delivery, but also as a new candidate for drug targeting in lipid-related disorders.

Summary: Dysfunctions of intestinal lipid transport can result in conditions such as dyslipidaemia and intestinal lymphangiectasia. Intestinal lymphatics also provide several therapeutic possibilities: molecular regulation of lacteal cell-to-cell junctioning and lymph flow could provide new ways of treating conditions like hyperlipidaemia and associated diseases such as atherosclerosis and other cardiovascular diseases, obesity, diabetes and fatty-liver disease. The intestinal lymphatic system can also be employed to deliver lipid nanoparticles as drug carriers to the venous circulation for improved treatment outcome. These findings highlight the importance and need for research on the different players of intestinal lymphatics in dietary lipid handling and therapeutic applications.

Keywords:
1. Cholesterol
2. Chylomicron
3. Lymphatics
4. Lacteal
5. Pathophysiology
INTRODUCTION

Dietary lipids are absorbed and packaged into chylomicrons in the intestines before being shipped forwards to the circulation. Chylomicrons are large, apolipoprotein B48 (apoB48)-containing lipoproteins. Even though they primarily consist of triglycerides, high chylomicron remnant levels have been associated with several pathologies, such as an increased risk for cardiovascular diseases (CVD) [1]. Interestingly it has been shown that altering lymphatic development induces high plasma cholesterol and altered lipoprotein metabolism [2]. Chylomicron movement from enterocytes into the lymphatic system has lately gained increasing interest as new features of intestinal lipid processing have been discovered. Understanding that lymph movement in intestinal lymphatic capillaries is an active process, that is regulated by several players has highlighted the need for more research on the different players affecting dietary lipid handling. In addition to cardiovascular effects, dysfunctions of lipid transport in lymphatics can result in dyslipidaemia, intestinal lymphangiectasia and predispose to conditions such as obesity, diabetes and fatty liver disease [3].

In healthy individuals, a majority of dietary lipids are absorbed in the small intestine. Before entering intestinal absorptive cells, or enterocytes, dietary triglycerides are broken into fatty acids and monoglycerides, and dietary cholesteryl esters are converted to fatty acids and free cholesterol by cholesterol esterase. Dietary lipids move through the intestinal brush border membrane by both active transport and passive diffusion. Cholesterol absorption is largely mediated by Niemann Pick C1-like 1 (NPC1L1) protein [1] and various apical proteins of enterocytes accelerate fatty acid transfer and lead fatty acids to their metabolic sites [4]. Inside enterocytes, dietary lipids are incorporated into specific lipoprotein particles called chylomicrons. Alternatively, triglycerides can also be stored into large cytoplasmic lipid droplets (CLDs) [5], and cholesterol can be secreted basolaterally into blood circulation through the apolipoprotein A1 (apoA1)/HDL pathway or it can be secreted back to the intestinal lumen through enterocytes in a mechanism called transintestinal cholesterol excretion.
Chylomicron biogenesis begins in the endoplasmic reticulum and it is finalised in the Golgi apparatus [1]. Mature chylomicrons containing dietary cholesterol, triglycerides, phospholipids and fat-soluble vitamins are secreted from the basal side of enterocytes into the intestinal lamina propria. Chylomicrons then travel into lymphatic capillaries called lacteals, and further through mesenteric collecting lymphatic vessels to the thoracic duct [3]. From there, chylomicrons are released to the blood stream and depleted of their lipids and form smaller chylomicron remnants, which the liver finally takes up through LDL receptor (LDLR) and LDL receptor-related protein (LRP).

This review will introduce recent findings concerning dietary lipid transport in chylomicrons in the lymphatic system and potential new therapeutic targets.

**LYMPHATIC TRANSPORT OF CHOLESTEROL IN CHYLOMICRONS**

**Chylomicron movement toward lacteals**

After chylomicron biogenesis, mature chylomicrons exit from the basal side of enterocytes into intestinal lamina propria through vesicle-mediated transport, as was described already decades ago [7]. However, the mechanisms directing these vesicles to the plasma membrane, causes fusion of the membranes and exocytosis of chylomicrons remain unknown. Factors controlling these processes would provide intriguing therapeutic targets to reduce dietary lipid access to circulation for the treatment of obesity and hyperlipidaemias.

To reach lacteals, chylomicrons must travel through the lamina propria. The intestinal lamina propria is a layer of loose connective tissue situated between the intestinal epithelium and the muscularis mucosae. Factors regulating chylomicron movement through the lamina propria remain largely unknown. To date, this movement is mainly proposed to occur by diffusion [8]. In a recent review,
Xiao et al. hypothesize that chylomicron movement in lamina propria could be connected to capillary blood flow, and affected by oxide (NO) and hypoxia signalling as well as vasodilation [9]. The gastrointestinal glucagon-like peptide 2 (GLP-2) is also known to affect intestinal release of chylomicrons [10], but the specific mechanism remains unknown.

**Entry of chylomicrons into lacteals**

Intestinal lacteals are crucial structures for dietary cholesterol transport. They drain chylomicrons together with interstitial fluid and immune cells from the intestinal lamina propria into the lymphatic system, forming initial lymph [3,11]. This chylomicron-rich lymph flow is known to increase significantly postprandially. Hence, lacteals are crucial structures for dietary lipid transport and correctly functioning intestinal lymphatics are key for a balanced lipid metabolism. Lacteals are blunt-ended thin-walled lymphatic capillaries that reside in the middle of each villus proliferation [3]. A blood capillary network that gathers dietary nutrients and provides the villi with oxygen surrounds each lacteal. Alterations of intestinal lymphangiogenesis, lymphatic structure and function have been shown to considerably affect health and homeostasis, and to contribute to the development of conditions such as obesity, fatty liver disease and hyperlipidaemias [12–14].

Intestinal lymphangiogenesis has been thoroughly covered in previous reviews [3,11,15]. Here, we will only focus on the most recent findings on the subject. Vascular endothelial growth factor (VEGF) receptor 3 (VEGFR3) is a key mediator of lymphangiogenesis also in the intestine [16,17]. VEGF-C and VEGF-D both bind to VEGFR3, but only VEGF-C has been shown to be indispensable for intestinal lymphatic maintenance [17,18].

Recently, the necessity of intestinal VEGFR3 signalling for lipid absorption was further asserted by demonstrating that impaired VEGFR3 signalling of Chy mice led to retention of chylomicrons in the small intestine [19**]. Chy mice have an inactivating mutation in the tyrosine catalytic domain of
VEGFR-3, which leads to defective VEGFR3 function and defective lymphatics. This study also showed that Chy mice had decreased plasma triglyceride levels and increased stool lipid content after feeding. Additionally, NO levels, which have previously been shown to be required for adequate chylomicron secretion [10], were significantly reduced in response to high fat consumption in Chy mice [19**]. This study demonstrated that VEGFR3 plays an important role in lipid absorption and chylomicron transport and altering intestinal VEGFR3 signalling could protect against obesity. Inhibiting the calcitonin receptor–like receptor (CALCRL) was also found to affect intestinal lymphatic structure and hinder intestinal lipid uptake into lacteals [20]. Impaired lymphatic CALCRL was also found to downregulate Notch signalling through Notch-ligand Delta-like 4 (DLL4), tying this finding to previously demonstrated lipid absorption defects upon inhibition of DLL4 [16].

Entry of chylomicrons into the lacteal lumen is a key limiting process in dietary lipid transport. The single endothelial cell layer of lacteals consists of initial lymphatic-specific oak leaf-shaped cells which overlap loosely and are connected on each side with cell-to-cell junctions [16]. These overlapping flaps, termed ‘primary lymphatic valves’, are thought to contribute to lymph formation [11], and according to current conception, chylomicrons are thought to reach the lacteal lumen by paracellular transport through the open cell-to-cell junctions between endothelial cells [7,16,21], even though a transcellular transport mechanism has also been described [22,23]. Cell-to-cell junctions of lacteals include both open button junctions or closed zipper junctions [16]. Button junctions are still present in submucosal lymphatic vessels, but they are absent from mesenteric collecting vessels and collecting lymphatic vessels only display zipper junctions, which prevent lymph leakage from vessels [16]. It is becoming increasingly clear that chylomicron entry into lacteals is an active process controlled by several factors, like transcription factor PLAGL2 and DLL4 [16,24]. Intestinal Notch signalling was previously shown to regulate lacteal junction zippering through VEGFR2/VEGFR3 [16]. A recent paper demonstrates that also the known angiogenic regulator VEGF-A affects lymphatic endothelial cell junctioning [25**]. Simultaneous deficiency for VEGFR1 and co-receptor Neuropilin 1 (Nrp1) was
shown to protect from obesity during high fat diet [25**]. This response was due to the inability of chylomicrons to enter collecting lacteals because of a shift of open button junctions to closed zipper junctions. This study proposes that lacteal junction zippering is mediated by VEGF-A/VEGFR2 interaction and that in wild type animals VEGF-A binds VEGFR1 and Nrp1, reducing bioavailability of VEGF-A for VEGFR2. In VEGFR1/Nrp1-deficient mice, VEGF-A is not trapped and is free to regulate lacteal junction zippering through VEGFR2.

Regulating lacteal junction opening presents with intriguing new therapeutic possibilities. Due to their size, chylomicrons cannot enter intestinal blood capillaries, and lacteals are the only route for draining intestinal chylomicrons. Intestine-specific control of lymphatic drainage could bring forth ways of controlling postprandial plasma lipid levels and preventing CVD. Common treatments for hypercholesterolemia include inhibiting cholesterol absorption through NPC1L1 with ezetimibe and inhibiting cholesterol synthesis with statins. However, several patients are not benefiting of these treatments, and new therapeutics are constantly sought for. Inhibiting chylomicron transport to lacteals also presents an intriguing option to fight obesity without affecting the nervous system or suppressing appetite.

**Lymph movement inside lacteals**

After reaching lacteals, lymph is pumped in an active process towards the mesenteric collecting lymphatic vessels. This was demonstrated by in an elegant *in vivo* study in which it was shown that lacteal contractile movement enhances fatty acid clearance rate [26]. Lacteals do not have mural smooth muscle cells (SMC) or pericytes [3], and indeed lacteal lymph motion was shown to be conveyed by lamina propria smooth muscle cells in a process under autonomic nervous system control [8]. Importantly, this study also demonstrated that dietary fatty acids affect the speed of lipid-release from enterocytes and their drainage to lacteals. This means that the different kinds of lipids digested are transported at different rates.
**Chylomicron transport in lymphatic mesentery**

Mesenteric collecting vessels from the intestines transport chyle, the chylomicron-rich milky lymph, to the mesenteric lymph node before reaching thoracic duct \[27\]. The collecting lymphatic vessels have bi-leaflet valves called secondary lymphatic valves in contrast to initial lymphatic endothelial cell flaps. These secondary valves ensure unidirectional lymph transport against a hydrostatic pressure gradient. Lymph propulsion is related to the SMC coverage of the vessels, causing phasic and tonic contractions. Mesenteric collecting lymphatic vessels’ contractility provides an active transport system to move chyle from collecting lacteals through the cisterna chyli to the proximal part of the thoracic duct \[27\]. Then, the thoracic duct transports chyle through the aortic hiatus in the diaphragm alongside the aorta before reaching the junction of the internal jugular and subclavian veins. Chyle is then emptied into the venous system, where it passes through the heart and lungs travelling all over the circulatory system before entering the liver. In addition to several known compounds like NO, prostaglandins and histamine, also high lipid loads in mesenteric lymphatic vessels were shown to reduce lymph pumping motion \[28\], suggesting that lymphatic lipid content itself can affect lymphatic vessel function.

The more detailed function of the thoracic duct in the context of lipoprotein metabolism is yet to be revealed. It is known that the primary function of the thoracic duct is to transport chylomicrons from the lacteals to the blood circulation. Lipoproteins are known to exchange apolipoproteins and undergo structural changes as they move through the circulation. Whether similar exchange of apolipoproteins or other processing occurs between chylomicrons and other lipoproteins already in the lymphatic vessels remains to be explored.

**New basolateral pathway for triglyceride uptake affecting chylomicron composition**
Intestinal triglyceride absorption has always been considered a one-way transport system. However, a recent study proposes a novel pathway similar to TICE, but for acquiring triglycerides from the basolateral side of enterocytes from triglyceride-rich lipoproteins (TRLs, namely chylomicrons and very low-density lipoproteins) and using these triglycerides for fatty acid oxidation within enterocytes [29*]. According to this study, apolipoprotein C3 (apoC3) on TRLs regulates the basolateral lipid substrate transport (BLST) pathway extracellularly. Importantly, high apoC3 levels induced less chylomicron secretion and smaller CLDs in mice. High apoC3 levels were also previously proved to induce the secretion of smaller chylomicrons containing less triglycerides [30,31]. According to the authors, this was caused by apoC3 inhibiting TRL use as a substrate, and the triglycerides used for enterocyte fatty acid oxidation were taken from chylomicron and CLD triglycerides [29*]. This mechanism is yet to be confirmed in humans, but several human conditions present with elevated plasma apoC3 levels [32] and these findings raise many questions on the possible role of BLST in atherogenesis. On the other hand, increased uptake of triglycerides from TRLs into enterocytes could present with new options in preventing hypertriglyceridaemia and obesity.

**Therapeutic delivery through intestinal lymphatics**

The intestinal lymphatic system circumvents the liver first pass metabolism and transports therapeutic reagents directly into systemic circulation via thoracic duct enhancing the bioavailability, site specificity and efficacy of therapeutic drugs. Recent advances in lipid nanoparticle and drug development have increased the therapeutic potential of the lymphatic vasculature as an alternative systemic drug delivery route [33,34]. Successful gastrointestinal delivery of intact nanoparticles could provide significant improvement to many therapeutics and drugs that suffer from poor bioavailability. Atorvastatin, a widely used drug for hyperlipidaemia, was shown to have several fold higher bioavailability in the plasma after gastrointestinal delivery as a nanoparticle form [35]. Also rosuvastatin loaded in a chylomicron mimicking carrier showed an improved drug absorption into the systemic circulation through lymphatic pathways [36]. In addition, siRNAs have been successfully
delivered through the lymphatic route indicating that novel nucleic acid therapies could be administered orally [37]. Research around the lipid-lowering RNA therapeutics has been intense and this could provide a beneficial administration route [38].

**Chylomicrons and atherosclerosis**

The number and size of chylomicrons increase markedly in postprandial state. Atherogenic properties of chylomicrons have long been debated, since chylomicrons are the largest lipoprotein class (diameter of 100-150nm) and they mainly consist of triglycerides. However, when depleted of their triglycerides in circulation, chylomicrons become cholesterol-enriched remnant lipoproteins [39*]. Chylomicrons contribute to total plasma lipid levels, and high apoB48 levels have been associated with both early atherogenic changes and more advanced disease progression [40], and increased numbers of intestinally derived lipoprotein remnants in plasma are also considered part of the atherogenic dyslipidaemia complex (ADC) [41]. Many factors speak for the atherogenic effect of chylomicrons and their remnants: chylomicrons are the preferred substrate for lipoprotein lipase compared to VLDLs, which leaves VLDLs circulating longer increasing potential atherosclerotic risk. Even though oxidized LDL containing apolipoprotein B100 (apoB100) is the canonical macrophage-attracting lipoprotein of atherosclerotic plaques [42], apoB48 has also been found in human atherosclerotic lesions [43]. This suggests that the smallest chylomicron remnants can penetrate vessel walls. Targeting intestinal chylomicron production and transport presents with a rather undermined treatment option for atherosclerosis progression.

Many factors also affect chylomicron turnover. A recent paper demonstrates that inhibition of TRL clearance by apoC3 is apolipoprotein E (apoE)-dependent [32]. Another recent study also showed that even though the lymphangiogenic factor VEGF-D appears be displaceable for dietary lipid absorption, it is required for hepatic clearance of chylomicron remnants through heparan sulphate proteoglycan syndecan-1 (SDC1) [44]. VEGF-D deficient mice in atherogenic background exhibited notable
hyperlipidaemia compared to atherogenic control mice. However, despite severe hyperlipidaemia, no signs of accelerated atherosclerosis were found in these mice and circulating lipoprotein size was shown to be increased in VEGF-D deficient mice suggesting that chylomicron remnants in this model remained too large to penetrate vessel walls. Additional research is required to investigate this mechanism in humans.

CONCLUSION

Many factors affect and regulate dietary lipid transport from the intestine. Chylomicron movement to and inside the lymphatic lacteals was long considered a passive process; however, recent studies have identified several molecules, such as hormones, transcription factors and growth factors that affect chylomicron transport in the intestine, and it is indeed a tightly regulated active process. Chylomicron formation and movement is regulated on several levels, from transcription factors and gene expression to diet content. Lacteals present an intriguing target for modulating lipid absorption and fighting obesity and hyperlipidaemia. Uncovering regulation of intestinal lipid absorption is also advantageous to fully make use of therapeutics’ delivery via intestinal lymphatics. Drug administration through intestinal lymphatics bypasses the liver and hence enhances specificity and efficacy of drugs. This review highlights the need to acknowledge intestinal lymphatics as both a potential target for novel therapies and as a mean for more efficient drug delivery. Despite on-going research, additional effort is still required to uncover the big picture of the molecular signalling pathways governing intestinal lymphatic function in lipid metabolism.

KEY POINTS

• Chylomicron formation in the intestine and their movement into lacteals is an active process.
• Lacteals and processes linked to chylomicron transport in the intestine provide interesting new targets for therapy and new options for drug delivery.

• VEGF-C, VEGF receptor-1 and -3, as well as Neuropilin 1 are important new players for lacteal function and chylomicron absorption.

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REFERENCES


This study shows that impaired VEGFR3 signalling of Chy mice leads to retention of chylomicrons in the small intestine, highlighting the role of VEGFR3 in lipid absorption.


This study reveals that VEGF-A/VEGFR2 signalling induces a change of lymphatic endothelial cell-to-cell junctions from closed state to open chylomicon-permeable state. This response is affected by Nrp1/VEGFR1, since their ablation in mouse endothelial cells resulted in obesity-resistance.


This article presents a novel pathway of basolateral lipid substrate transport (BLST) for triglyceride trafficking from lipoproteins back to enterocytes. ApoC3 levels were shown to affect this process.


This study, using nuclear magnetic resonance spectroscopy, emphasizes the contribution of TRL remnants to non-fasting total plasma cholesterol levels in Danish population, and hence underlines the role of chylomicron remnants as potentially atherogenic particles. *N.B.* In this article, chylomicrons and their remnants were included in the VLDL fraction.


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