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The Intestinal Lymphatic System: Functions and Metabolic Implications

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SUMMARY

This review will highlight recent progress and current information supporting an active role for intestinal lymphatic vessels in transport and distribution of dietary lipids. Modulation of gut lymphatics might represent a novel therapeutic strategy to prevent diet-induced obesity and the metabolic syndrome.

The lymphatic system of the gut plays important roles in the transport of dietary lipids, as well as in immunosurveillance and removal of interstitial fluid. Historically, despite its crucial functions in intestinal homeostasis, the lymphatic system has been poorly studied. In the last 2 decades, identification of specific molecular mediators of lymphatic endothelial cells (LECs) growth together with novel genetic approaches and intravital imaging techniques, have advanced our understanding of the mechanisms regulating intestinal lymphatic physiology in health and disease. As its metabolic implications are gaining recognition, intestinal lymphatic biology is currently experiencing a surge in interest. This review describes current knowledge related to molecular control of intestinal lymphatic vessel structure and function. We discuss regulation of chylomicron entry into lymphatic vessels by vascular endothelial growth factors (VEGFs), hormones, transcription factors and the specific signaling pathways involved. The information covered supports the emerging role of intestinal lymphatics in etiology of the metabolic syndrome and their potential as a therapeutic target. (Cell Mol Gastroenterol Hepatol 2019;7:503–513; https://doi.org/10.1016/j.jcmgh.2018.12.002)

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The lymphatic vasculature plays crucial roles in tissue fluid homeostasis, immune surveillance, and transport of dietary fat from the intestine to the bloodstream.1-3 The latter function was already noted in 1662 by the Italian physician Gaspare Aselli when he described the lacteal vessels, or chyliferous vessels, in the intestine of a well-fed dog.6 However, relatively modest attention has been given to dietary lipid transport by the lymphatic system because it was generally considered to involve passive unregulated drainage. In the last 2 decades, identification of specific markers for lymphatic endothelial cells (LECs) allowed the generation of lymphatic-reporter mouse models, that combined with state-of-the-art imaging of lymphatic structure have enhanced our understanding of gut lymphatic physiology in health and disease. Currently, the field of gut lymphatics is experiencing a surge in interest as the metabolic implications of intestinal lymphatics and their potential as a target in the management of obesity and its complications are becoming more appreciated. Numerous studies have now shown that lipid transport by lacteals is a tightly regulated active process, and that impairment of this regulation could lead to systemic metabolic consequences. The molecular mechanisms regulating lipid uptake and transport by lacteals will be discussed with a focus on their impact in control of energy metabolism.

Functional Organization of Intestinal Lymphatics

Unlike the closed loop formed by the blood-vascular network, the lymphatic system is a unidirectional transport system designed to return fluid to the blood circulation. The lymphatic network consists of blind-ended capillaries located in most tissues that funnel into progressively fewer and larger precollecting and collecting vessels. Lymph from the gastro-intestinal and lumbar region drains into the cisterna chyli at the posterior end of the thoracic duct and enters the circulation at the level of the subclavian vein.5,6

In the intestine, lymphatic capillaries, or lacteals, are located exclusively in intestinal villi, whereas collecting lymphatic vessels are present in the mesentery.7 The term gut lymphatics used throughout this review refers to both lacteals in the intestinal villi and lymphatic vessels in the submucosa. The structural organization of intestinal stroma has been elegantly described by Bernier-Latmani et al.9

Abbreviations used in this paper: ABC, adenosine triphosphate–binding cassette; CM, chylomicron; CR, chylomicron remnant; DLL4, delta-like ligand 4; ERK, extracellular signal-related kinase; FAO, fatty acid β-oxidation; LEC, lymphatic endothelial cell; NO, nitric oxide; NRP, neuropilin; Prox1, prospero homeobox protein 1; TG, triglyceride; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

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et al., who also provide detailed protocols for generating high-resolution 3-dimensional images of gut cell types including blood and lymphatic vessel cells, smooth muscle cells, fibroblasts, and immune cells.

Lacteal length normally reaches 60%–70% of villus length and this proportion does not differ along the different intestinal segments. Lacteals are surrounded by a highly organized cage-like structure of arterial and venous blood capillaries and by a treelike set of smooth muscle fibers (Figure 1) usually identified by staining for α-smooth muscle actin and desmin. Extracellular matrix proteins in the intestinal villus interact with LEC membrane proteins, notably the integrins, to regulate lymphangiogenesis and aspects of lacteal function. For example, integrin β1, expressed in intestinal blood and LEC vessels, is required for the proliferative response of LECs to fluid accumulation and cell stretching. Integrin α9, preferentially expressed on LECs, is important for providing fibronectin matrix support during lymphatic valve morphogenesis. Additional active matrix components located within the intestinal stroma include tenasin C, found in the villus but almost undetectable in the submucosa, which functions in tissue stretching, injury, and inflammation, and periostin, important for tissue remodeling in response to injury, present mostly in pericytroph fibroblasts. Of interest, the matrix protein Mfge8 (milk fat globule epidermal growth factor–like 8), a ligand for integrins αvβ3, αvβ5, and αvβ1 has been reported to influence intestinal lipid absorption. However, additional studies are needed to elucidate whether Mfge8 might influence chylomicron (CM) transfer to lacteals.

Adult lacteals undergo continuous remodeling; presence of fine cytoplasmic, actin-rich cellular extension, or filopodia, is observed in the majority of lacteal tips, indicating active regeneration in contrast to what is observed in other lymphatic beds that are more quiescent. Lymphatic ECs use glycolysis and fatty acid β-oxidation (FAO) for energy production during proliferation and migration. FAO generates acetyl-coenzyme A used by the histone acetyltransferase p300 for histone acetylation of key genes involved in lymphangiogenesis. In vitro, LECs as compared with microvascular ECs have higher expression of fatty acid binding protein FABP4 and FABP5, fatty acid transport proteins FATP3 and FATP4, and long-chain fatty acid translocase CD36, supporting the importance of FAO in LECs.

Important components of lacteal structure and function are cell-cell junctions. LECs are connected by functionally specialized junctions containing both adherens and tight junction proteins, such as vascular endothelial cadherin and claudin-5. LECs in initial lymphatics are interconnected by discontinuous button-like junctions, whereas downstream the collecting lymphatics display continuous zipper-like junction located at the cell borders with no openings. The transition of button to zipper junction pattern (Figure 2) reflects functional differences between regions specialized for fluid uptake (button) or for lymph transport (zipper). The flap-like openings between adjacent “buttons” allow access of interstitial fluid and immune cells into the lymphatic capillary lumen. The collecting vessels are less permeable because of the continuous cell-cell junctions, which avoids lymph leakage during transport from capillaries to lymph nodes. Lacteals display a mix of continuous and discontinuous junctions, demonstrating features of both sprouting and quiescent lymphatic capillaries. Lacteal junctions have been recently shown to regulate CM entry into the lacteals, as discussed subsequently.

**Molecular Regulation of Intestinal Lymphatics**

Recent studies have shown that defective lacteal lymphangiogenesis impairs absorption of dietary lipid. In this section, we review the molecular regulation of lymphangiogenesis and discuss rodent models of dysfunctional gut lymphatics and mesenteric lymphatic vessels, due to genetic deletion of prospero homeobox protein 1 (Prox1), vascular endothelial growth factor (VEGF)-C, notch ligand delta-like ligand 4 (DLL4), and adrenomedullin, which causes impaired absorption of lipids.

During embryonic development, lymphangiogenesis is primarily guided by VEGF-C via binding of its tyrosine kinase receptor VEGFR-3. VEGFR-3 is expressed in lymphatic endothelial cells in a manner that is distinct from that of its close relative VEGFR-2, which is expressed in many other endothelial cells types. VEGF-C is produced by resident mesenchymal cells in the developing lymphatic system and is required for the initial formation of lymphatic vessels. VEGFR-3 is also activated by VEGF-D, which is produced by mesenchymal cells in the surrounding tissue. This activation leads to proliferation and survival of lymphatic endothelial cells, as well as to their migration and tubulogenesis. VEGF-C and VEGF-D are both produced by resident mesenchymal cells in the developing lymphatic system and are required for the initial formation of lymphatic vessels. VEGFR-3 is also activated by VEGF-D, which is produced by mesenchymal cells in the surrounding tissue. This activation leads to proliferation and survival of lymphatic endothelial cells, as well as to their migration and tubulogenesis. VEGF-C and VEGF-D are both produced by resident mesenchymal cells in the developing lymphatic system and are required for the initial formation of lymphatic vessels.
kinase VEGF receptor 3 (VEGFR-3) which is highly expressed in LECs. The fully processed form of VEGF-C can also bind to VEGFR-2. In the absence of VEGF-C, the development of lymphatic vessels is arrested during their initial spouting from embryonic veins as VEGF-D, the second VEGFR-3 ligand, does not compensate for absence of VEGF-C. Lacteal vessels grow into the intestinal villi around embryonic day E17.5 to facilitate postnatal lipid absorption. These lymphatic plexuses and capillaries in the intestinal tube form through an active branching process associated with activation of VEGFR-3 by VEGF-C, and involvement of lymphatic vessel endothelial hyaluronan receptor 1-positive macrophages. Interstitial flow is also critical for guiding lymphangiogenesis through the generation of a VEGF-C gradient that dictates direction of vessel growth. VEGF-C is synthetized as a pre-pro-peptide (58 kDa) that undergoes a first cleavage in the C-terminal part by furin, resulting in pro-VEGF-C (29/31 kDa), and subsequently in the N-terminal part, yielding its active form (21/23 kDa). Pro-VEGF-C is secreted and can bind VEGFR-3 in LECs but fails to activate phosphorylation of VEGF-C or that of downstream signaling proteins; extracellular signal-related kinases (ERK1/2), AKT, or endothelial nitric oxide (NO) synthase. Mature VEGF-C can also activate VEGFR-2. Upon binding with VEGF-C, VEGFR-3 forms homodimers and undergoes intrinsic autophosphorylation on at least 5 cytoplasmic tyrosines (Y1230, Y1231, Y1265, Y1337, and Y1363). VEGF-C activation leads to protein kinase C-dependent activation of ERK1/2, implicated in cell proliferation. VEGF-C can also induce formation of heterodimers between VEGFR-3 and VEGFR-2 through a different phosphorylation pattern. The VEGFRs signaling cascades are also regulated by co-receptors such as neuropilin 1 and 2 (NRP1 and NRP2), non-tyrosine kinase transmembrane proteins that bind VEGFs. Recent studies in LECs show that VEGF-C activates AKT signaling via formation of VEGFR-2/VEGFR-3/NRP1 complex while ERK is activated by VEGFR-3 homodimers without contribution of NRP1 or NRP2. These studies were done in cultured LECs in vitro, but NRP1 is not detected in intestinal LECs in vivo. Comprehensive recent review articles are available detailing VEGF signaling pathways in lymphangiogenesis.

**Prox1**

Homeobox gene **Prox1** is a transcription factor critical for lymphatic lineage commitment and the formation of the

**Figure 2. Regulation of chylomicron uptake by VEGF-A.** Schematic model of cell-cell junctions in intestinal blood ECs and LECs regulating chylomicron absorption. (A) VEGF-A binding to NRP1/Fms-related tyrosine kinase 1 (FLT1) on blood ECs limits VEGF-A bioavailability for VEGFR-2, resulting in continuous cell junctions in blood ECs and in discontinuous ones on LECs. The discontinuous button-like LEC junctions allow lacteal to take up CMs. (B) Inducible genetic deletion of Nrp1 and Flt1 increase bioavailability of VEGF-A and subsequent signaling through VEGFR-2. High level of VEGF-A promotes transition of button-to-zipper junctions in the lacteals, which inhibits chylomicron entry into the lymphatic capillaries causing lipid malabsorption and reduced weight gain during high-fat feeding. These data support the hypothesis that NRP1 and FLT1 function together as a double decoy receptor system in intestinal blood ECs to limit VEGF-A signaling.
lymphatic vasculature. Homozygous genetic deletion of Prox1 in mice results in absence of lymphatic vessels, severe edema and death at around embryonic day 14.5 (E14.5). Prox1+/− pups often die after birth due to presence of pulmonary edema or lymphangiectasia, similar to the phenotype observed in mice with a deletion in the gene Ccbe1, Collagen And Calcium Binding EGF Domains 1, a regulator of the cleavage of pro-VEGF-C into its active form. The surviving Prox1+/− pups that reach adulthood develop spontaneous obesity. Heterozygous loss of Prox1 leads to defective lymphatic vascular integrity and subtle leakage of lymph in the visceral area. The accumulated lymph induces de novo differentiation of fat cell precursors, fat cell hypertrophy, and eventually adipocyte proliferation, and with age, Prox1+/− mice became progressively obese. Prox1+/− mice display dilated and dysfunctional submucosal and lymphatic vessels in the mesentery, increased mesenteric fat deposition and accumulation of inflammatory cells. These findings represent the first definitive studies highlighting importance of Prox1-mediated loss of gut lymphatic vessel integrity in the etiology of obesity.

VEGF-C and VEGFR-3/VEGFR-2

In the intestine, VEGF-C is secreted by smooth muscle cells located in the inner circular muscle layer of the intestinal wall, in arterial smooth muscle, and in a subset of the SMC fibers in the villus within the intestinal wall and surrounding the lacteals. Additional cell types in the gut are equipped to produce VEGF-C, such as macrophages and fibroblasts, but their contribution to VEGF-C secretion in the intestine is unclear. Postnatal inducible global deletion of Vegfc in mice associates with defective lipid absorption and increased excretion of cholesterol and free fatty acid in feces. Despite no difference in food intake, Vegfc−/− mice are protected from diet-induced obesity and insulin resistance, compared with littermate control mice, although the link between defective lacteal lymphangiogenesis and improved glucose sensitivity was not investigated. Vegfc deletion causes lacteal regression without affecting maintenance of other lymphatic beds, although the VEGF-C/VEGFR-3 pathway also plays critical functions also in the heart and brain. The same study found that, similar to Vegfc deletion, inducible global deletion of Vegfr3 postnatally affected lacteal maintenance and function. Of interest, lymphatic vessel density in the intestinal wall was not altered, suggesting that VEGF-C can signal via VEGFR-2 to stabilize the lymphatic plexus. The role of Vegfr2 in intestinal lymphatic function has not been investigated yet.

Recently, Shew et al showed that inactivation of VEGFR-3 tyrosine kinase motif in mice leads to retention of triglycerides (TGs) in the enterocytes of the small intestine, with decreased postprandial levels of TGs in the plasma and increased excretion of lipids into the stools. The study further showed that after a fat bolus levels of NO, which is required for CM mobilization into the bloodstream, are significantly reduced in intestines of mice with the mutant VEGFR-3. This observation suggested a critical role for VEGFR-3 signaling in the generation of NO during lipid absorption.

Delta-Like Ligand 4

Lacteal regeneration was recently shown to be regulated by DLL4, a Notch ligand. Activation of VEGFR-3/VEGFR-2 signaling cascade by VEGF-C was required to promote DLL4 expression in the lacteals. DLL4 was highly expressed in tip cells of sprouting lacteals and had a lower expression in stalk cells. Inducible deletion ofDll4 in LECs affected lacteal tip cell phenotypes and survival. Dll4−/− lacteals were shorter than those of wildtype mice because of decreased filopodia, impaired cell survival and migration. Deletion of lymphatic Dll4 promotes a transition from mixed adherens junctions to mostly continuous junctions, causing inefficient CM uptake and transport.

Adrenomedullin

Adrenomedullin is a small (52 amino acid) peptide highly expressed in LECs and regulated by Prox1 that is required for normal development of the lymphatic system. Adrenomedullin binds the G protein–coupled calcitonin gene-related peptide type 1 receptor (CALCRL) and its signaling directly promotes endothelial cell growth and survival through activation of MAPK/ERK pathways. Adrenomedullin regulates lymphatic permeability via junctions ZO-1 and vascular endothelial cadherin at the cell membrane. Inducible deletion of Calcrl in LECs results in dilated intestinal lymphatics and protein-losing enteropathy that are characteristic of clinical intestinal lymphangiectasia. Calcrl-deficient mice have smaller body weight compared with littermate control mice, and impaired lipid absorption possibly due to lacteal defects.

Role of Gut Lymphatics in Lipid Absorption

Digestion and absorption of dietary lipid in the gastrointestinal tract involves multiple steps. First, hydrolysis of neutral lipids in the gut lumen generates fatty acids and monoglycerides, which transfer through the apical membrane of mucosal enterocytes, where endoplasmic reticulum enzymes re-esterify them into TGs. Second, the TGs together with cholesterol, cholesteryl esters, phospholipids, and ApoB, are assembled into CMs that are released from the enterocyte’s basolateral membrane and enter the intestinal lymphatic lacteals. The molecular mechanisms regulating CM uptake by lacteals are emerging and support the concept that CM entry into lacteals is an active process, as opposed to the notion of passive draining from the interstitium of particulates too large to enter blood capillaries. CM uptake by lacteals is regulated by the transcription factor pleomorphic adenoma gene-like 2 (PlagL2). PlagL2 null mice have functional enterocytes that secrete CMs but the CMs fail to enter the lacteals; however, the mechanisms remain unknown.

VEGF-A Regulates CM Uptake by Lacteals via Modulation of Cell-Cell Junctions

A recent study showed that VEGF-A signaling regulates CM uptake through modulation of lacteal
junctiions. VEGF-A is the prototype member of the VEGFs family, and has key roles in hematopoiesis, vasculogenesis, angiogenesis, and vascular permeability, mainly mediated through VEGFR-2 signaling. VEGF-A binds with higher affinity to VEGFR-1, although this interaction results in limited downstream signaling. VEGFR-1 (also known as Flt1) is considered to be a decoy receptor that limits VEGF-A availability for VEGFR-2 and therefore activation and signaling of the VEGF-A/VEGFR-2 axis (decoy-or-sink hypothesis).

VEGF-A also binds to semaphorin receptor NRP1, a single spanning non-tyrosine kinase transmembrane protein that functions as a VEGFR-2 co-receptor. NRP1 regulates VEGFA-mediated endothelial permeability via VEGFR-2 phosphorylation and activation of downstream signaling. Zhang et al showed that inducible genetic deletion of Nrp1 and Vegfr1 (also known as Flt1) increase VEGF-A bioavailability and signaling through VEGFR-2. High level of VEGF-A induces button-to-zipper junction transformation in the lacteals, which inhibits CM entry into the lymphatic capillaries causing lipid malabsorption and reduced weight gain during high-fat feeding (Figure 2A and B). These observations suggested that NRP1 and Fms-related tyrosine kinase 1 function together as a double decoy receptor system in intestinal blood endothelial cells to limit VEGF-A signaling in lymphatic vessels. Interestingly, the Vegfa gene is highly expressed in intestinal villi as compared with Vegfc and Vegfb; however, expression of its decoy receptor Flt1 increases at birth, concomitant with expression of the CM processing proteins Mtp and ApoB, which induces Flt1. Thus, postnatal generation of CMs leads to increased expression of Fms-related tyrosine kinase 1 in intestinal blood ECs, which together with NRP1 prevents excessive VEGF-A signaling at the level of the lymphatics, allowing lacteal junction maturation and CM absorption. The study demonstrates that high VEGF-A signaling has opposite effects on blood and lymphatic vessels: it increases leakage of blood capillaries by opening normally closed cell-cell junctions, and reduces permeability of lacteals by closing junctions via transformation of buttons into zippers (Figure 2A and B). In addition, VEGFR-2 activation on LECs inhibits vascular endothelial cadherin cytoskeletal anchoring, which helps maintain lacteal button junctions as open. VEGF-A gain of function for a short period is sufficient to induce a switch in junction morphology and therefore lipid uptake. The opposite effects of VEGF-A on lymphatic and blood vasculature is possibly explained through inhibition of Rho-associated protein kinases, which promote zipping of endothelial cell junctions and suppress CM uptake into lacteals. Rho-associated protein kinase inhibitors have been previously reported to reduce leakage in blood vessels. Overall, the findings support importance of Nrp1 and VEGFR-1 in limiting VEGF-A activity during CM generation for promoting lymphatic transport of the absorbed lipid. Of interest, Khalifeh-Soltani et al showed that ligation of αβ3/1 by the milk protein M1ge8 reduces antral smooth muscle contractile force by preventing RhoA activation through a phosphatase and tensin homolog-dependent mechanism leading to malabsorption of dietary fats and carbohydrates protecting from diet-induced weight gain.

**Lymphatic Flow During Lipid Absorption**

Spontaneous lacteal contraction, in concert with adjacent smooth muscles, is also essential for drainage of dietary lipids. Choe et al investigated lacteal drainage of dietary lipids and other lipophilic molecules in vivo by using intravital imaging and a lymphatic-green fluorescent protein reporter mouse. Lacteal contraction was shown to be regulated by the autonomic nervous system, and to be increased by acetylcholine and decreased by norepinephrine. Ganglion-blocking agents, such as mecamylamine and pentolinium, which reduce smooth muscle motility in the intestine, decrease lacteal contraction, supporting enteric nervous system control of lacteal contraction and drainage.

Previous studies have suggested that drainage of the lipid-rich lymph through gut lymphatics is driven by the intrinsic pumping activity of the collecting vessels equipped with smooth muscle cells and 1-way valves that prevent backflow. However, a recent study showed that collecting lymphatic vessels isolated from murine visceral cavities display minimal contractile behavior suggesting that their contribution during lipid absorption is not significant. Lymphatic pumping activity is regulated by intraluminal pressure and wall shear stress via differential release of prostaglandins, NO, histamine. Release of NO and histamine and contraction frequency of intestinal collecting lymphatics were shown to increase following olive oil administration in an early work with rats. However, when lipid levels are very high they can exert negative effects on lymphatic vessels as shown in a recent study. This study where a 30% intralipid emulsion was infused in the duodenum, showed that as the lipid started rising (within 10–20 minutes) in the mesenteric collecting duct, the vessel responded by reducing contraction frequency and amplitude and constricting the overall vessel diameter, events that could be mediated by release of prostaglandins. In vitro, treatment of LECs with very-low density lipoproteins increased intracellular calcium accompanied by a significant loss of diastolic relaxation suggesting that, as in the blood vasculature, lipids can directly alter shear-mediated events in the lymphatics. These findings suggest that high lipids could negatively impact lymphatic function and warrant further studies to understand the mechanisms involved.

**CD36**

CD36/FAT (SR-B2), is a 75- to 88-kDa heavily glycosylated transmembrane protein expressed in several tissues and cell types including small intestine, muscle, the capillary endothelium, adipocytes and macrophages. CD36 binds a range of ligands including long chain FA, native or modified lipoproteins, pathogen-associated lipids and thrombospondin-1. CD36 facilitates fatty acid transport and plays a role in intestinal lipid absorption and transport into the lymph. CD36 null (Cd36−/−) mice have impaired TG and cholesterol secretion into the lymph after lipid infusion via a duodenal fistula despite producing smaller CMs. In humans, single nucleotide polymorphisms in the CD36 gene
that decrease CD36 protein level\textsuperscript{87,88} associate with defective CM production and clearance,\textsuperscript{89} but there is no information on their potential influence on lymphatic transport during lipid absorption.

Recent findings showed that conditional deletion of C\textit{d}3\textit{6} in endothelial cells (using a Tie2 promoter), results in a leaky epithelial barrier with neutrophil infiltration and inflammation, and associates with abnormal extracellular matrix remodeling in the proximal small intestine, as observed in germine C\textit{d}3\textit{6}\textsuperscript{-/-} mice.\textsuperscript{90} Systemically, endotheliosis is observed after an intragastric bolus of triolein consistent with presence of a leaky gut. These results show that endothelial CD36 is important for vascular homeostasis and highlight potential role of the blood and lymphatic endothelium in initiating gut inflammation.\textsuperscript{90}

CD36 has established functions in cellular signaling\textsuperscript{91,92} related to lipid metabolism, angiogenesis, and inflammation.\textsuperscript{84} For example, CD36 binding thrombospondin-1 promotes antiangiogenic signaling in microvascular endothelial cell via interaction of CD36 with VEGFR-2\textsuperscript{93} and decreases VEGF-mediated AKT activation.\textsuperscript{94} CD36 is highly expressed in LECs\textsuperscript{1} but molecular regulation of lymphatic signaling by CD36 is unknown. Proliferation of LECs also relies on FAO rather than glycolysis, and associates to higher expression of FA transport proteins, including CD36.\textsuperscript{17} Unpublished data from our laboratory show that CD36 enhances proteolytic cleavage of pro–VEGF-C to the active form and that it regulates VEGF-C-mediated survival, function and signaling in LECs (Cifarelli V et al, unpublished data).

**Lymphatic Transport of Cholesterol and Lipoproteins**

The lymphatic vasculature regulates removal of cholesterol from peripheral tissues\textsuperscript{95,96} and defective lymphatics impair reverse cholesterol transport from atherosclerotic plaques.\textsuperscript{97} The molecular mechanisms bridging lymphatic biology to chronic inflammatory disease such as atherosclerosis are emerging,\textsuperscript{98,99} but little is known on the regulation of cholesterol transport by gut lymphatics during overfeeding and the metabolic syndrome. In the intestine, key players in cholesterol transport and absorption across the enteroctye brush border are Niemann-Pick C 1-like protein 1, a cholesterol uptake transporter, and the adenosine triphosphate–binding cassette (ABC) proteins ABCG5 and ABCG8, cholesterol efflux transporters.\textsuperscript{3} The FA translocase CD36, expressed in epithelial cells lining the small intestine, has also been implicated in cholesterol transport on brush-border membranes.\textsuperscript{55,86} Once in the enterocyte, cholesterol is either incorporated into CMs, or released via ABCG8 to bind apolipoprotein A1 in high-density lipoproteins. Deletion of ABCG5 or ABCG8 reduces dietary TGs and cholesterol in the lymph,\textsuperscript{99} but the regulation of dietary cholesterol transport via gut lymphatics remains poorly studied.

**Role of VEGF-D in Lipoprotein Metabolism**

Similar to VEGF-C, VEGF-D is a ligand of VEGFR-3, but it does not appear to play a role in the regulation of lactic CM absorption. VEGF-D is produced by several tissues including the small intestine.\textsuperscript{100} A recent study showed that VEGF-D plays an important role in metabolism and uptake of CM remnants (CR) by the liver.\textsuperscript{101} VEGF-D deletion on a hyperlipidemic background (mice deficient of low density lipoprotein [\textit{ld}l] receptor and expressing only apoB100; \textit{ldl}-/-Apob100/-/) markedly increased cholesterol and TG accumulation in CMs and CR particles. Thus, VEGF-D regulates hepatic CR clearance rather than intestinal absorption, which is not affected in VEGF-D knockout mice.

**Conclusions and Perspective**

Accumulating evidence clearly show that impaired lactic lymphangiogenesis or modulation of lactic permeability via cell-cell junctions protects against diet-induced obesity and improves insulin sensitivity. However, the link between gut lymphatics and the metabolic syndrome is not completely understood and likely to involve multiple mechanisms. For example, enterendocrine cells of the small intestine secrete 2 incretin hormones such as glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 to promote glucose-stimulated insulin secretion in response to the absorption of fat and carbohydrate. Changes in lactic permeability and/or lymphangiogenesis could redirect glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 toward the blood circulation where they would be rapidly degraded. This would likely contribute to affect adiposity and improve insulin sensitivity observed in models of defective lacteals. The lymph fistula rat model is well suited for the study of incretin secretion by the gastrointestinal tract\textsuperscript{58} and offers new research opportunities to address these fundamental questions. In addition, more research is required to elucidate roles and mechanisms of VEGFs and their respective receptors in regulating lipid trafficking and flux in peripheral tissues such as the liver whose lymphatics are poorly studied.\textsuperscript{102} Nrp1;Flt1 mutants had reduced liver TG content and did not develop hepatic steatosis after 8 weeks of high-fat-diet feeding compared with the control group.\textsuperscript{10} However, whether defective gut lymphatics leads to less accumulation of lipid in the liver has not been comprehensively studied and it is likely to be an important contributor to the improved insulin sensitivity observed in models of defective lacteals during diet-induced obesity.

The importance of gut lymphatics in lipid metabolism is now emerging. Numerous studies have advanced our understanding of the regulation of lipid uptake by gut lymphatics, emphasizing that modulation of lactic permeability and proliferation could reverse diet-induced obesity in rodent models. However, more research is needed to address the specific link between gut lymphatics and systemic metabolism. The development of experimental models for studying intestinal lymphatics will be important in examining its role and molecular mechanisms in physiological and pathophysiological conditions.

Metabolic pathways impact lymphangiogenesis; glycolysis provides energy for proliferating lymph vessels, while FAO regulates lymphangiogenesis.\textsuperscript{102} However, the molecular
pathways activated in gut lymphatics during lipid intake and how they are altered with overfeeding are still unknown. A better understanding of these pathways could help develop new therapies for the treatment of obesity by reducing the absorption of excess dietary lipids and regulating insulin resistance.

A bidirectional crosstalk exists between the lymphatic vasculature and adipose tissue. The effect of obesity and associated complications such as hyperinsulinemia and inflammation on gut lymphatic function is largely unexplored. Chronic inflammation, endotoxemia and changes in the gut microbiota, key features of the metabolic syndrome are now emerging as factors that impair the gut microbiota, key features of the metabolic syndrome are now emerging as factors that impair the gut microbiota, key features of the metabolic syndrome.

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The authors regret not being able to cite the work of all studies related to this topic because of space restrictions.

Conflicts of interest
The authors disclose no conflicts.

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